NONVITAMIN AND NONMINERAL NUTRITIONAL SUPPLEMENTS

Edited by

Seyed Mohammad Nabavi and Ana Sanches Silva

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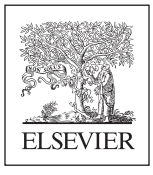

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Dedication

With memory of Seyed Ali Asghar Nabavi, I dedicate this book to my family.
Seyed Mohammad Nabavi

To my children, Inês and João, and my husband, Ricardo.
Ana Sanches Silva

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Foreword

The industry of nutritional supplements is increasing worldwide, and health professionals and consumers demand evidence that supports the benefits and safe use of these supplements. This book aims to assess the clinical evidence of many nonvitamin and nonmineral (NVNM) nutritional supplements available in the market, in order to be a valuable decision-making tool.

This book aims also to be an instrument for medical doctors, pharmacists as well as all health practitioners who did not receive comprehensive education regarding supplementation in order to assure their rational and safe use of NVNM supplements.

The book firstly addresses the history, definition, and legislation of dietary supplements. In the second part, it focuses on nonessential nutrients: *S*-adenosyl-methionine, astaxanthin, lutein, and zeaxantine; chondroitin and glucosamine; choline; carnitines and L-cysteine; creatine; coenzyme Q10 and embelin; quercetin; lipoic acid; methylsulphonylmethane; melatonin; resveratrol; and rutin and curcumin. The third part of the book is devoted to the extracts from plants and algae. Forty-six plant and algae extracts are addressed, including: artichoke (*Cynara scolymus*); beet (*Beta vulgaris*); bilberry (*Vaccinium myrtillus*); tailwort (*Borago officinalis*); brown algae (Phaeophyceae); cruciferous vegetables (Brassicaceae); elderberry (*Sambucus nigra*); fenugreek (*Trigonella foenum-graecum*); feverfew (*Tanacetum parthenium*); ginseng (*Panax ginseng*); goji berry (*Lycium barbarum*); horse chestnut (*Aesculus hippocastanum*); ispagula (*Plantago ovata*); konjac (*Amorphophallus konjac*); mangosteen (*Garcinia mangostana*); orthosiphon (*Orthosiphon stamineus*); rhodiola (*Rhodiola rosea*); St. John's wort (*Hypericum perforatum*); turmeric (*Curcuma longa*); and pomegranate (*Punica granatum*). The fourth part of the book is dedicated to animal extracts including bee products (royal jelly and propolis), chitosan, and shark cartilage, while the fifth part addresses extracts from yeast and fungi (e.g., *Saccharomyces cerevisiae*, *Monascus purpureus*, Reishi mushrooms, and *Ophiocordyceps sinensis*).

Chapters dedicated to NVNM nutritional supplements give information on sources, availability, relation with health, and possible interactions of these with other supplements, drugs, or foods, based on clinical evidence. Finally, the challenges of food supplements are discussed and future trends are presented.

We are conscious of many other NVNM nutritional supplements that could have been addressed. We hope that this edition will be well received and that in the future, other NVNM nutritional supplements can be addressed.

Seyed Mohammad Nabavi
Ana Sanches Silva

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Preface by Maria Daglia

As of 2016, the global food supplement market was valued at USD 132.8 billion, and it is expected to reach USD 220.3 billion in 2022, growing at a compounded average growth rate of slightly less than 9% over the next 5 years. While vitamins and mineral salts are the best-selling food supplements, and vitamins represent the largest ingredient segment in the dietary supplement market, there is an increasing market for substances with physiological effects and botanical extracts, including certain herbal drugs commonly found in traditional medicine, and this market is expected to grow rapidly in the next few years. This increasing trend is due to a growing interest in the healthy properties of nonnutrient minor food components (i.e., carotenoids, polyphenols, etc.) and botanical extracts, which, as reported by the World Health Organization, are the most important source of healthcare for many millions of people, and sometimes the only accessible and affordable source of care.

In particular, growing interest in plant extracts has resulted in a significant rise in scientific investigations on their biological activities. The results of these studies have led to the discovery that, besides pharmacological effects, many botanical extracts exert beneficial effects in maintaining the physiological functions and homeostasis of the human body, as well as reducing the risk factors responsible for physical and mental disorders, suggesting their possible use as ingredients in food supplements. Moreover, in this context, improvements in analytical techniques made over the last decade have led to better knowledge of the complex chemical composition of plant extracts, especially plant secondary metabolites, such as phenols, polyphenols and tannins, sulfur-containing compounds, alkaloids, and terpenes, to which the beneficial effects of botanical extracts are often ascribed. Thanks to progress in molecular biology, the molecular-level mechanisms by which some of these plant components exert their protective effects in humans have been found, reinforcing the arguments for their use.

In addition to these points, I would also like to emphasize that the market success of food supplements containing nonnutrient minor food components and plant extracts has likely been improved by their low intrinsic toxicity, at least at the concentrations to which people would be exposed by the consumption of food supplements, confirmed by the traditional use of botanical extracts, which reinforces the argument for their safety.

On that basis, considering the increasing number of nonnutrient minor food component and plant extract food supplements marketed worldwide, and considering the large body of literature data on the chemical composition and biological activities of nonessential nutrients and plant extracts published over the last few years, it would be very useful to have a text providing scientific information on these topics.

This book responds to this need, reporting the biological activities, traditional uses, and safety of the bioactive compounds and extracts from algae, animal, and fungi used most commonly as food supplement ingredients.

Maria Daglia

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Preface by Maurizio Battino

As a student, a researcher, and a professor of biochemistry and of nutrition, I have always desired a handbook where I could rapidly and efficiently find all up-to-date information regarding nonvitamin and nonmineral nutritional supplements, as these are usually very difficult to find in peer-reviewed literature in a summarized and useful format.

Moreover, legislation on much of these compounds is rare, or changes rapidly, and in any case is never considered in a scientific paper.

Therefore, I am very happy to welcome this idea led by very esteemed colleagues like Dr. Ana Sanches Silva and Seyed M. Nabavi, who decided to give the scientific and academic community a book extremely rich in information for the daily use of students, researchers, practitioners, and academics. As an enthusiastic user of such a resource, I am very grateful to all the authors for their efforts and the great result.

The book begins with a very interesting overview of dietary supplements in which great attention is devoted also to legislation aspects that are very important for daily research and work.

The book primarily covers 21 nonessential nutrients that are commonly used or investigated for their relevance in metabolism, as well as about 50 plant and algae extracts which are gaining attention daily and are of growing interest due to their high content in bioactive compounds.

Nowadays, it is very easy to find books or reviews in peer-reviewed journals with high impact centered and devoted to the role played by plant-derived bioactive compounds; however, these resources are often too specialized and rarely useful on a daily basis. Moreover, animal extracts or fungi or yeast extracts, even lower in number, are also of utmost importance with great involvement often as adjuvant in several pharmacological co-treatments. These aspects are critically and exhaustively discussed here, giving the reader an additional important tool for daily practice.

The challenges and foresight involved with food supplements are finally discussed in detail by the editors, who should be congratulated for having had such a good idea, and for having created a new, interesting, and useful book in a landscape of too many and often limited products.

I am delighted to present and support this book, which will represent a milestone for all of us.

Maurizio Battino

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Part I

Overview of Dietary Supplements

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Chapter 1.1

History, Definition, and Legislation

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INTRODUCTION TO NUTRITIONAL SUPPLEMENTS

The consumption of nutritional supplements has increased drastically in these past two decades. This parallels the accessibility of supplements to the consumer. Most of these supplements are advertised on the internet, newspapers, magazines, television, and radio. Out of all these social media, particularly the internet has attracted the attention of most consumers. Unfortunately, there is an issue with how much information is provided on these sites as well as whether the actual products are reliable and safe to the consumer. The consumption of nutritional supplements may be perceived as safe, but there is no proof that such products do not contain adulterants and sometimes toxic additives. Perhaps, more reliable nutritional supplements are available through retail outlets, particularly pharmacies, supermarkets, gymnasiums, and health-food shops.

PAST AND MODERN PERSPECTIVES ON FOOD SUPPLEMENTATION

During the past 2000 years, from Hippocrates till the advent of modem medicines, there was no distinction between food and medicine. In fact Hippocrates advocated that "Let food be thy medicine and medicine be thy food" (Smith, 2004). Careful selection of food and herbs helped to maintain the health of individuals and the general public. Today there are still residues of this philosophy, for example, the consumption of a high soy diet and reduced premenstrual syndrome in young Chinese women (Ho and Jiayi, 2012). With the advent of modern medicines and with the hope that conditions could be treated with synthetic agents, little attention was given to food intake and supplementation of diet with health-promoting natural products. Consequently, this new philosophy led to better prospects for controlling and curing conditions and diseases, but at the same time led to an increase in the incidence of diseases linked to unhealthy eating. With the reemergence of natural products as health-promoters, supplementing the normal diet, more care is now being given to ensure a correct and balanced diet, and healthy living. Today nutritional supplements form an integral part of the normal diet. So much so that these have become part of a holistic diet that complements a healthy lifestyle.

DEFINITIONS AND OTHER TERMS

The term "nutritional supplements" may cover a range of consumables fit for human consumption. These supplements may be categorized by composition or function. Some manufacturers assign a particular name to these nutritional supplements, in order to emphasise a particular characteristic of the product. Some supplements are termed as nutritionally functional foods. The term "functional food" refers to foods which contain additional beneficial supplements, and was first mentioned in Japan (Hasler, 1998).

Likewise, the word "nutraceutical" from "nutrition" and "pharmaceutical" was coined in 1989 by Dr. Stephen DeFelice (Brower, 1998). However, this is a loosely used word since one may further subdivide these functional foods into medicinal and nutritional functional foods. In principle, the first category deals with cases where patients suffering from a condition consume foods which reduce the negative impacts of a disease, possibly reducing drug intake. The second category deals more with consumers who take functional foods to mitigate disease and promote their health. Some authors define functional foods as products which supply the body with proteins, fats, carbohydrates, and vitamins amongst other nutrients required for healthy living (Kalra, 2003). There are other foods which incorporate nutritional supplements. Among these, medical foods, designer foods, genetically modified foods, and fermented foods, should be mentioned. Considering the different terminology used, their definitions suggest different compositions and functions. Medical foods contain a mixture of fats, carbohydrates, amino acids, vitamins, and minerals which yield therapeutic products in support of various metabolic conditions (Acosta et al., 1996). On the other hand, designer foods are manipulated traditional foods with added health

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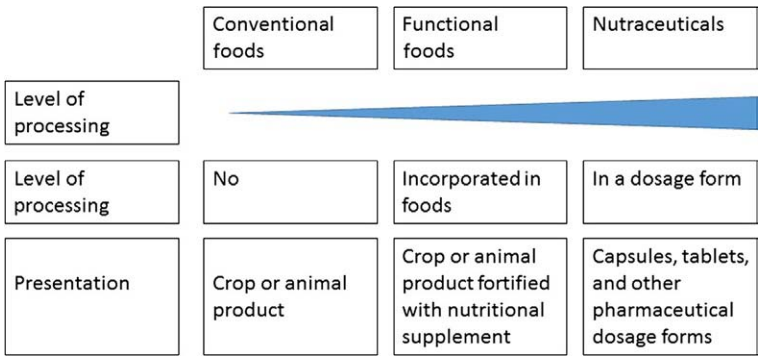

FIG. 1.1.1 Categorization of different foods and derivatives with respect to their properties. *Functional foods include medical foods, designer foods, genetically modified foods, and fermented foods.

benefits and may aim to reduce the risk of chronic diseases (Rajasekaran and Kalaivani, 2013). Medical foods, designer foods, and genetically modified foods contain health-promoting phytochemicals, which may be considered as supplementation to nutrition. However, genetically modified foods are derived from live organisms, mainly crops that have been modified to produce amounts of phytochemicals that are superior to those found in normal foods derived from conventional crops. All three categories may be considered as part of the normal diet. In spite of this, genetically modified crops and nutritional supplements are not legally accepted in every country. Fermented foods on the other hand usually contain live cultures as well as the health-promoting nutrients that may be produced by the organisms in these cultures. Fig. 1.1.1 shows the categories of different foods and derivatives with respect to their properties.

COMPOSITION OF NUTRITIONAL SUPPLEMENTS

Nutritional supplements are made up of bioactive substances derived from plants and animals. Those derived from plants are generally called phytochemicals. Most bioactive substances are usually part of whole foods. These may be present either individually or in a group of closely related substances. The later may have additive or synergistic effects within its biological systems. The role of these bioactive substances is to protect, prevent, and possibly cure several forms of diseases and chronic conditions (Lachance, 2008).

The amount of a natural product that needs to be consumed by an individual may not be theoretically feasible if it needs to be consumed directly as a plant or animal product. For example, to achieve the recommended daily intake of 60 mg of lycopene for a 60-kg adult an individual would need to have a daily consumption of approximately 600 g of ordinary tomatoes (Ilahy et al., 2011).

Phytochemicals, bioactive compounds derived from plants, are categorized into two main categories, products of primary metabolism and products of secondary metabolism. Primary metabolites encompass carbohydrates, fats, proteins, vitamins, and minerals. Secondary metabolites include terpenoids, glycosides, flavonoids and phenylpropanoid derivatives, tannins, and alkaloids, among others. In general, primary metabolites are related to the normal physiological functions of the body. However, some vitamins and minerals may also be considered as support substances against diseases, for example, vitamin C for colds and calcium for osteoporosis. However, vitamins and minerals are outside the scope of this book. Secondary metabolites are bioactive compounds that may exert a pharmacological or toxicological effect beyond the physiological effects of primary metabolites. In fact, these metabolites may be found as nutritional supplements and/or medicinal supplements (Fig. 1.1.2). However, these two categories fall under different legal classes, which may not be explicitly distinct from each other.

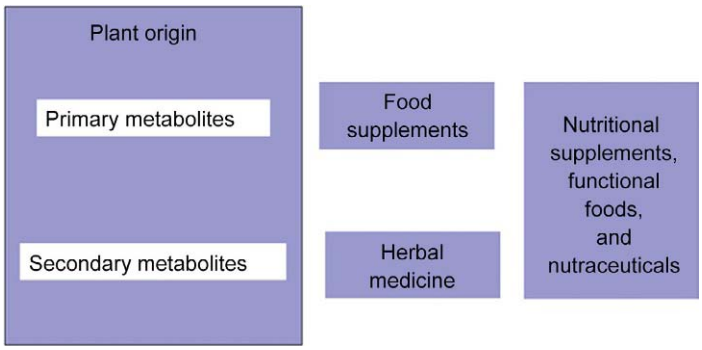

FIG. 1.1.2 Categorization of products with respect to their content of primary and secondary metabolites (Attard, 2009).

LEGAL IMPLICATIONS: FOOD SUPPLEMENTS OR MEDICINAL SUPPLEMENTS?

Although nutritional supplements are widely used in marketing, there is no regulatory definition for these products (Zeisel, 1999). The presentation of a supplement primarily determines the classification of a product as a food or as a medicine (Attard, 2009). In the United States, medical foods and dietary supplements are regulated, while functional foods and nutraceuticals are subject to publicity and consumer trends (Aarts, 1998; Hasler, 1998). In Canada and the European Union, functional foods are food products while nutraceuticals are usually considered as medicinal products.

There are several regulators bodies worldwide, controlling the manufacture and importation of food and food supplements. The main authorities include the United States Food and Drug Administration, the European Food Safety Authority, Health Canada's the State Food and Drug Administration, the Food Safety and Standards Authority of India, and Ministry of Health, Labour and Welfare in Japan. In spite of all these authorities, only one country has a regulatory framework for functional foods, known as the Foods for Specified Health Use. In fact in Japan, around 100 products bear a special licence approved by the Health Ministry (Arai, 1996). Within the European framework, some nutritional supplements fall under Directive 2002/46/EC (EU, 2002) while medicines are regulated by Directive 2001/83/EC, as amended, and Directive 2004/24/EC (Attard, 2011a). Natural substances that may be classified as food supplements are not fully supported by the aforementioned directives, so much so that the annexes for the food supplements directive includes mainly vitamins, minerals, and related substances only (Attard, 2009). Placing a nutritional supplement on the market is less costly than placing a natural medicine. Manufacturers may still include health-promoting claims on the product's description but minimal research is necessary for the marketing of the product. In the case of medicines, manufacturers need to abide by the rules and regulations that cover medicines, and so it is more time consuming and costly to place a medical product on the market. Consequently, manufacturers tend to market their products as food supplements (Brower, 1998). The judgement of a product as a food supplement or medicine is not yet consistent between authorities within countries. This provides a problem which usually places these products in a "gray area," sitting between foods and medicines. As a matter of fact, the competent authority within a country has to decide on whether the product should be considered as a food or a medicine.

SUPPLEMENT CONSUMERS

In recent years, several studies have been conducted to identify the main consumers of nutritional supplements. Consumers of supplements tend to fall into one of three categories: the ageing population; those following an alternative philosophy to medical control and treatment of diseases; and those that have the perception that green products are healthy and safe to consume.

The major consumers of nutritional supplements are children between the ages of 1 and 5 years (Bowering and Clancy, 1986; Kovar, 1985). However, in the majority of cases these include vitamin and mineral preparations. On the other hand, according to Ervin et al. (1999), the consumption of nonvitamin and nonmineral preparations was mainly attributed to females and those with higher incomes and a higher educational status.

Adolescents are more interested in sports performance and supplements which allow them to consume more energy. This is usually achieved through the consumption of nutritional supplements and sports and energy drinks (O'Dea, 2003). Most of the adolescents obtain these supplements from their parents. Other adolescents consider these sports drinks a way of maintaining good health by building more muscle tissue and making them feel more energetic (O'Dea, 2003). Some adolescents believe that nutritional supplements may prevent trivial conditions such as common colds. Considering older age groups, it has been reported that the majority of people who maintain a healthy lifestyle are actually middle-aged females, who are educated and who dedicate sufficient time to their well-being. A healthy lifestyle is linked to the consumption of fruit and vegetables as well as taking exercise. One study suggests that individuals who wish to pursue a healthy lifestyle, but due to commitments and a lack of time cannot afford to plan meals or take exercise, may be helped by the intervention of food companies providing attractive, healthy lifestyle products for consumption (Divine and Lepisto, 2005). The beverage industry has investigated this and confirmed that there are several products, including beverages, which are supplemented with ingredients such as guava and pomegranate (Beverage Industry, 2004).

Recently, a study carried out by Garcia-Alvarez et al. (2014) revealed that in a survey of 2359 consumers, a third of them take supplements periodically with almost another third taking supplements when the need arises. The majority of these consumers are relatively health conscious, that is, they did not smoke or drink alcohol, particularly at the time they started consuming nutritional supplements. On the other hand, more than one-half admitted that they never reverted to complementary and alternative medicines as a form of treatment. This shows that consumers tend to control their health using

nutritional supplements rather than resorting to therapeutic measures. The main nutritional supplements reported were ginkgo, evening primrose, and artichoke. Other studies noted ginseng and Echinacea as commonly consumed nutritional supplements (Perkin et al., 2002). As consumers of nutritional supplements are spread across all age groups, industry should deal with the needs of these groups accordingly.

EVIDENCE-BASED TRIALS AND CLINICAL TRIALS

Nutritional supplements have been implicated in the prevention and/or control of several medical conditions and diseases. Though the use of certain supplements has been advocated since ancient times (Smith, 2004), traditional evidence may not justify the use of certain supplements. Recently, researchers and the supplement industry itself have dedicated time and money to research the science behind nutritional supplements in human health. Although nutritional supplements are not intended to be used as a treatment for a particular disease or condition, their role may be either less specific or else implicated in delaying the onset of a disease or reducing the impacts of a disease or condition in an individual. This research has dealt with two categories of individuals: healthy individuals who wish to maintain a healthy lifestyle and diseased individuals who wish to control their condition, perhaps by reducing their medication and their dependence on conventional therapies.

However, to justify the use of nutritional supplements to prevent or control several medical conditions means that in vitro and in vivo studies are required along with clinical trials (Piersen et al., 2004). Nutritional supplements are derived from complex nutrient matrices, usually from botanical sources. Therefore, any experimental elaboration should be conducted on the isolated botanical extract itself, and in some cases their constituents should be considered too. Some researchers argue that the isolation of single constituents may not provide the best information regarding the mechanisms of action of these nutritional supplements (Liu, 2003).

Many botanical nutritional supplements are claimed to provide antioxidant protection to the body. Oxidants and free radicals are known to be generated either endogenously by cell metabolism (Finkel and Holbrook, 2000) or else exogenously through pollution (Risom et al., 2005), radiation (Robbins and Zhao, 2004), cigarette smoking (Van der Vaart et al., 2004), and xenobiotics, including pesticides (Abdollahi et al., 2004). In such cases an overload of oxidants may occur as they are not normally destroyed by the body (Pham-Huy et al., 2008).

This state is termed oxidative stress, and plays a very important role in the development of chronic and degenerative illnesses, such as cardiovascular and neurodegenerative diseases (Mariani et al., 2005), diabetes (Giacco and Brownlee, 2010), cancer (Reuter et al., 2010), autoimmune disorders (Bashir et al. 1993), and rheumatoid arthritis (Wruck et al., 2011) amongst others.

Clinical trials associated with nutritional supplements are always a point of debate, particularly when they are claimed to delay or prevent disease. It is impractical to consider whether a large number of healthy individuals would develop a particular condition over a number of decades whilst utilizing a specific nutritional supplement. However, scientists have studied the regular consumption of particular fruits and vegetables in a particular population and correlated the lack or low incidence of a disease to the consumption of such foods. Metaanalytical methods have been used to collate and analyze a number of studies in order to determine the statistical justification of a particular nutritional supplement for a disease. Such correlations include lycopene and breast cancer (Dorgan et al., 1998), flavonols and coronary heart disease (Huxley and Neil, 2003), and soy (isoflavones) and prostate cancer (Yan and Spitznagel, 2009), amongst others. However, these correlations should be treated with caution (Egger et al., 1998).

SAFETY OF NUTRITIONAL SUPPLEMENTS

The safety of nutritional supplements is generally unchallenged when marketed, unlike the case of herbal medicines which undergo rigorous testing prior to their market placement (Attard, 2011b). There have been cases, in recent years, where a nutritional supplement has been responsible for a number of patients being diagnosed with severe hepatitis and liver failure. The problem with these products may be intrinsic, that is, they may contain phytochemicals which may provoke liver damage, such as pyrrolizidine alkaloids. However, intentional addition of adulterants by companies has also been observed. There are more than 500 nutritional supplements adulterated with synthetic drugs and analogues. Such adulterants include anabolic steroids, stimulants, antidepressants, and banned weight-loss medications (Cohen, 2014). Some studies also interpreted the presence of very minute quantities of adulterants emanating from cross contamination on production lines. This may have resulted from insufficient cleaning after producing one supplement, before moving on to producing another (Geyer et al., 2008). Although pharmacovigilance is well structured and applies to conventional and natural medicines prescribed by doctors, in the community or hospitals, nutritional supplements are not monitored likewise. Therefore, adverse effects are not diagnosed early, and by the time an adverse effect is discovered a number of patients will have

already developed undesired symptoms In addition, as nutritional supplements are sold over the counter, it is likely that such products are consumed more abundantly than prescribed and controlled conventional and natural medicines. Reports have shown that some nutritional supplements, used by athletes, contained stimulants such as caffeine, ephedrine, and even methylenedioxymethamphetamine (De Hon and Coumans, 2007).

Although the discovery of the presence of doping agents in nutritional supplements used in athletics may be valuable, the number of products used and number of events, with doping testing, are insufficient to provide information on the introduction of such illegal substances within such products. The problem arises with those nutritional supplements that contain new "designer" steroids, which are not on the list of banned steroids (Geyer et al., 2008). Therefore, such substances cannot be legally controlled.

Nutritional supplements may interact with medical treatment (Cassileth et al., 2009). Such effects have been discovered either from the viewpoint of the nutritional supplement or the medication. For example, on the one hand, this may occur when a nutritional supplement is found to interfere with cytochrome P450 and/or P-glycoprotein systems, hence resulting in an increase or decrease in the plasma levels of a conventional medicine (Attard, 2012; Mallet et al., 2007). For example, genistein, a soy isoflavone, interferes with the efficacy of tamoxifen in animal models of breast cancer (Ju et al., 2002) and epigallocatechin gallate, in green tea, interacts with bortezomib (Golden et al., 2009). On the other hand, some medications are said to interact with a number of nutritional supplements. The classical example is warfarin which is affected by a number of nutritional supplements either directly through their effects on platelets or indirectly by interacting with liver enzymes (Nutescu et al., 2006).

Therefore, food supplements should be taken with caution by patients who are suffering from chronic conditions (Elinav et al., 2007). There have been cases where an adverse reaction in an individual has been misinterpreted as a new medical disorder (Mallet et al., 2007). This may be avoided if the patient seeks advice from a pharmacist or mentions to the doctor about the use of food supplements prior to the doctor prescribing medication.

CONCLUSION

Nutritional supplements are widely used by the general public worldwide. These supplements provide beneficial metabolites which may be effective in disease prevention or may be useful as adjuncts to clinical medication. Therefore, the public should be advised on the potential benefits but also of any potential hazards that these supplements may incite to their health. On the other hand, manufacturers should seek to provide safer nutritional supplements, with proper labelling and claims. Therefore, quality and safety should be a priority during the manufacturing process. Consideration should also be given to a proper declaration of the efficacy of the product. This should be done whilst acknowledging that some nutritional supplements are classified as medicines in some countries. Finally, some nutritional supplements, already present on the market, still lack the necessary research and development. This should be strengthened in the near future to reduce the incidence of nutritional supplement—drug interactions and the adverse effects of these supplements in vulnerable patient groups such as those suffering from kidney or liver problems, pregnant/breast feeding women, children, and the elderly.

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Part II

Nonessential Nutrients

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Chapter 2.1

S-Adenosylmethionine (SAMe)

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INTRODUCTION

S-adenosylmethionine (SAMe, also known as AdoMet, Fig. 2.1.1) is one of the rare naturally-occurring sulfonium ions. The positively charged sulfonium center provides SAMe with a chemical versatility comparable only to a few other biomolecules such as adenosine triphosphate (ATP). Thus, in addition to being used in a myriad of metabolic pathways, the types of biochemical processes in which it participates are highly diverse, ranging from acting as an alkyl donor to a generator of free radicals. SAMe, along with its metabolites play important roles in the metabolism of a plethora of organisms.

STRUCTURE

The sulfur present in SAMe is a part of L-methionine, and in SAMe is connected to the 5′-carbon of 5′-deoxyadenosine through a sulfonium linkage. The 5′-deoxyadenosine moiety is derived from ATP. The stereochemical configuration at the sulfur in enzymatically formed adenosylmethionine has the absolute configuration of (S) which however, racemizes to its enatiomeric counterpart (R) with a half-life of several days. The carbons attached to the positively charged sulfur are electrophilic in nature and are susceptible to nucleophilic attack. The α-amino group of SAMe has an unusually low pKa of 7.8, compared to 9.2 for methionine, due to the proximity of the sulfonium cation. For the same reason, the carboxylic acid present in the SAMe side chain also has a slightly reduced pKa of 1.8 compared to 2.2, atypical for methionine.

OCCURRENCE

SAMe, found in all living organisms, was originally reported by Cantoni (1952). Humans synthesize nearly 7 g of SAMe per day, mainly in the liver. SAMe biosynthesis was found to result from ATP and methionine (Eq. (2.1.1)) catalyzed by SAMe synthetase [also known as methionine adenosyltransferase (MAT)].

$$\text{L-Methionine} + \text{ATP} \underset{}{\overset{\text{MAT}}{\rightleftharpoons}} \text{SAMe} + \text{PP}_i + \text{P}_i \tag{2.1.1}$$

The inorganic pyrophosphate (PP_i) formed in addition to SAMe is hydrolyzed by inorganic phosphatase to two equivalents of phosphate (P_i). Therefore, the synthesis of SAMe is metabolically costly due to consumption of all three phosphoryl groups present in ATP. The requirement for pyrophosphate hydrolysis has been attributed to the necessity of forming a thermodynamically favored product owing to the "high energy" nature of the sulfonium moiety (McQueney et al., 2000).

AVAILABILITY

SAMe was first reported in Italy. It has been marketed in some European countries since the mid-1980s for the treatment of depression and for other medical conditions such as osteoarthritis, fibromyalgia, liver disease, and migraine headaches (Chavez, 2000; Di et al., 2000; Papakostas et al., 2003; Shippy et al., 2004). In the United States, it is not approved by the food and drug administration (FDA) and therefore has not been classified as a drug. However, it is available as a nonprescription (over the counter) dietary supplement under the Dietary Health and Supplement Act of 1999 (Papakostas et al., 2003).

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00002-3

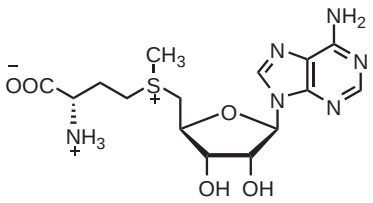

FIG. 2.1.1 Chemical structure of *S*-adenosylmethionine (SAMe).

COST

Commensurate with most drugs, the price of SAMe varies between countries. In the USA, CVS pharmacy sells 40 SAMe 400-mg tablets for USD 39.49. In Italy, the price of 20 SAMe 400-mg capsules costs in the range EUR 24–28. In the UK, 60 such tablets can be obtained for GBP 45.95. In some countries such as Italy, Germany, and Russia, pharmaceutical-grade SAMe is available on physician prescription only.

DOSE

The daily recommended doses of SAMe range from 200 to 1600 mg in a divided dosing scheme. However, this depends on the route of administration (ROA) and the condition for which it is being taken, as well as the severity of the condition (Chavez, 2000; Delle et al., 2002; Morelli and Zoorob, 2002). Exogenous, orally administered SAMe usually has a short half-life, undergoing first-pass effects and rapid metabolism. However, a nonlethal oral dose of SAMe at 1600 mg/day is significantly bioavailable (Gören et al., 2004). Since SAMe is best absorbed on an empty stomach, it should be administered 30–60 min before meals or 2 h after meals; people should be instructed to adhere strictly to these directions. It could also be administered parenterally (nonoral) or using intramuscular (IM) or intravenous (IV) routes (Williams et al., 2005).

BIOLOGICAL FUNCTIONS

Major biological functions of SAMe are related to its involvement in various metabolic pathways as described in Fig. 2.1.2, some of which are discussed below.

The Role of *S*-Adenosylmethionine Acting as Methyl Donor

Methylation is the most common route of utilization of SAMe, depleting almost 80% of the SAMe occurring in mammals. SAMe is the most widely used methylating agent in living creatures, modifying nucleic acids, proteins, and a plethora of small molecules giving rise to most of the one-carbon metabolism. The coproduct of these methylation reactions is *S*-adenosylhomocysteine (SAH), which is a potent inhibitor of most methylases. This process is regulated by the relative intracellular concentrations of SAMe and SAH ratio though the modulation of activities of the enzymes catalyzing these processes.

DNA and RNA Methylation

The intermolecular methyl transfer from SAMe to various nucleic acids has important implications for various biological process including DNA replication, transcription, and on RNA function. In humans, methylation of "CpG islands" is associated with the transcriptional inactivity of specific genes, in processes that include tissue-specific gene expression, and in epigenetic phenomena such as hereditary imprinting and X-chromosome inactivation (Rice and Allis, 2001).

RNA methylation leads to a range of diverse modifications, with varied biological significances which are sometimes unclear. Methylation of the N7 of guanine by SAMe leads to "capping" of eukaryotic messenger RNA, which is important for the stability of mRNA and nuclear export.

Protein Methylation

SAMe is associated with the methylation of a variety of polar side chains, including the sulfhydryls of cysteine and methionine, carboxylates of aspartate and glutamate, the imidazole of histidine, the amides of glutamine and asparagine, the guanidinium of arginine, the ε-amino group of lysine, and terminal amino groups (Clarke, 1993). For arginine or lysine side chains, multiple methylations by SAMe can occur, resulting in symmetrical and unsymmetrical dimethylarginine and dimethyllysine or trimethyllysine.

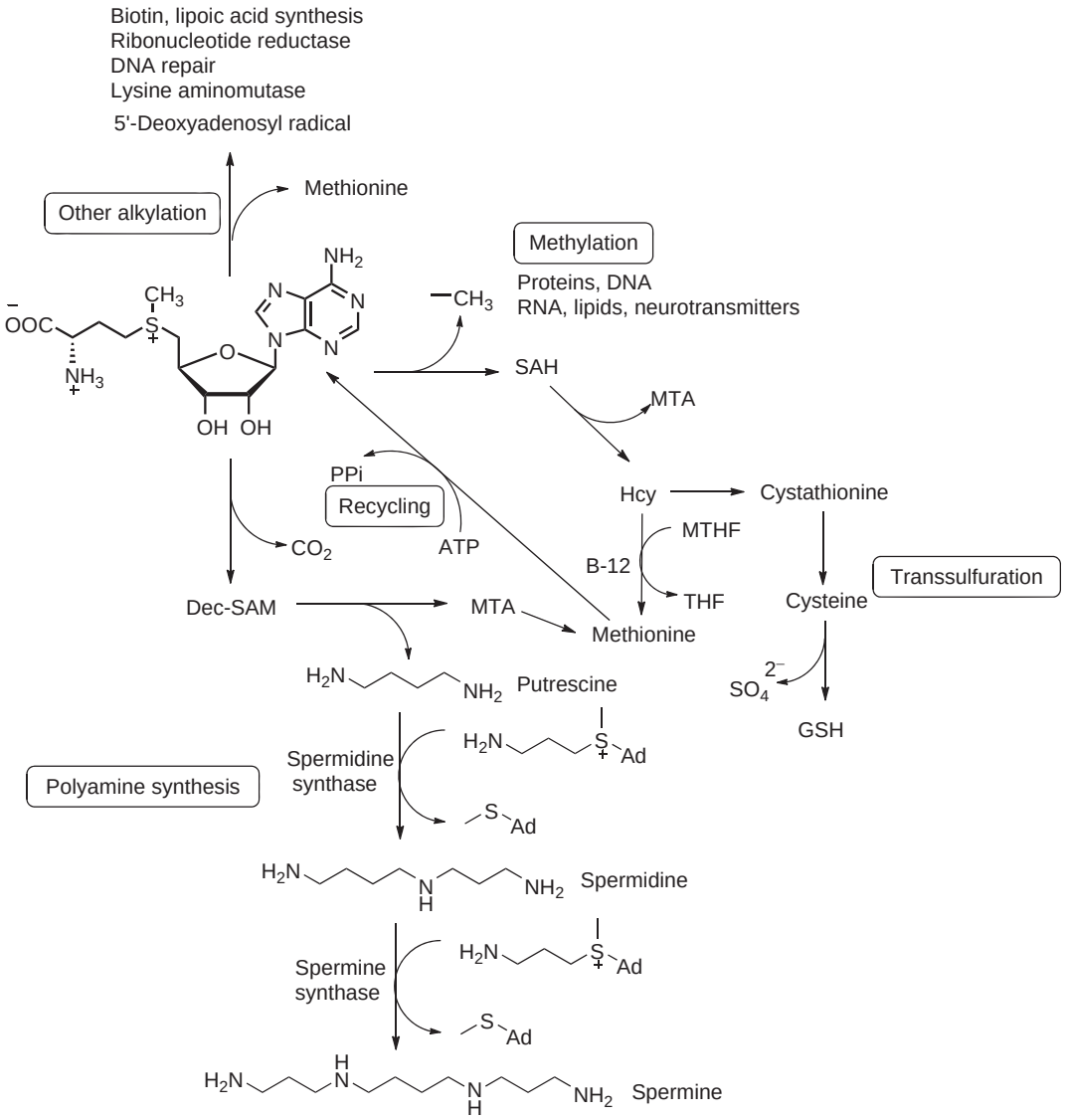

FIG. 2.1.2 Metabolic pathways for SAMe. *SAH*, *S*-adenosylhomocysteine; *Hcy*, homocysteine; *MTHF*, methyltetrahydrofolate; *THF*, tetrahydrofolate; *GSH*, glutathione; *Dec-SAMe*, decarboxylated SAMe; *MTA*, methylthioadenosine; *ATP*, adenosine triphosphate; *PPi*, pyrophosphate; *Ad*, 5′-adenosyl.

SAMe methylation on carboxylate is associated with the regulation of a variety of metabolic processes in humans. The rapid hydrolysis of carboxymethyl esters make the carboxylate methylations suitable for transient signaling. Many "G-proteins," such as RAS, have a carboxy-terminal CAAX signal sequence that elicits a multistep modification to yield a C-terminal cysteine that undergoes double alkylation–carboxymethylation and *S*-isoprenylation, both having SAMe as the alkyl donors. These modifications have important implications in targeting the protein towards membrane localization.

Nitrogen methylation on lysine, histidine, or arginine are irreversible in nature due to high energy cost of the nitrogen–carbon bond. Regulation of methylation and acetylation of lysine residues in histones by SAMe results in altering protein–protein interactions which leads to modification of the chromatin structure and finally to a modulation of eukaryotic gene expression (Rice and Allis, 2001).

Miscellaneous Methylation

SAMe leads to the production of many biomolecules in its metabolic pathway. It dimethylates the electron transport cofactor ubiquinone (coenzyme Q) and monomethylates guanidinoacetate forming creatine, which is used in energy storage. Amino groups present in the membrane constituent choline, the osmolyte betaine (*N*,*N*,*N*-trimethylglycine), as well as the neurotransmitter adrenaline (epinephrine) also serve as the substrates for *N*-methylation by SAMe. Glycine methylation in

liver provides an indirect source of homocysteine, which is one of the precursors of cysteine synthesis. SAMe-dependent methylation of sulfide precursors is also responsible for the formation of other sulfonium ions containing biomolecules such as *S*-methylmethionine (vitamin U) and the osmolyte dimethylsulfoniopropionate (DMSP).

Other Alkylations

A Baldwin allowed 3-exo-tet cyclization of the carboxy-bearing carbon on to the Cγ of the methionyl side chain of SAMe leading to 1-amino-1-carboxycyclopropane. Fatty acids containing cyclopropane are ubiquitous in the lipid membranes of many bacteria and eukaryotes and are used to regulate membrane fluidity. These rings are formed by methyl addition, from the side chain of SAMe to the double bond of a *cis*-unsaturated fatty acyl chain, and concomitant rearrangement.

Queuosine, the tRNA-based hypermodified nucleoside, incorporates the ribose moiety of SAMe into an epoxycyclopentane-modified 7-deazaguanine. On the other hand, the carboxylaminopropyl side chain of SAMe acts as the alkyl donors for the biosynthesis of several tRNA bases, as well as the hypermodified histidine residue diphthamide (2-[3-carboxyamido-3-(trimethylammonio)-propyl]histidine) in the protein EEF2 (eukaryotic elongation factor 2), and in the biosynthesis of secondary metabolites such as nocardicin β-lactam antibiotics.

The modified amino acid hypusine (N^{ε}-(4-amino-2-hydroxybutyl)lysine), found in protein elongation factor 5A of eukarya and archaea, is synthesized indirectly from SAMe through incorporation of the butylamine moiety from spermidine.

Transsulfuration

All the SAMe-dependent methylation reactions produce SAH (Fig. 2.1.2), which is metabolized rapidly to homocysteine. The latter then gets converted to cystathionine in a reaction catalyzed by pyridoxal phosphate (vitamin B6). This marks the initiation of the transsulfuration pathway which furnishes glutathione as the final product. Glutathione, a tripeptide, has important cellular functions including combating oxidative stress, aging, neurodegeneration, depression, and inflammation. Homocysteine could also act as the methyl acceptor for methionine synthetase and betaine-homocysteine methyltransferase reactions. Homocysteine metabolism is closely regulated by the intracellular methionine concentrations. When methionine synthesis becomes essential, homocysteine is remethylated by methyltetrahydrofolate (MTHF, Fig. 2.1.2). On the other hand, consumption of homocysteine via cystathionine synthetase is accelerated when methionine is in surplus.

Biosynthesis of Polyamines

The carboxyaminopropylamine side chain of SAMe is employed in the biosynthesis of the polyamines spermidine and spermine (Fig. 2.1.2). These cationic polyamines are built upon putrescine, which is derived from ornithine or agmatine, and are widely distributed in nature (Tabor and Tabor, 1984). Although polyamines take part in the regulation of cellular proliferation, their relatively low affinities for nucleic acids and other complexes reflect ready dissociation, which has rendered their exact molecular function elusive. In this pathway, SAMe is initially decarboxylated to furnish *S*-adenosylmethioninamine (Dec-SAMe, Fig. 2.1.2). This serves as the donor of the propylamine group required to convert putrescine to spermidine, and then finally to spermine (Pegg, 2009). The 5′-methylthioadenosine, the by-product formed in this synthesis, is recycled in some organisms into adenine and methionine through a complex set of reactions. While adenine can be freed by hydrolases or phosphorylases, and the corresponding 5-methylthioribose (or 5-methylthioribose 1-phosphate) converted to methionine in a sequence of steps (Miyazaki and Yang, 1987). This salvage pathway is essential for conserving the amount of reduced sulfur in the body.

In addition to propylamines, SAMe also serves as an amino donor in transamination reactions in the synthesis of the biotin component 7,8-diaminopelargonic acid (Berger et al., 1996).

Role of *S*-Adenosylmethionine as a Radical Generator

SAMe undergoes C5′-S bond cleavage to form the 5′-deoxyadenos-5′-yl radical which gives rise to a broader appreciation of SAMe's diverse biological roles (Fig. 2.1.3; Frey and Booker, 2001). In some radical reactions, SAMe acts as a free radical–carrying cofactor, similar to the function of coenzyme B12. Radical formation from SAMe was thought to be quite energetically demanding since the C–S bond is quite strong, approximately twice as strong as the C–Co bond in cobalamins. Iron–sulfur clusters, especially [4Fe–4S] electron transfer proteins, are postulated to be the electron donor in this radical formation reaction (Broderick et al., 2014).

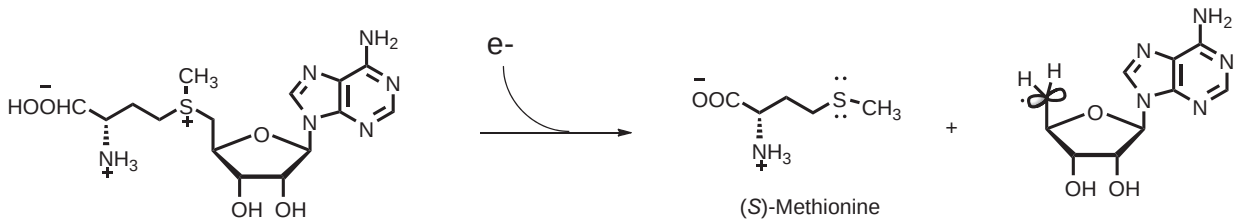

FIG. 2.1.3 Cleavage of SAMe into methionine and the 5′-deoxyadenos-5′-yl radical.

Role of *S*-Adenosylmethionine as a Regulator

SAMe acts as an important regulatory molecule in the metabolic pathways of amino acid, nucleotide, and sulfur metabolism. In yeast and some bacteria, SAMe transcriptionally regulates the gene for methionine biosynthetic genes which have been characterized genetically and biochemically in yeast and enteric bacteria. The SAMe-binding MetJ repressor protein of *Escherichia coli* has a three-dimensional topology that is unique among known protein structures. As examples of its more direct control of metabolism, SAMe allosterically regulates the eukaryotic cysteine biosynthetic enzyme cystathionine β-synthase, and the plant threonine synthase.

Medical Aspects

Use as an Antidepressant

SAMe is widely used for depression in adults in some countries due to its antidepressant activity. SAMe has been shown to demonstrate superior efficacy to placebo and efficacy equivalent to the first-line therapeutics such as tricyclic antidepressants (TCA; Arroll et al., 2009; Bressa, 1994; Williams et al., 2005).

To date, the mechanism of any antidepressant effect of SAMe remains elusive. It has been suggested that SAMe may increase the activity of the monoamine systems strongly associated with the etiology and treatment of depression. Animal studies demonstrated an association between SAMe treatment and increased brain concentrations of noradrenaline (norepinephrine) and serotonin (5-HT; Algeri et al., 1979; Curcio et al., 1978; Otero-Losado and Rubio, 1989a). In humans, dosing SAMe has been shown to increase concentrations of 5-hydroxyindole acetic acid (the main metabolite of serotonin) in cerebrospinal fluid (CSF; Agnoli et al., 1976). In addition, through stimulation of phospholipid methylation, SAMe may increase the fluidity of cell membranes, something which is linked to an increase in β-adrenoceptor and muscarinic (M1) receptor density (Bottiglieri, 2002). Further, SAMe may regulate the expression of key genes in the brain affecting memory, behavior, learning, and cognition (Sugden, 2006).

In spite of the clear need for new treatments for depression, and the apparent evidence for its efficacy, SAMe is not formally approved or widely used as an antidepressant treatment in many countries. It is important to consider SAMe as a potential treatment for depression management given the incremental costs for managing the same using existing therapeutic methods.

S-Adenosylmethionine Therapy for Chronic Liver Disease

The effectiveness of SAMe therapy has been investigated in a variety of chronic liver conditions. The effects of SAMe treatment in vivo in rat models of surgical cholestasis (bile duct ligation) have shown several benefits. Rats treated with SAMe and subsequently subjected to bile duct ligation for 7 days were found to show diminished oxidative stress, as measured by thiobarbituric acid reactive substances (TBARS), and to have a reduced amount of oxidized glutathione compared to its total amount (Gonzalez-Correa et al., 1997). The two largest studies that have examined the utility of SAMe therapy in this setting have both been conducted in patients with features of intrahepatic cholestasis (IHC) due to a mixture of different aetiologies (Fiorelli, 1999; Frezza et al., 1990). The first was a double-blind placebo-controlled trial conducted among 220 IHC patients, many of which did not have a well-established etiology and presented a range of disease stages (68% cirrhosis, 26% chronic viral hepatitis, 6% primary biliary cholangitis (PBC); Frezza et al., 1990). This study, with a 1600-mg/day PO (oral) treatment of SAMe, exhibited a significant reduction in clinical biochemical indices of cholestasis and ameliorated symptoms of fatigue and pruritus. This study also exhibited that significantly more SAMe-treated patients reported a >50% increase in general well-being over the placebo group (SAMe 84% vs. placebo 29%, $P < .01$; Frezza et al., 1990). This was further corroborated by a subsequent study in which 640 IHC patients were allocated to one of two different parenteral dosing schedules (500 mg/day IM or 800 mg/day IV) for 15 days in a nonrandomized, nonplacebo

controlled, observational study (Fiorelli, 1999). The majority of patients recruited had chronic viral hepatitis with or without concomitant excess alcohol consumption, and approximately 60% were cirrhotic at enrolment. A majority (over 60%) of the patients was found to exhibit significant improvements in subjective symptoms of pruritus and fatigue using a visual analog scale with the diminished serum markers of cholestasis also noted (Fiorelli, 1999).

Side Effects

Due to its prevalent usage, SAMe is thought to be safe for most adults. Some medicine may cause adverse drug–drug interactions. However, no specific interactions are known at this time. Before taking SAMe, it is advised the patient consult with a doctor or pharmacist if they have any medical conditions, especially if any of the following apply to them:

- If they are pregnant, planning to become pregnant, or breast feeding.
- If they are taking any prescription or nonprescription medicine, herbal preparation, or dietary supplement.
- If they have allergies to medicines, foods, or other substances.

SAMe should be avoided if the patient has used the following medications in past 14 days.

- Monoamine oxidase inhibitors (MAOIs). These include isocarboxazid, linezolid, methylene blue injection, phenelzine, rasagiline, selegiline, tranylcypromine, and others.
- Any narcotic medicine such as meperidine (Demerol), pentazocine or tramadol (Ultram and Ultracet).
- Any prescription cough medicine such as dextromethorphan (Robitussin).

In several studies, SAMe has been attributed to trigger mania (Carney et al., 1989; Lipinski et al., 1984). In one study, 9 of 11 individuals with bipolar disorder experienced a transition to an "elevated mood state" (hypomania, mania, or euphoria; Carney et al., 1989). Reports of induced mania and hypomania were found even in individuals with no prior history of bipolar disorder (Kagan et al., 1990). A short-term mania along with suicidal thought was reported in one person with no previous psychiatric history on SAMe; recovery followed discontinuation (Gören et al., 2004). These findings must be interpreted with caution as bipolar II disorder (diagnosed by the presence of a hypomanic episode) is sometimes misdiagnosed as major depressive disorder when hypomanic episodes are overlooked.

Theoretically, a long-term use of SAMe and dysregulation of its metabolism could lead to hyperhomocysteinemia, a medical condition characterized by an abnormally high level of homocysteine in the blood. However, in a 4-week study of SAMe treatment of healthy participants, no elevation in homocysteine levels was found (Gören et al., 2004); instead a mild headache and gastrointestinal disturbances were reported (Gören et al., 2004; Lipinski et al., 1984).

SUMMARY

SAMe is now sold as a nutraceutical and is utilized as a remedial for several human disorders. It has demonstrated an ability to treat diverse human disorders such as liver cirrhosis and arthritis. In addition, many tumor cells require methionine for growth, a characteristic that seems to be related to the perturbed metabolism of SAMe. Several inhibitors of SAMe decarboxylase have been explored clinically in the contexts of anticancer (Ham et al., 2013) and antiparasitic (Birkholtz et al., 2004; Reguerra et al., 2007) therapies. Moreover, there is enough experimental evidence of SAMe being very effective against depression and chronic liver disease, as discussed in detail in this chapter. For all these reasons, SAMe has made a successful transition from the laboratory to the supermarket.

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Chapter 2.2

Astaxanthin, Lutein, and Zeaxanthin

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INTRODUCTION

Carotenoids are a group of natural, fat-soluble pigments produced by bacteria, algae, yeasts, fungi, and higher plants. Fish and crustaceans cannot synthesize carotenoids endogenously. However, they can absorb them from their diet and store them in their bodies (Rüfer et al., 2008).

Astaxanthin is the main carotenoid found in aquatic organisms such as salmon, shrimp, and lobsters. The chlorophyte algae *Haematococcus pluvialis* and the yeast *Phaffia rhodozyma* accumulate high levels of astaxanthin (Ambati et al., 2014). In addition, astaxanthin is related to other carotenoids such as zeaxanthin and lutein. These pigments share many metabolic and physiological functions attributed to carotenoids (Guerin et al., 2003).

Astaxanthin can act as an antioxidant, with more activity than other carotenoids (Campoio and Oliveira, 2011). The antioxidant activity of astaxanthin and other carotenoids is attributed to the oxygenated groups in each ring present in their structure (Fig. 2.2.1). Diverse clinical studies have reported that the intake of carotenoids can decrease the risk of macular degeneration, cancer, and heart disease, as well as protecting against diverse microbial infections (Lorenz and Cysewski, 2000).

Lutein and zeaxanthin are two very well-known antioxidant carotenoids present in the retina, which protect the eyes against inflammation and oxidative stress (Melo van Lent et al., 2016); these carotenoids are found in the human brain from the first year of life (Bovier et al., 2014). Recently, it has been reported that these compounds can inhibit lipid peroxidation in membranes and protect the skin against high energy sources (Juturu et al., 2016). Both compounds are present in high concentrations in egg yolk, fruit, and in leafy green vegetables (Kalariya et al., 2012).

Astaxanthin is safe when it is consumed with other food; to increase its biovailability it can be mixed with vegetable oils (Ambati et al., 2014). The main source of natural commercial astaxanthin is the microalga *H. pluvialis*, although synthetic astaxanthin is also available. Lutein and zeaxanthin supplements are produced from *Tagetes erecta* extracts (Juturu et al., 2016).

The astaxanthin-based supplements (Miyawaki et al., 2008; Park et al., 2010; Tominaga et al., 2012; Zanotta et al., 2014) zeaxanthin (Schwartz et al., 2016) and lutein (Zhang et al., 2017) used in clinical studies are produced and commercialized in different countries.

Diet and nutrition are important for maintaining health and preventing diseases; commercial dietary supplements are a source of essential nutrients. According to Block et al. (2007), dietary supplements containing the carotenoids lutein, zeaxanthin, astaxanthin, lycopene, and β-carotene are among the most highly consumed.

This chapter presents a detailed literary review of diverse human clinical trials involving the consumption of astaxanthin, lutein, and zeaxanthin as nutritional supplements, based on their biological properties and therapeutic value.

CHEMICAL STRUCTURE AND PROPERTIES

Astaxanthin, zeaxanthin, and lutein belong to the xanthophyll family of carotenoids. The presence of a terminal hydroxyl and ketones in the ionone rings underlies the esterification ability, antioxidant activity, and greater polar configuration of these compounds compared to other carotenoids. Carotenoids act as antioxidants by quenching singlet oxygen and free radicals (Campoio and Oliveira, 2011). The antioxidant activity of astaxanthin is 10-fold greater than the antioxidant activity of other carotenoids, such as zeaxanthin, lutein, canthaxanthin, and β-carotene, and 100–500-fold greater than the activity of α-tocopherol.

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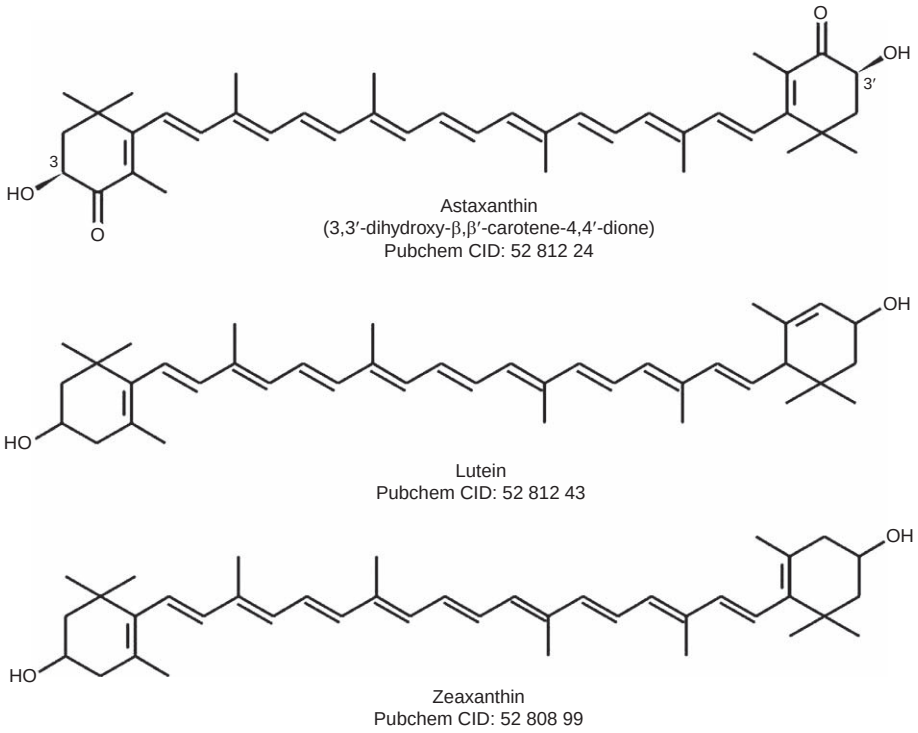

FIG. 2.2.1 Chemical structure of astaxanthin, lutein, and zeaxanthin.

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4-dione) is an oxycarotenoid; its name is derived from the crustacean *Astacus astacus*. This molecule is highly unsaturated and sensitive to high temperatures, light, and oxidative conditions that can cause the isomerization of astaxanthin to the *cis* form, which has lower activity than the *trans* configuration (Kittikaiwan et al., 2007). Because of the two stereogenic carbon atoms at positions C-3 and C-3′, astaxanthin exists in nature as a mixture of two enantiomers (3S, 3′S- and 3R, 3′R-astaxanthin) and a meso compound (3R, 3′S); the all-E-configuration is predominant (Grewe et al., 2007). Astaxanthin can exist in a free state or be esterified by fatty acids, forming monoesters and diesters (Núñez-Gastélum et al., 2016); it can also be associated with a protein, which is known as a caroprotein.

Lutein (β,ε-carotene-3,3′-diol) is a carotenoid belonging to the xanthophyll family (Kalariya et al., 2012). Its name is derived from the word *luteus*, meaning yellow. Structurally, lutein is comprised of a large carbon chain with a cyclohexenyl at each end containing a hydroxide group. The carbon chain has alternate double and single bonds, with lateral methyl groups (Kijlstra et al., 2012). Zeaxanthin (β,β-carotene-3,3′-diol), is a positional isomer of lutein. In both carotenoids, the hydroxyl groups allow orientation in cellular membranes and lipoproteins (Roberts et al., 2009).

NATURAL SOURCES

Astaxanthin

Astaxanthin is widely and naturally distributed in marine animals including, crustaceans, such as shrimp and crabs, and in fish such as salmon and trout (Yamashita, 2013). Astaxanthin and its esters have been isolated from the shells of lobsters (Maoka and Akimoto, 2008), river crabs (Meyers and Bligh, 1981), and shrimp exoskeletons (López-Cervantes et al., 2006; Sachindra and Mahendrakar, 2005). In crustaceans, astaxanthin accumulates primarily in the shell with its associated red–orange coloration only released following thermal or solvent treatment.

Astaxanthin is the main carotenoid in the microalga *H. pluvialis*, forming complex esters with several fatty acids. *Monoraphidium* sp., another microalga that produces astaxanthin, has a 10-fold greater concentration than *H. pluvialis* (Fujii et al., 2010). These microalgae could serve as continuous sources of astaxanthin following cultivation in large-scale bioreactors.

The red yeast, *P. rhodozyma*, is surprisingly different from other pigmented yeasts, because it synthesizes and accumulates astaxanthin as its main carotenoid pigment. The majority of astaxanthin produced is found in the free

form (Parajo et al., 1998). The astaxanthin concentration in *P. rhodozyma* ranges from 5 to 200 μg/g depending on the nutrients found in the growth medium (Johnson and Lewis, 1979). However, the highest astaxanthin yield produced by yeast is lower than levels found in microalgae, which are similar to those found in crustaceans.

Lutein and Zeaxanthin

Similar to other carotenoids, these two lipophilic pigments must be consumed in the diet because the human body cannot synthesize them. Lutein and zeaxanthin give egg yolks, animal fat, and the macula of the human retina their yellow color (Kalariya et al., 2012). Perry et al. (2009) determined that the main sources of lutein are spinach, cilantro, parsley, kale, unshelled pistachios, cooked egg yolk, and corn tortillas. They also reported that yellow corn flour, peppers, oranges, chives, and cooked egg yolks are sources of zeaxanthin.

BIOAVAILABILITY

Currently, only a few studies have examined the bioavailability of astaxanthin in humans. Carotenoids can be absorbed from the diet by passive diffusion into cells in the intestinal mucosa, where transportation is associated with plasmatic lipoproteins. Carotenoids have individual absorption patterns, metabolisms, and plasma transport mechanisms, all of which include geometric isomerization (Østerlie et al., 2000). Non-polar carotenoids (β-carotene and lycopene) are transported by low-density and very low-density lipoproteins; while polar carotenoids (zeaxanthin and lutein) are transported by low-density and high-density lipoproteins (Guerin et al., 2003). A considerable number of human studies have centered on the absorption, transport, and metabolism of carotenoids; however, limited information is available regarding xanthophylls.

Østerlie et al. (2000), investigated the distribution of astaxanthin isomers E/Z and R/Z in the plasma fractions and lipoproteins of three male subjects (aged between 37 and 43 years) following the ingestion of a 100-mg dose of astaxanthin. The maximum concentration of astaxanthin (1.3 mg/L) was reached at 6.7 h post administration and the half-life of astaxanthin was 21 h. Astaxanthin was found in very low-density lipoproteins (36%–64%), low-density lipoproteins (29%), and in high-density lipoproteins (24%). The isomer distribution of the lipoprotein fractions was not affected by time. The authors reported that astaxanthin from the diet was absorbed easily and incorporated into human plasma lipoproteins. In addition, they detailed the different capture mechanisms of astaxanthin E/Z isomers.

Because astaxanthin is a very lipophilic compound with low oral bioavailability, Mercke Odeberg et al. (2003) studied the bioavailability of astaxanthin in the presence of fats; the study included 23 healthy men aged between 20 and 46 years. All treatments included 40 mg of astaxanthin administered in one dose. Three formulations were prepared with different lipids and surfactant agents; the main source of astaxanthin in the formulations was the green microalga *H. pluvialis* as a powder rich in fats. Plasma concentrations were monitored throughout the assay and the absorption velocity of each formulation was evaluated. All formulations showed improved bioavailability; however, the formulation from glycerol monooleate and dioleate, with polysorbate-80 as the tensioactive agent, exhibited a 4-fold higher bioavailability than a commercial reference formulation.

In a recent study, Rüfer et al. (2008) examined the bioavailability and distribution of astaxanthin configuration isomers in human plasma following the ingestion of wild and cultivated salmon. The study involved 28 healthy men who consumed 250 g of wild or aquacultured salmon daily for 4 weeks. The level of the astaxanthin isomer standard in human plasma was found to be similar to that of the ingested salmon throughout the study. However, at day 28, the relative isomer proportion (3S, 3′S) was slightly higher and (3R, 3′R) was lower in human plasma than in the salmon meat. When participants were administered 1.25 mg of astaxanthin, the plasma concentration at day 3 ranged from 27.3 to 42.0 nmol/L. This result shows a nonlinear response to astaxanthin concentration in human plasma, possibly due to the saturation of absorption mechanisms and enterocyte transport at high doses.

ASTAXANTHIN IN HUMAN HEALTH

A series of studies have demonstrated that astaxanthin acts as a health promoter, specifically, in the prevention and treatment of cardiovascular, gastrointestinal, hepatic, neurodegenerative, ocular, and dermatological diseases, as well as diabetes, diabetic neuropathy, metabolic syndromes, cancer, and chronic inflammation (Yamashita, 2013). A number of human clinical studies are detailed in Table 2.2.1.

TABLE 2.2.1 Human Clinical Studies Using Astaxanthin as a Dietary Supplement

Reference/Study	Subjects and research design
Yoshida et al. (2010) Administration of natural astaxanthin increases serum HDL cholesterol and adiponectin in subjects with mild hyperlipidemia	Placebo-controlled study 61 nonobese subjects with moderate hypertriglyceridemia 0, 6, 12, and 18 mg/day of commercial astaxanthin for 12 weeks Aged between 25 and 60 years
Zanotta et al. (2014) Cognitive effects of a dietary supplement made from extract of *Bacopa monnieri*, astaxanthin, phosphatidylserine, and vitamin E in subjects with mild cognitive impairment: a noncomparative, exploratory clinical study	Prospective cohort, noncomparative, multicenter trial 104 subjects with minimental state One tablet daily containing astaxanthin (2 mg), vitamin E (30 mg), *Bacopa monnieri* dry extract (100 mg), and phosphatidylserine (30 mg) for a 60-day period Aged 71.2 ± 9.9 years
Katagiri et al. (2012) Effects of astaxanthin-rich *Haematococcus pluvialis* extract on cognitive function: a randomized, double-blind, placebo-controlled study	Randomized double-blind placebo-controlled study 96 healthy subjects One astaxanthin-rich capsule (6 mg/day or 12 mg/day) for 12 weeks Aged 55.7 ± 3.7 years
Kaneko et al. (2017) Protective effect of astaxanthin on vocal fold injury and inflammation due to vocal loading: A clinical trial.	10 nonsinger male subjects who consumed 24 mg/day of astaxanthin Aged between 23 and 30 years
Miyawaki et al. (2008) Effects of astaxanthin on human blood rheology	Single-blind method 10 adult men (57.5 ± 9.8 years) for a food study and 10 adult men (50.8 ± 13.1 years) for a placebo food study Two astaxanthin capsules once daily (6 mg astaxanthin/day) 10-day period
Tominaga et al. (2012) Cosmetic benefits of astaxanthin on human subjects	Two human clinical studies: Open label noncontrolled study with 30 healthy female subjects (aged between 20 and 55 years) for 8 weeks, 6 mg/day as oral supplement and 2 ml/day (78.9 μM/day) astaxanthin as topical application Randomized double-blind placebo-controlled study with 36 healthy male subjects (aged between 20 and 60 years) for 6 weeks, 6 mg/day as oral supplement
Park et al. (2010) Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans	Double-blind placebo-controlled study 14 healthy female subjects (aged 20.2–22.8 years) 0, 2 or 8 mg/day astaxanthin for 8 weeks

Improvements in Memory and Learning

It is widely accepted that many people experience a gradual cognitive decline as they age (Zanotta et al., 2014). Brain health during mild cognitive decline, senile dementia, and Alzheimer's disease is related to oxidative stress. Katagiri et al. (2012) reported that the brain tends to produce reactive oxygen species (ROS) and free radicals when high levels of glucose and oxygen are consumed. Given these findings, it has been proposed that antioxidants protect the brain from ROS, thus maintaining mental health. Lobos et al. (2016) confirmed the neuroprotective effects of astaxanthin found in an in vitro model, showing that a daily intake of astaxanthin is a beneficial strategy for managing Alzheimer's disease and other neurological disorders.

Katagiri et al. (2012) studied the effect of an astaxanthin-rich extract (*H. pluvialis*) on the cognitive function of healthy middle aged and elderly subjects, with age-related forgetfulness, by evaluating cognitive performance. The CogHealth scores improved in the high-dosage group, while the Groton Maze Learning test scores improved in the low-dosage group. These results demonstrate that astaxanthin-rich extract from *H. pluvialis* can improve cognitive function in healthy individuals.

Zanotta et al. (2014) designed a clinical study to investigate the cognitive effect of a commercial phytotherapeutic supplement containing astaxanthin (*H. pluvialis*) in subjects diagnosed with mild cognitive decline. Participants were evaluated using the Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) and the clock drawing test. Both scores showed statistically significant improvement. Memory tests consisting of individual components of

the ADAS-cog showed greater improvement. The perceived efficacy was evaluated as excellent or good for 62% of the subjects in the study.

Amelioration of Dyslipidemia

The coexistence of hypertension, deficient tolerance to glucose, and dyslipidemia represent a known metabolic syndrome, which increases the risk for developing type 2 diabetes and cardiovascular diseases (Yamashita, 2013).

Yoshida et al. (2010) presented the first study regarding the effect of astaxanthin on the lipid profile of humans. Comparisons between the assays showed that 12–18-mg/day doses significantly reduced triglycerides and increased the adiponectin hormone content in serum. In addition, a 6–12-mg/day dose significantly increased high-density lipoprotein (HDL) cholesterol.

Protection of the Vocal Fold

Professionals using their voices excessively, such as singers, priests, and teachers, can develop vocal disorders with symptoms such as fatigue, lethargy, and dysphonia. Kaneko et al. (2017) designed a clinical study to evaluate the protective effect of astaxanthin on vocal fold lesions. These lesions are known to be aggravated by ROS generated by inflammatory cells. The results of the study showed that astaxanthin has the potential to protect mucous membranes against lesions and inflammation. The antiinflammatory effect of astaxanthin led to a significant improvement in the aerodynamics, acoustic function, and GRBAS (grade, roughness, breathiness, asthenia, strain) scale associated with the vocal fold. Astaxanthin can prevent scaring of the mouth fold by regulating oxidative stress during the early stages of cicatrization.

Relief From Eye Fatigue

Significant improvement in relief from eye fatigue has been observed following the ingestion of astaxanthin (Yamashita, 2013). In addition, promotion of visual fatigue or asthenopia recuperation has been shown to be attributable to improved blood circulation in the peripheral systems. Based on these findings, Miyawaki et al. (2008) studied the effect of a continuous ingestion of astaxanthin on blood rheology by conducting a test that measured the total time of blood transit using heparinized blood. A reduction in blood transit time, varying from 52.8 ± 4.9 s to 47.6 ± 4.2 s, was observed, thus confirming an improvement in blood rheology.

Recovery of Skin Elasticity and Recovery From Skin Dryness

A number of studies have examined the dermatological activity of astaxanthin. Tominaga et al. (2012) studied the cosmetic effects of astaxanthin based on two factors, administration technique and gender. They found that astaxanthin derived from *H. pluvialis* can improve the status of all skin layers by combining oral supplementation with topical treatment in both women and men.

Oxidative Stress, Inflammation, and Immune Response

Chronic inflammation is a determining factor for diseases such as hypertension, diabetes, and atherosclerosis (Kishimoto et al., 2016). In this study, the authors established that astaxanthin possesses preventive action against atherosclerosis due to its potential to improve inflammation, lipid, and glucose metabolism.

According to a hypothesis proposed by Park et al. (2010), astaxanthin, acting as an antioxidant and antiinflammatory agent, can improve the immune response. This study examined the immunostimulant, antioxidant, and antiinflammatory activity of astaxanthin in young, healthy women. The immune response was evaluated at week 0, 4, and 8, with a tuberculin test carried out on day 8. Depending on the concentration, plasma astaxanthin increased after week 4 and week 8. Subjects who received 2 mg of astaxanthin showed a higher tuberculin response than subjects with no supplementation. In general, this study demonstrated that astaxanthin can improve the immune response and decrease biological markers of DNA damage and inflammation in participants.

The levels of fatty acids in plasma can increase as a result of obesity, excessive exercise, and diabetes. In addition, the production of free radicals can increase in diabetic patients and thus generate conditions of oxidative stress. Given that fatty acids are instigators of oxidative stress, and astaxanthin has antioxidant activity, Campoio and Oliveira (2011) evaluated oxidative stress in human lymphocytes caused by a mix of fatty acids, as well as the protective action of astaxanthin using an in vitro assay. The results of the study showed that fatty acids can increase the production of superoxide anions, hydrogen peroxide, and nitric oxide. Additionally, astaxanthin decreased the production of ROS and the prolific growth of

cells treated with fatty acids. Lastly, the authors mentioned that astaxanthin partially prevents oxidative stress in human lymphocytes by controlling the production of free radicals.

THE ROLE OF LUTEIN AND ZEAXANTHIN IN HUMAN HEALTH

In adults, cognitive damage and dementia tend to increase with age. According to Johnson et al. (2008), this impairment in elderly individuals is related to a low consumption of lutein and docosahexaenoic acid (DHA). Based on the fact that lutein and DHA accumulate in the cellular membranes of the central nervous system, the authors designed a clinical assay to evaluate the effects of lutein and DHA in various cognitive domains in women (aged between 60 and 80 years). The participants received 12 mg of lutein or 800 mg of DHA, a mixture of both, or a placebo, daily for 4 months. The results demonstrated that verbal fluidity, memory, and processing speed improved significantly in patients who received lutein, DHA, or a mixture of both. These findings suggest that lutein and DHA improve cognitive function in elderly people.

It is well known that lutein is transported through a hematoencephalic barrier and accumulates in the macula of the retina and other nervous tissue. Based on previous findings, Bovier et al. (2014) hypothesized that an increase in lutein and astaxanthin concentrations in the visual system can improve visual processing speed. In this clinical study, the subjects (young men and women, aged between 18 and 32 years) were pooled in three groups to evaluate two commercial supplements and a placebo over a period of 4 months. One of the supplements contained zeaxanthin (20 mg) and the other zeaxanthin (26 mg) mixed with lutein (8 mg) and omega-3 fatty acids. Supplementation increased the concentration of macular pigment and improved visual processing speed.

CONCLUSIONS

This chapter reviewed astaxanthin, lutein, and zeaxanthin and has demonstrated that these natural carotenoids are bioactive compounds with biological activities than benefit human health. Currently, astaxanthin is the lipophilic pigment most widely used in clinical assays for disease prevention, based on its properties as an antioxidant and ameliorator of chronic inflammation. Specifically, astaxanthin, lutein, and zeaxanthin have shown effects against neurodegenerative, cardiovascular, and dermatological diseases. However, more clinical assays are required to warrant the protective effects and safe use of astaxanthin-, lutein-, and zeaxanthin-based dietary supplements.

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Chapter 2.3

Chondroitin and Glucosamine

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INTRODUCTION

As defined in the National Institutes of Health, 1994 Dietary Supplement Health and Education Act of 1994, a dietary supplement is destined to be taken by mouth to reinforce the diet, and contains a "dietary ingredient," based on one or any mixture of the following components: amino acids, minerals, vitamins, enzymes, glandulars, organ tissues, herbs, or other botanics, a concentrate, metabolite, constituent, or extract. The use of dietary supplements is prevalent worldwide and may be related to many factors including age, gender, income, educational level, and health conditions.

Some nonvitamin, nonmineral dietary supplements, such as glucosamine (G) and chondroitin sulfate (CS), may have antiinflammatory and chondroprotective properties (Bottegoni et al., 2014; Henrotin et al., 2014; Nakamura, 2011; Sterzi et al., 2016). Being basic components of cartilage, glycosaminoglycans are produced naturally by the human body but can be also supplemented in the diet. CS and G, alone or in combination, are the most common oral supplements taken to improve joint health in people with arthritis. Arthritis is a widespread disease in the world and osteoarthritis (OA) represents its most common form. Arthritis causes disability and morbidity and results in high individual, societal, and economic costs (Wilson, 2016). CS and/or G are taken by many people mainly to manage joint pain and delay joint destruction and cartilage loss. They are considered as symptomatic, slow-acting drugs for OA (SYSADOA) in Europe and Asia, while they are commercialized as over-the-counter dietary supplements in the USA and Australia (Fransen et al., 2015). Moreover, CS and G are more available, better tolerated, and safer than antiinflammatory drugs, which may cause side effects and interact with other medicines (Huskisson, 2008). Many clinical studies, metaanalysis, and systematic reviews have analyzed the safety and effectiveness of CS and G (Bishnoi et al., 2016; Bottegoni et al., 2014; Hochberg et al., 2016; Machado et al., 2012; Munarolo et al., 2011; Towheed et al., 2005; Vangsness et al., 2009). However, the obtained results appear conflicting and controversial.

The main objectives of this chapter are to review the main sources, the structural and biochemical characteristics of CS and G, to summarize the results of mainly clinical trials, to discuss health-related issues, and finally to identify the main factors associated with the limitations of clinical trials.

CHONDROITIN SULPHATE: BIOCHEMICAL CHARACTERIZATION AND SOURCES

CS, a linear and negatively charged polysaccharide, consists of disaccharide-repeating units of differently sulphated glucuronic acid and N-acetylgalactosamine (Volpi, 2007). These natural glycosaminoglycans, synthesized intracellularly from glucose or G (Bottegoni et al., 2014), are structural constituents of cells, tissues, and organs, and play a key role in regulating many biological processes (Gandhi and Mancera, 2008; Schiraldi et al., 2010). The chemical structure of CS depends on many factors including CS source characteristics (organism origin, species, tissue, and age) and extraction and purification processes (Bishnoi et al., 2016; Novoa-Carballal et al., 2017; Volpi, 2009) as shown in Fig. 2.3.1. The number of disaccharide units and sulfate groups per disaccharide unit (charge density), the molecular weight, the number and position of sulfate groups, and the ratio of 4-sulfated to 6-sulfated disaccharide units are among the main chemical properties affected by CS source (4s/6s) (Fig. 2.3.1). The molecular weight of CS and its charge density have a great impact on intestinal absorption which is facilitated by the low molecular weights of these polysaccharides (Bishnoi et al., 2016).

The structural complexity and heterogeneity of CS lead to a broad range of biological activities, and consequently various nutraceutical, therapeutic, and pharmacological applications (Novoa-Carballal et al., 2017). There are around 25 CS depending on sulfate content and sulfation position (Lamari and Karamanos, 2006). Many mammalian and invertebrate

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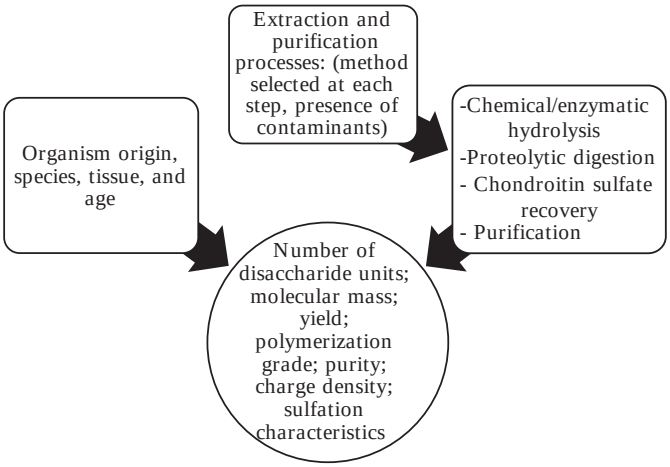

FIG. 2.3.1 Main factors affecting biological and pharmacological activities of chondroitin sulfate.

sources can be used to extract this polysaccharide. However, CS available commercially is mainly obtained from porcine, bovine, poultry, and shark cartilage (Volpi, 2007; Maccari et al., 2015).

Zang et al. (2012) proposed a method for CS origin identification by near-infrared spectroscopy, and found that shark cartilage is mainly composed of disaccharides monosulfated at position 6, while bovine and porcine cartilage are mainly made up of disaccharide units monosulfated at position 4. Likewise, Li et al. (2016) achieved the same results (Table 2.3.1). Using different degradation methods (HCl, enzymatic, microwave-assisted alkali, and oxidative) to prepare different low molecular weight CSs from shark, porcine, and bovine cartilage, these authors proved the dependence of biological activity on molecular weight and sulfate content.

Religious restrictions on the consumption of pork may limit the use of CS from porcine cartilage in some countries. Moreover, the use of CS obtained from animal tissues may expose consumers to bovine spongiform encephalopathy and other diseases. In addition, the limited availability of some shark species threatened with extinction, and their rising costs, promoted the use of safe and harmless alternative sources for CS extraction. Among the available raw materials currently used for CS isolation are included: fishbone species (Maccari et al., 2015), shellfishes (Cao et al., 2015; Volpi and Maccari, 2007), and by-products from processing industries (Blanco et al., 2015; Murado et al., 2010; Nakano et al., 2012; Novoa-Carballal et al., 2017).

The presence of disulfated disaccharides, especially those disulfated in position 2 and position 6, is a typical characteristic of CS from cartilaginous fishes (Table 2.3.1) and may be used for origin identification (Volpi, 2007; Maccari et al., 2015). Bony fishes have been proposed as a valuable alternative CS source to shark cartilage which needs long and complex purification steps to obtain CS with the required purity (Maccari et al., 2015). Cao et al. (2015) characterized glycosaminoglycans from mollusks through acid hydrolysis. They reported a predominance of CS in 20 species of edible Bivalvia and Gastropoda. By optimizing environment-friendly extraction and purification processes based on enzymatic hydrolysis, alkaline hydroalcoholic precipitation, and membrane ultrafiltration, Vázquez et al. (2016) obtained a high-purity CS (>98%) from *Prionace glauca* head wastes. High-purity CSs are required for clinical applications, while low-purity CSs can be used for nutraceutical and dietary applications.

GLUCOSAMINE: BIOCHEMICAL CHARACTERIZATION, SOURCES, AND PRODUCTION

Synthesized from glucose, G (2-amino-2-deoxy-β-D-glucopyranose) is an amino sugar constituting the main component of the cell wall. This abundant biomolecule is mainly used as a dietary supplement, but also can be employed for the manufacture of fortified food and beverages as well as dairy products (Grand View Research Inc., 2016). Many published papers have reported the role of this amino monosaccharide in OA treatment, wound healing, and dentistry (Nakamura, 2011; Reginster et al., 2012). Its world production may reach 46,000 tons by 2017 and is expected to increase in the future. Commercial forms of G include *N-acetyl*-D-glucosamine, hydrochloride, sulfate, or chlorohydrate salt, and dextrorotatory isomer (Dahmer and Schiller, 2008). Having the same therapeutic activity, *N-acetyl*-D-glucosamine is sweet and is suitable for oral administration, while the other commercial forms of G, hydrochloride and sulfate salt, have a bitter taste (Sinha et al., 2016).

TABLE 2.3.1 Chemical Characterization of Chondroitin Sulfate From Different Sources

					Disaccharide composition (%)							
Source	Yield/ content(w/w dry tissue)	Charge density	Molecular weight (kDa)	4s/6s ratio	Nonsulfated	4-monosulfated	6-monosulfated	2,6-disulfated	4,6-disulfated	2,4-disulfated	Trisulfated	Reference
Bovine milk	22%	0.94		2.2	6	60	27	–	–	–	–	Coppa et al. (2011)
Parmigiano-Reggiano cheese	72%	0.91	15.4	2.5	9	65	26	–	–	–	–	Coppa et al. (2012)
Bovine cartilage	–	–	18.15	2.92	7.30	68.82	23.54	–	–	–	–	Li et al. (2016)
Porcine cartilage	–	–	10.07	2.07	0.88	66.68	32.12	–	–	–	–	
Shark cartilage	–	–	31.30	0.57	0.00	31.31	54.77	12.66	–	–	–	
Sturgeon bone	0.28%–0.34%	0.93	37.5	0.69	7	38	55	–	–	–	–	Maccari et al. (2010)
Monkfish bones	0.011%–0.34%	0.95	48.68	1.81	12.9	51.0	28.2	1.3	6.3	0.4	–	Maccari et al. (2015)
Cod bones spiny		1.06	18.12	2.19	3.4	59.8	27.3	9.5	Trace	Trace	–	
Dogfish bones		1.04	13.46	0.45	7.4	25.3	55.7	10.5	0.8	0.3	–	
Salmon bones		0.96	20.07	1.37	7.7	51.2	37.3	3.5	0.2	0.1	–	
Tuna bones		1.05	32.94	2.23	1.6	63.2	28.3	4.6	1.1	1.2	–	
Skins of grey triggerfish (GTSG)	8.62%	1.17	41.72	3.3	3.5	59	18.2	5.48	5.66	7.0	1.0	Krichen et al. (2017)
Skins of smooth hound (SHSG)	9.86%	1.26	23.8	3.2	5.5	47	14.5	4.56	5.45	20.1	0.4	
Ray fish cartilages	–	–	142	0.43	2.7	27	61.9	8.5	–	–	–	Hashiguchi et al. (2011)

(Continued)

TABLE 2.3.1 Chemical Characterization of Chondroitin Sulfate From Different Sources—cont'd

					Disaccharide composition (%)							
Source	Yield/ content(w/w dry tissue)	Charge density	Molecular weight (kDa)	4s/6s ratio	Nonsulfated	4-monosulfated	6-monosulfated	2,6-disulfated	4,6-disulfated	2,4-disulfated	Trisulfated	Reference
Scyliorhinus canicula fin	3.9		43	0.64	22.5	31.5	32.4	16.3	–	–	–	Novoa-Carballal et al. (2017)
Scyliorhinus canicula head	5.8		45	0.76	14.1	35.5	28.7	18.1	–	–	–	
Scyliorhinus canicula skeleton	1.9		43	0.82	15.1	39.5	29.7	16.7	–	–	–	
Prionace glauca head	12.1		60	0.15	16.3	10.1	64.2	9.3	–	–	–	
Raja clavata skeleton	13.7		44	0.26	19.5	16.0	55.9	8.6	–	–	–	
Hemolymph of the freshwater snail *Planorbarius corneus*	0.9 µg/mL	0.75	31	3.4	25	58	17	–	–	–	–	Volpi and Maccari (2007)

G is mainly derived from chitosan, a linear biopolymer containing copolymers of D-glucosamine and *N-acetyl*-D-glucosamine, produced by deacetylation of chitin that is predominately extracted from the shell wastes of crustaceans. Many papers have reported the biological activities of chitin, chitosan, and their derivatives, as well as their applications in various fields such as food industry, agriculture, pharmaceutical, biomedical, cosmetic, textile, and enzyme immobilization (Bottegoni et al., 2014; Hamed et al., 2016; Muzzarelli, 2009; Sinha et al., 2004). The extraction of G from crustacean by-products by chemical or enzymatic hydrolysis may present several disadvantages, including seasonal variability, possible allergic and toxic reactions, chemical composition variability, a reduction in its use due to a rise in vegetarianism, and negative environmental impacts (Sitanggang et al., 2012). To overcome the main limitations of chemical hydrolysis described in Table 2.3.2, and allow G production from nonanimal sources, Lv et al. (2017) proposed a highly effective and selective biocatalytic process for G production from crude solid mushroom fractions using a two-enzyme system (*N*-acetylhexosaminidase from *Zobellia galactanivorans* and *N*-deacetylase from *Cyclobacterium marinum*). G can also be produced from fructose and ammonia or from plants and plant extracts. Different fermentation processes have been proposed to produce G using microbial sources (Table 2.3.2). Sitanggang et al. (2012) discussed the different methods used to produce, isolate, and purify G from microorganisms with a special emphasis on fungal G derived from the submerged or solid-state fermentation systems.

CLINICAL EVIDENCE

Several possible modes of action of CS and G in OA have been proposed. They act synergistically to increase proteoglycan synthesis by chondrocytes and delay cartilage degeneration by inhibiting cleavage enzymes (Huskisson, 2008). G represses the proinflammatory mediator production in chondrocytes activated with interleukin-1β, and consequently prevents OA progression (Nakamura, 2011). CS, known for its antiinflammatory and chondroprotective properties is reported to reduce the stimulation and translocation of nuclear factor-κB in chondrocytes and synovial membrane and to enhance viscosity of the synovial fluid at the afflicted sites (Bottegoni et al., 2014).

TABLE 2.3.2 Chemical and Biotechnological Methods for Glucosamine Production

Source	Extraction method	Observations	Reference
Shell waste of crustaceans	Chemical hydrolysis	Severe hydrolysis conditions, modification of glucose ring, reduced yield, increased by-product formation and toxic wastes, high cost, harmful effects on human health and the environment	Sinha et al. (2016)
	Enzymatic hydrolysis	Mild hydrolysis conditions, high specificity and selectivity, high yield, no modifications of glucose ring, high cost	Pan et al. (2011)
Fructose and ammonia	Solvents	By-product formation, reduced yield, glucosamine instability	Hubbs (2007)
Plants	Hydroponic plant production and addition of a glucosamine precursor (nitrogen-based fertilizer) before harvest, heating the harvested plant materials, extraction with water	Time consuming, elevated temperatures, high yield	Petiard et al. (2013)
Microorganisms	Fermentation processes		Sitanggang et al. (2012)
Bacteria	Direct secretion of glucosamine into the fermentation medium or production of *N-acetyl*-D-glucosamine which necessitates chemical hydrolysis to obtain glucosamine	Rapid degradation of glucosamine during bacterial fermentation Time-consuming and expensive processes	
Fungi	Conversion of fungal biomass to glucosamine by chemical or enzymatic hydrolysis	Variation of fungal biomass and chitosan yield as a function of fungal species, fungal cultivation, fermentation system and conditions, environmental conditions	

In fact, CS and G are endorsed as disease-modifying OA drugs by the European League Against Rheumatism (EULAR) and the Osteoarthritis Research Society International (OARSI) guidelines for treating knee, hip, and hand OA (Davies et al., 2013). Although the clinical efficacy of these two natural substances has received particular scientific attention, the obtained results are conflicting and controversial.

Usha and Naidu (2004) evaluated the safety and effectiveness of G and methylsulfonylmethane, taken individually or in combination, and a placebo in knee OA. They measured clinical symptoms, in 118 patients with mild to moderate OA, through the pain rating index, pain intensity, the swelling index, the Lequesne index, 15-minute daily walk, and use of rescue remedy. These authors found that the combined therapy was well tolerated and more efficient in relieving pain symptoms and in enhancing the functional capacity of joints more quickly than single supplements. Likewise, Munarolo et al. (2011) demonstrated the efficacy of the combined use of G and CS supplements in association with physical therapy in improving pain during movement from baseline to endpoint, Lequesne's index, and the intensity of crepitus in knee OA patients. They also highlighted the safety profile of these supplements, which did not induce any adverse reactions in the treated patients. In another study, Clegg et al. (2006) tested glucosamine hydrochloride (GH) and CS alone or in combination in a blind, 6-month, multicenter and large-scale trial and demonstrated their efficacy in decreasing knee pain only for patients suffering severe OA. Roman-Blas et al. (2017) tested the combined intake of CS and glucosamine sulfate (GS) in a randomized multicenter, double-blind, 6-month, placebo-controlled study. They concluded that a CS–GS combination was not found to be significantly better than the placebo in alleviating pain and functional disability in 164 patients suffering from knee OA. These findings were supported by those of Fransen et al. (2015) who showed that CS–GS treatment achieved a significant decrease in tibio–femoral joint space narrowing over a period of 2 years without any significant symptomatic benefits when compared with the placebo. Hochberg et al. (2016) examined patients with different stages of knee OA. These patients were randomly selected to receive CS plus GH (3× daily) or celecoxib (200 mg, 1× daily) for 6 months. The findings of this multicenter trial reported that the combined CS–GH supplement therapy was safe and tolerable, had a similar efficacy to celecoxib in reducing stiffness, swelling, and functional disability, and consequently may constitute an effective solution for patients with contraindications for analgesics and nonsteroidal antiinflammatory drug (NSAID) treatments. In another randomized, double-blind, parallel-group comparative trial, Kanzaki et al. (2015) assessed the efficacy of a dietary supplement incorporating GH, CS, vitamin D, peptide derivatives, and quercetin on knee-joint and physical functions. They found that the tested supplement improved the Japanese Knee OA Measure score, walking ability, and knee extension strength in patients suffering from mild to severe knee pain more effectively than the placebo. Recently, Peluso et al. (2016) demonstrated that the combined treatment of GS and therapeutic mud baths positively affects the determinants of quality of life in knee OA patients. Using the commercially available dietary supplement, CartiJoint Forte, based on GH, CS, and Bio-Curcumin, in a clinical trial, Sterzi et al. (2016) reported its efficacy in improving "on moving" visual analogue scale and Lequesne's index in knee OA patients. Likewise, Merolla et al. (2015) conducted a comparative and randomized clinical trial for patients with a full-thickness supraspinatus tendon rupture to assess the efficacy of a dietary supplement incorporating G and CS, in combination with other nutraceuticals, versus a placebo during two months and found that the oral supplement significantly affected short- and mid-term pain. Regarding the management of temporomandibular joint osteoarthrosis, randomized, double-blind clinical tests reported the efficacy of G and CS in improving the symptoms of this degenerative disease (Nguyen et al., 2001). G (500 mg, 3× daily) was found to be better than Ibuprofen (400 mg, 3× daily) in reducing pain (Thie et al., 2001). In their systematic review, Machado et al. (2012) noted some limitations of the few studies dealing with the use of supplement therapy for the osteoarthrosis of the temporomandibular joint, related to the studies small sample sizes, short trial durations, and other methodological criteria including randomization, calculation of the sample size, masking level, assessment of tested factors, and calibration.

SAFETY AND INTERACTIONS

Many clinical studies, metaanalysis, and systematic reviews have demonstrated the safety and effectiveness of CS and G and discussed their possible contribution in reducing the intake of NSAIDs and analgesics which are delivered to OA patients without medical control in some countries (Bishnoi et al., 2016; Bottegoni et al., 2014; Hochberg et al., 2016; Towheed et al., 2005; Vangsness et al., 2009). However, some adverse gastrointestinal effects, such as abdominal pain and nausea, were reported in some studies on G and CS (Bottegoni et al., 2014; Huskisson, 2008). Moreover, shellfish allergic subjects should consult their physicians prior to supplement intake. However, the tropomyosine protein responsible for shellfish allergies is not present in chitosan which is the main marine source of G (Muzzarelli, 2009). Despite the fact that studies reported no effect of G on blood glucose levels, glucose metabolism, and insulin sensitivity in healthy and diabetic subjects, caution should be taken for type 2 diabetes patients requiring a constant monitoring of their blood glucose levels when receiving G (Simon et al., 2011). As it has anticoagulant activity, CS supplementation may interfere with blood-thinning drugs when taken simultaneously. Moreover, caution is advised when using G or CS during pregnancy and

breastfeeding due to the lack of scientific evidence. Due to the dependence of the safety profile of these two supplements on their origin, physicochemical properties, and presence of contaminants, the use of pharmaceutical-grade compounds may prevent safety concerns. To the extent of our knowledge, no study has been published on the interactions of G or CS with foods, herbs, or other supplements.

LIMITATIONS OF CLINICAL TRIALS AND CONCLUDING REMARKS

It is clear from the results of the clinical studies provided above that the effects of G and CS vary among trials. This variability may be attributed to many factors:

1. Poor-quality studies performed with low-quality supplements, undefined dosing, small sized samples, and short follow-up times.
2. The use of several heterogeneous formulations having great variations in source, composition, form, grade, purity, and availability.
3. A difficulty in generalizing the results obtained on a characterized and specific OA patient population to other different age groups or patients with different disease severities.
4. The combined use of G and CS supplements in association with other therapies or medications may have an influence on the results.
5. Potential conflicts of interest and the integrity of published results from industry-sponsored clinical trials.
6. A general tendency to publish positive trials.

As the safety and efficacy of CS and G formulations are highly affected by the origin and quality of their constituents it is necessary to develop specific and standardized procedures, especially for nonpharmaceutical-grade CS and G, to control their origin, properties, purity, and overall quality of formulation constituents and resulting products. Because of the prominent place occupied by supplements in alternative and complementary medicine it is imperative that health practitioners communicate with OA patients about the potential benefits and risks of CS and G intake as well as the variability and controversy of clinical trials. Further clinical trials are needed to determine the interactions of G or CS with foods, herbs, or other supplements and the effects of these supplements on healthy subjects.

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Chapter 2.4

Choline

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INTRODUCTION

In 1850, lecithin was first identified in brain tissue and fish eggs by Gobley. The word lecithin was derived from a Greek word, *lekithos*, meaning egg yolk. In 1862, Strecker isolated lecithin from ox bile and reported on a molecule that was formed after boiling lecithin. He named the molecule choline (derived from a Greek word, *chole*, which means bile). In later years, the chemical structure of lecithin was defined as phosphatidylcholine (PC) (Zeisel, 2012). In 1968, choline was mentioned as an important nutrient in the 7th edition of the *Recommended Dietary Allowances*. In 1998, choline was considered an essential nutrient and its adequate intake (AI) established (550 mg/day for adult men and 425 mg/day for adult women) (Hollenbeck, 2010). After discovering choline's nutrient functions, researchers considered it as a kind of vitamin (vitamin J) and also classified it in the group of B vitamins (Biasi, 2011). Choline is necessary for various human physiological functions. As a component of phospholipid (e.g., PC and sphingomyelin), it critically contributes to cell membrane structural integrity and signaling events. As a methyl-group donor, it plays an important role in homocysteine reduction into methionine. Also, it is crucially required for acetylcholine synthesis (neurotransmission) and lipoprotein formation (lipid transport). Therefore, choline deficiency can lead to critical impairments in the functioning of many body organs such as the liver, muscles, kidneys, brain, and nervous system (Sanders and Zeisel, 2007; Zeisel and Da Costa, 2009).

PC plays an important role in the hepatic formation of very low-density lipoprotein (VLDL) and its secretion from the liver. Betaine, the oxidized metabolite of choline, has two important physiological roles: contributing to osmoregulation and one-carbon metabolism. As an osmolyte, it helps with water reabsorption in the kidneys and intracellularly ensures the regulation of cell volume and integrity. Like folate, betaine serves as a source of methyl group and is involved in methionine biosynthesis. The conversion of homocysteine to methionine is catalyzed either by ubiquitous methionine synthase (MS) or by betaine homocysteine methyltransferase (BHMT), which is mainly active in the liver and kidneys. MS utilizes 5-methyltetrahydrofolate (the active form of folate) as a methyl donor and cobalamin (vitamin B12) as a cofactor, while BHMT needs betaine as its methyl donor. Thereby, choline and/or betaine supplementation can be beneficial in its ability to compensate folate deficiency (Ueland, 2011) (Figs. 2.4.1 and 2.4.2).

CHOLINE DIETARY SOURCES

Choline and choline-related compounds such as glycerophosphocholine, phosphocholine, lecithin (PC), and sphingomyelin are found in varying amounts in most foods. The US Department of Agriculture (USDA) Nutrients Databases have provided comprehensive data about the amount of choline and choline-related compounds in foods. Based on these data, the choline content of eggs are noticeably higher than other foods. Total choline content of fresh raw whole egg and fresh raw egg yolk are 250 and 680 mg of choline moiety/100 g of food, respectively. Meat and fish, whole grain, cereal, vegetables and fruits, milk, and fat and oils are other main classes of food written in order of their choline contents (Patterson et al., 2008).

BIOSYNTHESIS AND METABOLISM OF CHOLINE

In animals, required choline is provided via both *de novo* biosynthesis and the diet (Figs. 2.4.1 and 2.4.2). Endogenous choline is biosynthesized via the phosphatidylethanolamine N-methyltransferase (PEMT) pathway. Through the pathway, the enzyme PEMT catalyzes methylation of phosphatidylethanolamine (PE) to PC.

Dietary choline is absorbed from the intestine via choline transporters. Then, after uptake into the cell, it passes through three steps to be converted to PC. In the initial step, it is quickly phosphorylated to phosphocholine by the enzyme choline

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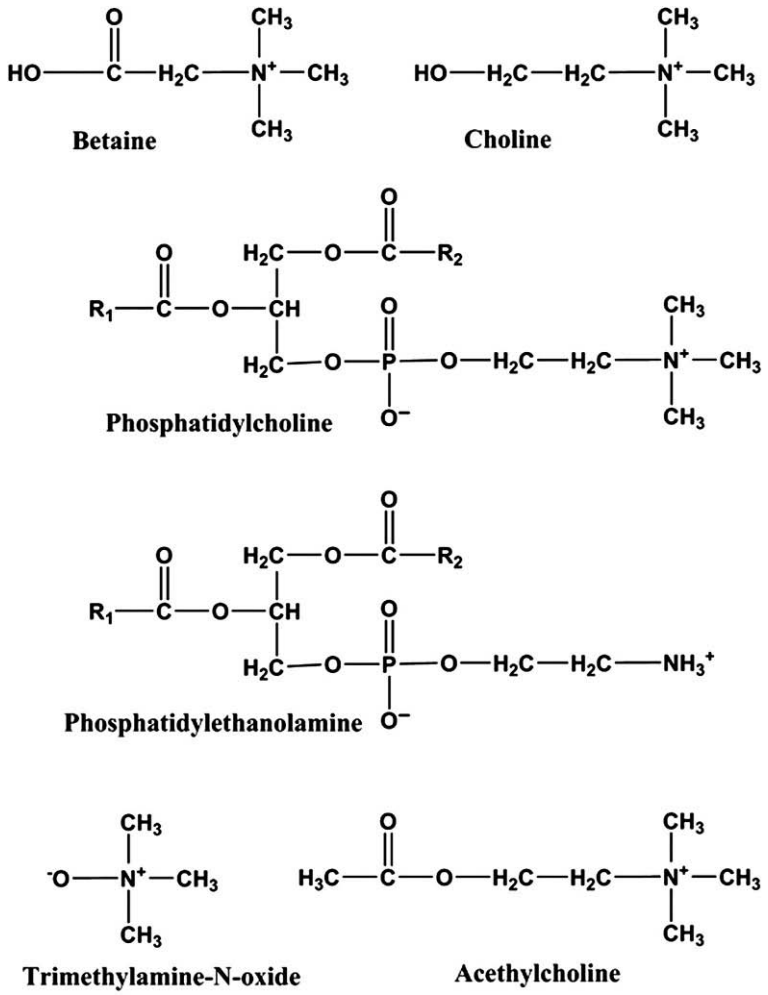

FIG. 2.4.1 Choline and metabolically related compounds. R_1, R_2, fatty acid residues.

kinase (CK). Then, in a rate-limiting step which is catalyzed by CTP:phosphocholine cytidylyltransferase (CCT), phosphocholine is changed into cytidine diphosphocholine (CDP-choline). In the final step, PC is produced via CDP-choline:1,2-diacylglycerol cholinephosphotransferase (CPT) action on its substrate, CDP-choline. PC is mostly produced through the second (CDP-choline) pathway and is the main metabolite of choline. The reverse conversion of PC to choline can also occur via the enzymatic action of phospholipases on PC. Choline also can be oxidized to betaine in the kidneys and liver, or changed into acetylcholine in the nervous system (DeLong et al., 1999; Li and Vance, 2008).

Furthermore, a part of the dietary choline content is metabolized by intestinal flora before absorption. Intestinal lipases convert PC (the main source of total choline in the diet) to some choline-containing products such as glycerophosphocholine, phosphocholine, and choline. Gut microbiota then metabolize these products to trimethylamine (TMA). After absorption, TMA is immediately oxidized to trimethylamine-*N*-oxide (TMAO) via the action of flavin-containing monooxygenases (FMO3) in the liver. There is growing evidence that TMAO is involved in the pathogenesis of atherosclerotic coronary artery disease (Tang et al., 2013; Wang et al., 2011).

CHOLINE DIETARY REQUIREMENT IN LIVER DISEASE

Animal studies have amply demonstrated the positive effects of choline supplementation in the reduction of nonalcoholic hepatic steatosis and alleviation of associated liver damage via subsiding oxidative stress and apoptosis. Further, results of human studies have shown that choline supplementation can prevent hepatic steatosis and probably its development into inflammation and fibrosis (i.e., cirrhosis and liver cancer) (Duric et al., 2012). Choline and its derived metabolite, PC, play an important role in the hepatic formation of VLDL and its secretion from the liver. Therefore, a choline-deficient

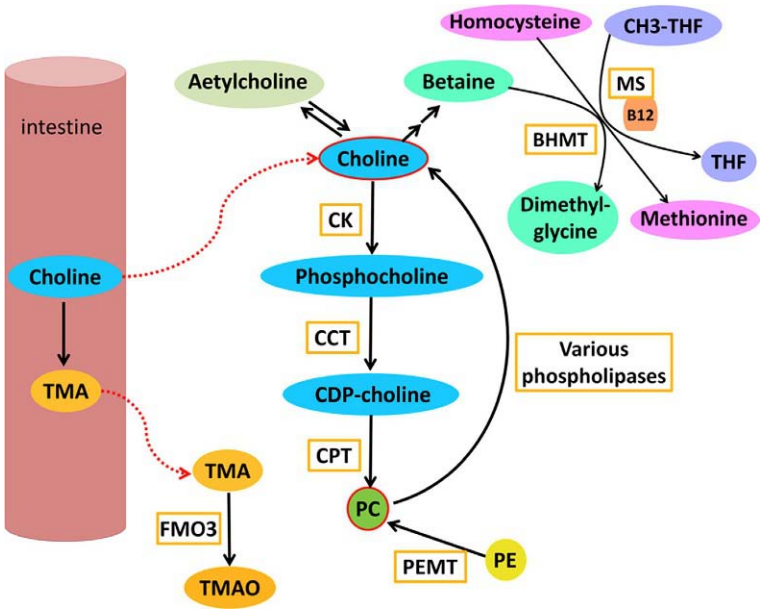

FIG. 2.4.2 Methabolic pathways of choline. *B12*, vitamin B12; *BHMT*, betaine homocysteine methyltransferase; *CCT*, CTP:phosphocholine cytidylyltransferase; *CDP-choline*, cytidine diphosphocholine ;*CH3-THF*, 5-methyltetrahydrofolate; *CK*, choline kinase; *CPT*, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; *FMO3*, flavin-containing monooxygenases; *MS*, methionine synthase; *PC*, phosphatidylcholine (lecithin); *PE*, phosphatidylethanolamine; *PEMT*, phosphatidylethanolamine N-methyltransferase; *THF*, tetrahydrofolate; *TMA*, trimethylamine; *TMAO*, trimethylamine-*N*-oxide.

diet leads to hepatic steatosis in animals while supplementing with choline or PC can reverse it (Duric et al., 2012; Yao and Vance, 1988). Recent studies indicate that when animals are fed with a choline-deficient diet, the elevation of lipolysis in white adipose plays an important part in the development of hepatic steatosis to liver injury and steatohepatitis (Jha et al., 2014; Tanaka et al., 2014). A methionine-deficient and choline-deficient diet was found to induce alterations to rodent liver in a similar manner to the hepatic changes in human nonalcoholic steatohepatitis (NASH) and hence has been used as a dietary animal model for NASH (Marcolin et al., 2011; Takahashi et al., 2012).

However, it has been shown that overdose of PC may lead to the development of hepatic steatosis. Excess dietary PC, via activating the peroxisome proliferator-activated receptor (PPAR), can increase hepatic lipid accumulation and also adipogenesis (Zhang et al., 2009).

Reductions in plasma choline concentration and muscle choline stores, along with related metabolic changes, have been revealed in children with cystic fibrosis, while choline supplements have improved these abnormalities (Schall et al., 2016). Choline or PC supplements have improved choline metabolism-related abnormalities such as increased plasma *S*-adenosylhomocysteine and decreased methionine, *S*-adenosylmethionine/*S*-adenosylhomocysteine, and glutathione:glutathione disulfide in children with cystic fibrosis (Innis et al., 2007).

CHOLINE AND RISK OF CANCER

Theoretically, betaine, the oxidized metabolite of choline, is an important methyl-group donor. Thus, choline and betaine play a critical role in nucleotide synthesis and DNA and RNA methylation. Accordingly, their low status in the body may result in inefficient DNA repair, chromosomal breaks, and subsequently cancer occurrence (Cho et al., 2010, 2007). However, published studies regarding the effect of choline supplementation in cancer, especially population-based, are inconsistent and further investigations are needed. A large prospective cohort study has found an association between high plasma choline and an elevated risk of prostate cancer (Johansson et al., 2009). In another retrospective study, a direct association between choline intake and occurrence of colorectal adenoma in women has been observed (Cho et al., 2007). A prospective study among postmenopausal women found no relationship between intakes of choline and betaine and risk of breast cancer (Cho et al., 2010). However, in another study, researchers have indicated the inverse relationship. They also suggested that the factors affecting choline metabolism, polymorphism of PEMT and choline dehydrogenase (CHDH), are associated with breast cancer risk (Xu et al., 2008). As the maturation of mammary glands begins before birth, an animal study investigated the effect of choline supplementation given to pregnant mothers on the prevention of breast cancer in their daughters. The results showed that the expression of breast cancer–related genes changed in rats if their mothers

prenatally received adequate choline, meaning they might be less susceptible to breast cancer (Kovacheva et al., 2009). PC has inhibited the growth of four human hepatic cancer cell lines and showed dose-dependently induced apoptosis in them. In addition, PC supplementation of hepatocarcinogenesis-induced rats led to a decrease in tumor nodules and the induction of apoptosis (Sakakima et al., 2007).

CHOLINE AND CARDIOVASCULAR DISEASES

Betaine, the metabolite of choline oxidation, plays an important role in the conversion of homocysteine to methionine (Fig. 2.4.1), thereby choline deficiency can result in elevated plasma levels of homocysteine (hyperhomocysteinemia). It has been demonstrated that there is an association between elevated plasma concentration of homocysteine and increased risk of cardiovascular disease (CVD). Meanwhile, many studies have shown that choline and/or betaine supplementations can reduce homocysteine plasma levels (Dalmeijer et al., 2008; Hollenbeck, 2010; Zeisel and Da Costa, 2009). Considering the mentioned mechanism, it seems that there is a relationship between choline intake and risk of CVD, however, several human studies have failed to confirm such a relationship (Hollenbeck, 2010). Rajaie and Esmaillzadeh reviewed the related epidemiological evidence on this subject. They found no significant association between dietary intakes of choline and betaine with the risk of CVD, while they indicated that when choline and betaine are consumed for a long time, CVD mortality may decrease owing to the effects of these nutrients on the reduction of inflammation and other risk factors (Rajaie and Esmaillzadeh, 2011). In a prospective cohort study among postmenopausal Dutch women, no association was observed between regular dietary intakes of betaine or choline and CVD incidence (Dalmeijer et al., 2008). A large population-based study has been conducted to investigate the association between choline and betaine plasma levels and some known risk factors of CVD in middle aged and elderly men and women. Researchers of this study found a direct relationship between choline plasma level and CVD risk factors, however, betaine plasma level has been inversely related to those risk factors (Konstantinova et al., 2008). In another large cohort study in the elderly, similar results have been reported for choline and betaine. However, the relationship between PC plasma level and CVD risk factors has not been consistent. Higher plasma PC concentrations have been associated with most of the favorable CVD risk factors and also some unfavorable ones (elevated low-density lipoprotein (LDL) cholesterol and triglycerides) (Roe et al., 2017).

On the other hand, there is growing evidence that TMAO is involved in the pathogenesis of atherosclerotic coronary artery disease. Gut flora metabolize PC to TMA which is absorbed and converted to TMAO in the liver (see earlier section of this chapter entitled "Biosynthesis and metabolism of choline"). TMAO may increase the expression of multiple macrophage scavenger receptors contributing to atherosclerosis. TMAO plays an important role in enhancing the accumulation of cholesterol in macrophages and accretion of foam cells in the artery walls (Tang et al., 2013; Wang et al., 2011). In a human study, a significant decrease in TMAO plasma concentration was observed after antibiotic administration (Tang et al., 2013). A prospective cohort study, in stable cardiac patients, revealed that high plasma choline and betaine levels, only when accompanied by increased plasma TMAO level, can be associated with an increased future incidence of adverse cardiac events such as mortality, myocardial infarction, and stroke (Wang et al., 2014). In another cohort study, after a 5-year follow-up of patients with stable heart failure, an association has been found between higher plasma levels of TMAO and increased mortality risk (Tang et al., 2014). Similarly, there is a relationship between increased choline, betaine, and TMAO levels and more advanced left ventricular diastolic dysfunction as well as risk of long-term adverse clinical events in patients with chronic systolic heart failure (Tang et al., 2015). To prevent or treat TMAO-related atherosclerosis, we need to clearly know about the type and abundance of gut microbiota metabolizing PC to TMA, human genotypes influencing TMA conversion in the liver, and the influence of the human diet on gut flora and gut flora on choline bioavailability (Romano et al., 2015).

WOMEN'S REQUIREMENT OF CHOLINE: PREGNANCY, BREAST FEEDING, AND POSTMENOPAUSAL STATUS

During pregnancy, choline concentration in the plasma decreases due to the high transfer of choline from mother to fetus and its storage in the placenta and amniotic fluid. This condition physiologically provides adequate choline for the fetus to grow and especially develop its neural tube. Therefore, adequate choline nutrition is important for the health of both mother and fetus (Sanders and Zeisel, 2007). However, women in most low-income countries and about one-quarter of women in high-income countries consume poor-choline diets (Zeisel, 2013). There is evidence that prenatal dietary intakes of choline decreases the risk of neural tube defects (NTDs) (Shaw et al., 2004, 2009) and breast cancer (Kovacheva et al., 2009) in children. Also, periconceptional choline supplementation has decreased the risk of orofacial clefts in neonates (Shaw et al., 2006).

In early pregnancy, dietary choline, in the same manner as dietary folate, plays an important role in neural tube closure. Therefore, inadequate choline intake may increase the risk of NTDs (Sanders and Zeisel, 2007). A case-controlled study has indicated that the risk of NTDs in neonates is reversely associated with the periconceptional choline intake of their mothers (Shaw et al., 2004). Likewise, in a prospective study, a strong inverse linear relationship has been observed between maternal total choline and risk of NTDs (Shaw et al., 2009).

In later stages of pregnancy, choline nutrition influences hippocampal development and subsequently cognitive function throughout a life (Zeisel, 2006). Blusztajn and Mellott have reviewed a considerable number of animal studies which investigated the influence of choline intake on learning and memory. They indicated that a high choline supplementation of rodents during pregnancy enhanced their cognitive function in later life and reduced the risk of age-related memory loss. The reviewers also considered choline epigenetic mechanisms and underlined the choline modulatory activity on the expression of synaptic plasticity-related genes via DNA and histone methylation (Krzysztof Blusztajn and Mellott, 2012). In spite of numerous animal studies, and a number of human studies, investigating the effect of choline on the central nervous system's development and function is very low. In a prospective cohort study, a relationship was found between higher prenatal choline intake of mothers and an increase in the visual memory of children aged 7 years (Boeke et al., 2013). However, in another prospective study, no association was observed between maternal physiological concentrations of choline (via regular dietary intake) at different times in pregnancy and the intelligence of children at age 5 years. Nevertheless, the influence of maternal "pharmacological" supplementation of choline on the intelligence of children may prove to be different in future studies (Signore et al., 2008).

Similarly, there is an increased maternal demand for choline during breast feeding. Maternal liver choline stores are depleted in order to enrich the milk with high amounts of choline. PC or choline supplements given to breast feeding mothers elevate choline content in breast milk and may improve neonate development (Davenport et al., 2015). An animal study showed that PC supplementation in rats during lactation led to the improved functioning of the immune system of the breast-fed neonates (Lewis et al., 2016). Accordingly, it seems that adjusting choline content in infant formulas, based on the choline content of human breast milk, should be taken into consideration.

The recommended AIs for pregnant women and breast feeding women are 450 mg/day and 550 mg/day, respectively (Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference et al., 1998; Zeisel and Da Costa, 2009).

Scientific evidence reveals that estrogen enhances the PEMT activity in humans; thus, premenopausal women obtain more of their choline from endogenous biosynthesis while postmenopausal women require more dietary choline. A study found that 80% of postmenopausal women who consumed a choline-deficient diet showed signs of fatty liver or muscle damage, whereas just 44% of premenopausal women showed such signs (Fischer et al., 2007). To prevent choline deficiency related organ dysfunction, choline supplementation of postmenopausal women should be given special attention (Fischer et al., 2010).

SPORT AND THE DIETARY NEED FOR CHOLINE SUPPLEMENTS

According to many sports studies, free choline stores may decrease below normal concentrations during prolonged and arduous physical activity. In such sporting activities choline supplementation can elevate endurance performance. The recommended dosage for PC (90%) suggested by sports studies is 0.2 g/kg body mass (equivalent to 2.1 g of choline for an 80-kg person) to be administered 1 h before exercise (Jäger et al., 2007; Penry and Manore, 2008). However, some studies have not confirmed the positive effect of choline on endurance performance (Mason and Lavallee, 2012).

Choline supplementation used by athletes as a nutritional strategy for rapid loss of body mass is also unproven. In a study on (just twenty two) female taekwondo and judo athletes, it was observed that choline supplementation, starting one week prior to competition (2.0 g daily), reduced leptin levels, body fat, and body mass index (Elsawy et al., 2014). However, further studies are necessary to evaluate the effectiveness and safety of choline consumption for this purpose.

TOXICITY AND ADVERSE EFFECTS OF CHOLINE

Acute toxicity of choline in mice has been investigated through intranasal (200 mg/kg, every other day), intraperitoneal (200 mg/kg, every other day), and oral (200 mg/kg, daily) administrations over a 28-day period. Evaluation of hematologic and clinical biochemical parameters, as well as the histopathology of various organs, showed that choline administration via any of these routes did not lead to toxicity in mice (Mehta et al., 2009). Former literature has reported a fishy body odor, hyperhydrosis, salivation, low blood pressure, and liver toxicity associated with higher doses of choline in humans.

Considering hypotension as the most critical adverse effect, the tolerable upper intake level (UL) of choline has been determined as 3.5 g/day (Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference et al., 1998). Fishy body odor, associated with high doses of choline, is due to TMA accumulation in boys and its secretion to corporal fluids.

Furthermore, some humans with defects in the FMO3 gene fail to convert TMA to odorless TMAO (see the earlier section in this chapter entitled"Biosynthesis and metabolism of choline"). This disorder is known as fish odor syndrome or primary trimethylaminuria. A choline-restricted diet is a nutritional strategy for reducing the severity of this condition (Messenger et al., 2013).

Plasma free choline levels can be a credible parameter for the assessment of toxicity and deficiency. Normal levels of plasma choline are defined as 10–15 nmol/mL with choline toxicity occurring when the concentration of plasma free choline is higher than 200 nmol/mL (Buchman, 2009). Higher doses of choline can result in a higher plasma concentration of TMAO which is associated with an elevated incidence of CVD (see the earlier section in this chapter entitled"Choline and cardiovascular diseases").

CONCLUSION

Choline is an essential and vitamin-like nutrient with diverse biological functions. Inadequate choline nutrition in a fetus can lead to NTD and defects in hippocampal development. After birth, a poor-choline diet may result in nonalcoholic hepatic steatosis, liver injury, and steatohepatitis. Choline consumption may need special attention and dietary recommendations for pregnant, breast feeding, and postmenopausal women, as well as children with cystic fibrosis, athletes, infants, and patients with primary trimethylaminuria. No clear association has been found between dietary intake of choline and risk of cancer or CVD. Anabolism and catabolism of choline are considerably influenced by personalized characteristics such polymorphism of the genes contributing to choline metabolism, the age and sex of individuals, and even their gut flora composition. There is a growing concern regarding TMAO, a metabolite of dietary choline, which researchers have associated with the development of atherosclerotic coronary artery disease.

Although many facts about choline and its derivatives have been revealed, the need for further studies, especially clinical ones, and a reconsideration of many research findings is required. Nutritionists may still not be able to clearly provide appropriate guidance based on the existing findings such as: the relationship between choline intake and diet-related chronic diseases, the benefits and disadvantages of choline supplementation for athletes, the outcomes of choline and PC overconsumption, the control and prevention of the adverse effects of TMAO, etc.

ABBREVIATIONS

AI	Adequate intake
BHMT	Betaine homocysteine methyltransferase
CCT	CTP:phosphocholine cytidylyltransferase
CDP-choline	Cytidine diphosphocholine
CHDH	Choline dehydrogenase
CK	Choline kinase
CPT	CDP-choline:1,2-diacylglycerol cholinephosphotransferase
CVD	Cardiovascular disease
FMO3	Flavin-containing monooxygenases
MS	Methionine synthase
NASH	Human non-alcoholic steatohepatitis
NTDs	Neural tube defects
PC	Phosphatidylcholine (lecithin)
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine N-methyltransferase
PPAR	Peroxisome proliferator-activated receptor
TMA	Trimethylamine
TMAO	Trimethylamine-*N*-oxide
UL	The tolerable upper intake level
VLDL	Very low density lipoprotein

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Chapter 2.5

Carnitines (Including L-Carnitine, Acetyl-Carnitine, and Proprionyl-Carnitine)

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INTRODUCTION

Carnitine (β-hydroxy-γ-trimethylaminobutyrate), a nonessential organic nutrient, is a quaternary ammonium compound, naturally occurring in nature. It is found in greater concentration in all animal species, and in numerous microorganisms and plants (Vaz and Wanders, 2002).

L-carnitine has a number of important roles in intermediary metabolism. L-carnitine is involved in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix, where β-oxidation takes place. Other physiological roles of carnitine include modulation of the acyl-CoA/CoA ratio (Carter et al., 1995; McGarry and Brown, 1997), storage of energy as acetyl-carnitine (Bremer, 1983; Carter et al., 1995), and the modulation of toxic effects of poorly metabolized acyl groups by excreting them as carnitine esters (Duran et al., 1990; Rebouche, 1996). Aside from assisting in fatty acid transport, carnitine has an antioxidant activity, protecting various cells against oxidative injury (Ribas et al., 2014).

In animal tissues, L-carnitine is maintained by absorption from dietary sources, endogenous synthesis, and efficient tubular reabsorption by the kidney. The main sources of dietary L-carnitine include animal products, particularly red meat with 500–1200 mg/kg, followed by fish, chicken, and dairy products, containing 16–64 mg/kg. On the other hand vegetables, fruits, and grains contain very little carnitine amount (<0.5 mg/kg). Unfortunately, only 60%–70% of available carnitine is absorbed from food sources and its content can be depleted if meat is cooked at high temperature over an open flame (Bloomer et al., 2013). Although animals obtain carnitine primarily from their diet, most mammals are capable of synthesizing carnitine endogenously.

Synthetized from essential amino acids, lysine and methionine, L-carnitine is involved in reversible transesterification reactions with distinct chain length acyl-CoAs, catalyzed by carnitine acyltransferases of distinct chain length specificities (carnitine acetyl-, octanoyl-, and palmitoyltranferases) and in the transportation of activated fatty acids through membrane systems within the cell, particularly into the mitochondrial matrix (long-chain fatty acid oxidation, known as mitochondrial β-oxidation) (Kerner and Hoppel, 2013). This latter process represents the repetitive oxidative cleavage of long-chain fatty acids into two carbon units, acetyl-CoA, which is further oxidized for energy production.

In addition to L-carnitine, the biologically active form, a variety of specific carnitine forms have been studied. Acetyl, propionyl, tartrate, and fumarate are some of the carnitine salts investigated with specific goals. Acetyl-L-carnitine has the ability to cross the blood–brain barrier and has been used for enhancing cognitive function, memory, and mood (Inano et al., 2003). Ho et al. (2010) reported that L-carnitine L-tartrate has an important impact in selected markers of exercise recovery. Synthetized by the esterification of propionic acid and carnitine, the propionyl-L-carnitine (PLC) is a novel form of carnitine with multiple physiological roles which has recently been used as a food supplement in the form of glycine propionyl-L-carnitine (GPLC) (Mingorance et al., 2011).

CARNITINE

The daily requirement of carnitine by humans is met by endogenous synthesis and dietary intake, mostly from meat and meat products. In the former case, protein bound lysine is first methylated to trimethylysine (TML) using S-adenosylmethionine. Availability of the intermediate TML limits carnitine biosynthesis with most TML stored in the body

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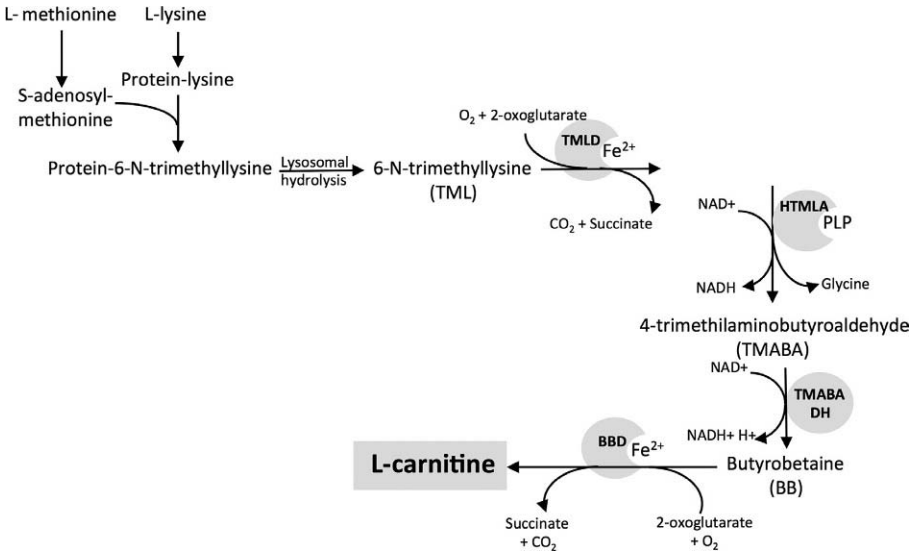

FIG. 2.5.1 Biosynthesis of L-carnitine.

being located in skeletal muscle protein. Following proteolytic liberation, free TML is converted by multiple reactions to butyrobetaine (BB) (not in cardiac and skeletal muscle), the ultimate carnitine precursor. In such process, lysine supplies the carbon skeleton to the carnitine molecule (Fig. 2.5.1) and, in turn, methyl groups come from methionine residue (Pekala et al., 2011). The *N*-metylation of lysine residues observed in many proteins (myosin and actin for example) is a kind of translational modification with such reaction being catalysed by a specific methyltransferase. S-adenosyl-L-methionine is a cosubstract with chemically reactive methyl groups attached to the sulfur atom, which makes it a methyl-group donor. The lysossomal hydrolysis of proteins containing TML releases the TML residues, which are then hydroxylated by mitochondrial dioxygenase TML (TMLD) to 3-hydroxytrimethyllysine (HTML). The next stages involve HTML cleavage to glycine and 4-trimethylaminobutyraldehyde (TMABA) catalysed by HTML aldolase (HTMLA) and the dehydrogenation of TMABA to give BB catalysed by TMABA dehydrogenase (TMABA DH). Although most tissues are capable of synthesizing BB, the hydroxylation of BB to carnitine is restricted to the liver and, to a lesser extent, in the kidneys and the brain (Berardi et al., 1998; Kerner and Hoppel, 2013), requiring the iron ion and ascorbate as cofactors (Paik et al., 1977; van Vlies et al., 2006).

Free L-carnitine, absorbed from dietary intake or synthesized in the liver and kidneys, reaches the blood stream and is then taken up by other tissues. Since the carnitine concentration in tissues is generally higher than in plasma, its body distribution is determined by a series of systems of active transport against a concentration gradient, an independent efflux process, and an exchange mechanism, specific to each tissue type. Under physiological conditions, plasma carnitine concentration is maintained within a narrow range by a modest rate of inner carnitine synthesis, dietary intake, and efficient management by the kidneys (Kerner and Hoppel, 2013). Carnitine is not metabolized in the human body, being filtered at the renal glomerulus with about 85% of it being reabsorbed by the proximal tubules (Rebouche and Engels, 1984). Less than 2% of the absorbed carnitine is excreted in urine or bile (Pekala et al., 2011), in the form of L-carnitine, acetyl L-carnitine, and other acylcarnitine esters (Rebouche and Engels, 1984). Nevertheless, longer chain carnitine esters are absorbed less than the other carnitine forms. Because tissues such as heart, muscle, liver, and kidney are very dependent on the energy generated by β-oxidation, it is crucial they have sufficient amounts of carnitine.

DIETARY SOURCES AND INTAKE OF L-CARNITINE

The carnitine reserves consist of nonesterified molecules (free carnitine) and multiple acylcarnitine esters (forms bounded to different fatty acids). About 99.5% of body carnitine is intracellular, while circulating plasma carnitine accounts for only 0.5%. Daily urinary carnitine excretion equals the sum of dietary absorption and endogenous synthesis (about 400 μmol/day) (El-Hattab and Scaglia, 2015; Rebouche, 1992; Stanley, 2004).

The body carnitine level is maintained by absorption from the diet, synthesis, and renal reabsorption. At normal physiological conditions, renal carnitine reabsorption is very efficient (90%–99% of the filtered load) being equal to the normal plasma carnitine concentration (approximately 50 μmol/L). Thus, when carnitine increases in the plasma circulation, the

efficiency of its reabsorption decreases and its clearance increases, which results in a rapid decline of carnitine concentration to its baseline. Therefore, as the dietary intake of carnitine varies, urinary carnitine excretion also varies to keep plasma carnitine within the normal range (Ramsay et al., 2001). Under normal circumstances, an adult (about 70 kg) can synthetize from 11 to 34 mg of L-carnitine each day (160–480 μg/kg body weight). This amount can be insufficient when living is stressful or physically exigent, namely in the case of men undertaking advanced sports training or athletes. About 75% of the carnitine present in the body is obtained from the diet (Flanagan et al., 2010). L-carnitine is mostly present in meats and dairy products and almost absent in vegetables (Rebouche et al., 1993). Among foods from animal origins, lamb and beef have higher L-carnitine contents than fish, pork, and poultry, followed by, in decreasing order, whole milk and cottage cheese. In fruits and vegetables, only avocado and asparagus have noteworthy amounts of carnitine (Pekala et al., 2011). Since carnitine is more concentrated in animal products, strict vegetarians, and lacto-ovo vegetarians, get very little carnitine from their diets. The rate of L-carnitine biosynthesis in vegetarians is estimated to be around 1.2 μmol/kg of body weight per day while omnivorous humans ingest 2–12 μmol/kg of body weight per day, which represents 75% of carnitine sources in the body (Vaz and Wanders, 2002). Regular supplementation is sometimes recommended but, in theory, makes sense only in individuals performing acute physically stressful tasks (muscle carnitine faster depletion). The bioavailability of oral carnitine dietary supplements is only in the order of 14%–18% of the dose (Rebouche, 2004).

CARNITINE FUNCTIONS

There are two forms of carnitine: L-carnitine (biologically active) and D-carnitine (inactive). Aside from the assistance in fatty acid transport, L-carnitine and its derivative salts (fumarate, acetyl, tartrate, propionyl, etc.) show antioxidant activity (Calo et al., 2006) and may participate in improving cognitive function (acetyl-L-carnitine) (Inano et al., 2003), exercise recovery (L-carnitine L-tartrate), (Ho et al., 2010) and nitric oxide (NO) production (PLC; GPLC) (Mingorance et al., 2011).

The main function of carnitine in intermediary metabolism is the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix. L-carnitine is yet involved in the transfer of peroxisomal β-oxidation products (acetyl-CoA) for Krebs cycle oxidation or in the modulation of the acyl-CoA/CoA ratio, storage of energy as acetyl-carnitine, and regulation of the toxic effects of poorly metabolized acyl groups by excreting them as carnitine esters (excretion in urine) (Pekala et al., 2011) (Fig. 2.5.2).

CELLULAR UPTAKE AND ACTIVATION OF LONG-CHAIN FATTY ACIDS

Long-chain fatty acids represent an unequivocal source of energy production for many organs, mainly for muscle and liver, but since most tissues have only residual levels of storage lipids, they depend on a continuous supply of fatty acids from adipose tissue following mobilization by lipolysis and transport in the blood bound to albumin. The fatty acids uptake by tissues is a process mediated by transport proteins located in the plasmatic membrane and once within the cell they are then bound to proteins existing in considerable amounts in the cytosol. Depending on the tissue demand for energy, fatty acids are transformed to triglycerides and stored for further oxidation in mitochondria. Before being sent into storage or oxidation, fatty acids are first activated to acyl-CoA esters, with such reactions being catalyzed by long-chain acyl-CoA synthetase. Cytosolic long-chain acyl-CoA is impermeable to the mitochondrial membranes and, in general, carnitine works as a

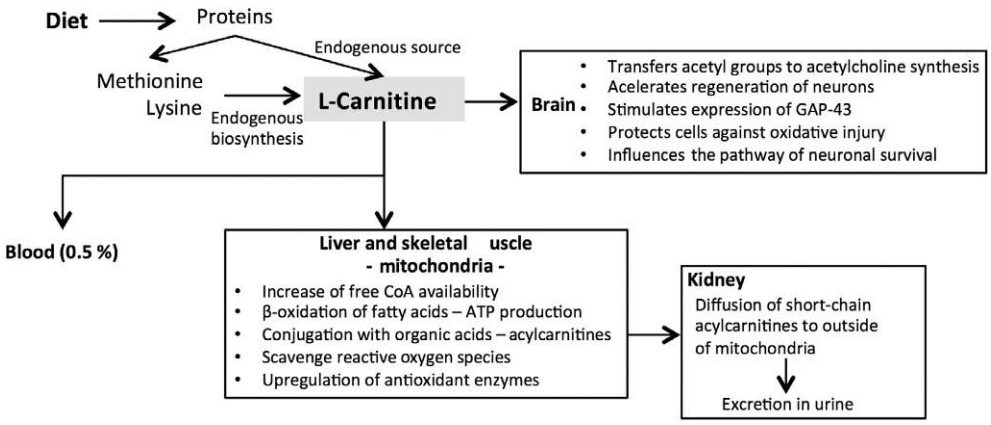

FIG. 2.5.2 Main functions of carnitine in the brain, liver, and muscle cells under physiological conditions.

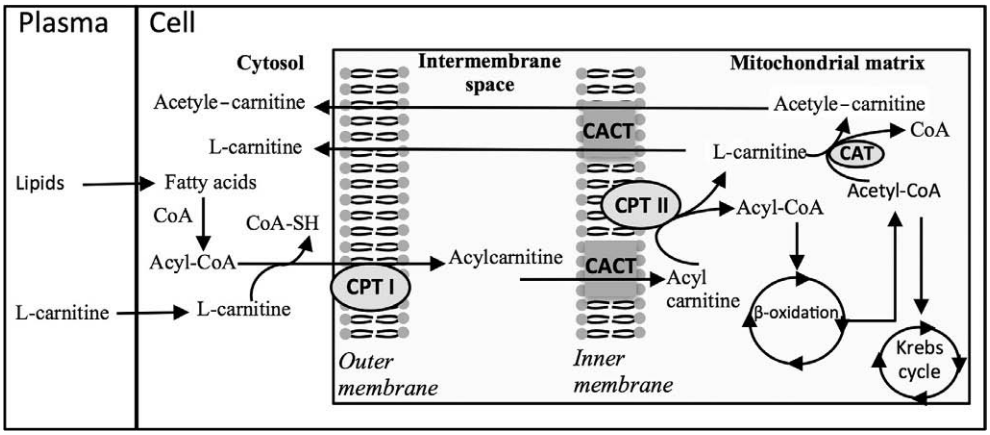

FIG. 2.5.3 Role of L-carnitine in the transport of long chain fatty acid into the mitochondria. *CACT*, carnitine-acylcarnitine translocase; *CAT*, carnitine acetyltransferase; *CPT I*, carnitine palmitoyltransferase I; *CPT II*, carnitine palmitoyltransferase II.

carrier for the acyl groups. Long-chain fatty acid acyl groups are transported exclusively as carnitine esters by translocase, which constitutes a transmembraneous protein located in the inner mitochondrial membrane. The impermeability of the mitochondrial membranes, particularly the outer membrane, can be overcome by a voltage-dependent mechanism, involving an anion-selective channel, called mitochondrial porin, which regulates the permeability of this membrane to ions and metabolites (Kerner and Hoppel, 2013).

The mitochondrial carnitine system plays a crucial role in the β-oxidation of long-chain fatty acids through their transport into the matrix, involving the malonyl-CoA-sensitive carnitine palmitoyltransferase-I (CPT-I, located in the outer membrane), the carnitine:acylcarnitine translocase (CACT) (an integral inner membrane protein), and carnitine palmitoyltransferase-II (CPT-II, localized on the matrix side of the inner membrane) (Fig. 2.5.3). CPT-I transfers activated long-chain acyl residues from acyl-CoA into carnitine. The resulting long-chain acylcarnitine esters are transported over the inner mitochondrial membrane via an integral inner membrane protein, CACT. Following the translocation of long-chain acylcarnitines into the mitochondrial matrix, the carnitine esters are converted to their respective intramitochondrial CoA esters by CPT-II, thus completing the carnitine-dependent uptake of activated fatty acids (Longo et al., 2016). Finally, the acyl-CoA undergoes β-oxidation with a release of energy in the ATP form. Fatty acid β-oxidation is a multistep process by which the activated long-chain fatty acids are broken down, with each cycle resulting in the removal of two carbon atoms from the fatty acyl residue in the form of acetyl-CoA (Kerner and Hoppel, 2013; Pekala et al., 2011).

The influence of L-carnitine on an exercise-altered metabolism may be explained by its relation to acetyl-CoA. Acetyl-CoA is a common product of glycolysis and fatty acid L-carnitine β-oxidation. Increased levels of acetyl-CoA can interfere with the conversion of pyruvate into acetyl-CoA by suppressing the L-carnitine-mediated increase (Kerner and Hoppel, 2013).

L-CARNITINE'S ANTIOXIDANT ROLE DURING OXIDATIVE STRESS

Despite the role of L-carnitine in fatty acid transport, many studies have suggested this compound as an antioxidant (Lohninger et al., 2005; Ribas et al., 2014; Surai, 2015). This role of carnitine seems to be an apparent contradiction, since L-carnitine increases the metabolism of fatty acids facilitating the formation of reactive oxygen species (ROS) by the electron transport chains of mitochondria. However, it has been reported that L-carnitine determines the formation of NO (Brown, 1999), activating oxidative damage defense enzymes (Kremser et al., 1995) and superoxide dismutase (SOD) as well as catalase against 3-nitropropionic acid-induced neurotoxicity (Kremser et al., 1995). According to the literature review we can conclude that there are several important mechanisms in the antioxidant action of carnitine (Bloomer et al., 2013; Kolodziejczyk et al., 2011; Ribas et al., 2014; Sung et al., 2016; Surai, 2015). L-carnitine is shown to directly scavenge free radicals and it can chelate transition metals (Fe^{2+} and Cu^{+}), preventing their participation in ROS formation (Surai, 2015). L-carnitine decreases free radical formation by inhibiting specific enzymes (e.g., xanthine oxidase and NADPH oxidase) responsible for free radical production, which have a high biological relevance in various stress conditions. In addition, carnitine participates in maintaining the integrity of mitochondria, including the electron-transport chain of mitochondria, in stress conditions. Indeed, carnitine can be considered as a mitochondria-specific antioxidant, responsible for the maintenance of mitochondria integrity and regulation of ROS production and ROS signaling (Surai, 2015). The

protective effect of L-carnitine and its derivatives on the antioxidant systems of the body is also shown in various models of oxidative stress/toxicity caused by a variety of toxicants and neurotoxic agents (Surai, 2015).

Determined under in vitro conditions, the antioxidant capacity of L-carnitine seems to be dependent on concentration, behaving similarly to α-tocopherol and trolox administered at a concentration of 30 μM, through scavenging effects (Gülçin, 2006). L-carnitine administration during exercise is expected to boost the activity of endogenous antioxidants, delaying fatigue by removing ROS (Wickens, 2001). In a study using human blood samples it was concluded that L-carnitine provided protective effects, including suppressing peroxynitrite-induced peroxidation and decreasing low molecular–weight thiols, glutathione and cysteine, through the oxidation of the arachidonic acid cascade and antioxidant mechanisms (Malaguarnera et al., 2009; Saluk-Juszczak et al., 2010).

Low-density lipoprotein (LDL) cholesterol is one of the major risks for cardiovascular diseases (Lembo et al., 2000), with the oxidized form being an essential element in atherosclerotic plaque formation (Boullier et al., 2001; Steinberg, 1997). Oral administration of L-carnitine in patients with diabetes with increased oxidized LDL levels reduced oxidized LDL, indicating that L-carnitine can effectively control diseases induced by ROS increase. Thus, L-carnitine is effective in a relatively wide range of ROS and ROS-induced lipid peroxidation, preventing inflammation by scavenging mechanisms. However, the precise mechanism by which L-carnitine acts as an antioxidant has not yet been confirmed (Boullier et al., 2001).

The increase of ATP synthesis by the electron transport chain and the production of ROS associated with physical exercise, promotes reduced muscle contraction, inducing fatigue and loss of performance. The administration of L-carnitine improves exercise performance since it accelerates ATP synthesis by fatty acid metabolism, removes and mutates ROS, and activates stabilization of endogenous antioxidants, improving muscle contraction efficiency and delaying fatigue (Sung et al., 2016).

OTHER ROLES OF CARNITINE IN METABOLISM

L-carnitine plays a crucial role in the maintenance of the acetyl CoA/CoA ratio in the cell during high-intensity exercise, which produces large amounts of acetyl CoA (Hoppel, 2003; Pekala et al., 2011). Such an increase inhibits the pyruvate dehydrogenase complex and consequently the rise of lactate. By reacting with acetyl-CoA carnitine suppresses the accumulation of lactic acid, forming acetyl carnitine and CoA, enhancing performance under high-intensity exercise. In some other metabolic conditions, for example, ischemia, fasting, and acute stress, characterized by increased pyruvate dehydrogenase activity and fatty acid supply from activated lipolysis, the capacity to oxidize acetyl-CoA may be exceeded, leading to an accumulation of acetyl-CoA and short chain acyl-CoA esters obtained from the degradation of branched-chain amino acids in skeletal muscle (Pekala et al., 2011).

Carnitine is also an activator of carbohydrate metabolism by promoting pyruvate oxidation associated with the decrease in acetyl-CoA content (Pekala et al., 2011).

SUPPLEMENTATION OF L-CARNITINE IN SPORTS NUTRITION

The performance enhancement of L-carnitine on exercise is due to glycogen-sparing effects, reduction in the accumulation of lactate, and an increase in fatty acid metabolism. However, the increased accumulation of ROS deteriorates the force of muscle contraction as well as the oxidation level of plasma components. Ergonomic aids in sports nutrition include dietary antioxidants such as vitamin C and E to improve exercise performance, by reducing oxidative stress (Bryant et al., 2003; Snider et al., 1992; Sung et al., 2016). Based on the few studies carried out so far, it is difficult to determine the optimal dosage of L-carnitine as well as its administration period. L-carnitine is probably beneficial when muscular function is impaired in metabolic diseases, but has little or no effect in healthy individuals. While exercise increases the metabolic rate, it can result concomitantly in excessive ROS formation. In such cases L-carnitine aids by reducing oxidative stress by attenuating ROS and accelerating endogenous antioxidant activity.

POTENTIAL EFFECT OF CARNITINE AS A THERAPEUTIC AGENT

It is well known that L-carnitine and its esters are able to improve metabolic functions, inclusively under pathological conditions (Nagesh et al., 2011; Ramsay and Zammit 2004; Shenk et al., 2009; Zhang et al., 2010).

The supplementation of L-carnitine seems to benefit conditions such as anorexia, chronic fatigue, coronary vascular disease, hypoglycemia, male infertility, and muscular myopathies, among others (Pekala et al., 2011). Clinical studies have demonstrated that L-carnitine favorably modulates oxidative stress through preventing membrane fatty acid peroxidation (Malaguarnera et al., 2009). According to Sayed-Ahmed et al. (2001) L-carnitine prevented the progression of atherosclerotic

lesions. The protective effects of L-carnitine against damage to the heart, caused by diabetes-induced alterations, and additional ischemia have been described by Schneider et al. (2005). L-carnitine may be an important agent in the protection of myocardial alterations in diabetes with additional ischemia, since it stabilizes mitochondrial and cellular functions and acts through its antioxidant or radical scavenging potential (Kolodziejczyk et al., 2011).

As a food supplement, carnitine is mostly available as L-carnitine or bound to either acetic or propionic acids (acetyl L-carnitine and propionyl L-carnitine, respectively). Acetyl-L-carnitine (ALC) is produced from L-carnitine and acetyl-CoA in mitochondria by carnitine O-acetyltransferase, and transported to the cytoplasm where it is converted back to L-carnitine and acetyl-CoA. Several studies have suggested that ALC may play a neuroprotective role in hypoxic-ischemic brain injury (Virmani and Binienda, 2004; Wainwright et al., 2006; Zanelli et al., 2005). ALC serves as a source of acetylcholine and L-glutamate, and also contributes to energy-producing reactions. The ALC appears to be the best form to use for brain disorders (Alzheimer's disease) while propionyl L-carnitine seems to be more effective for heart and peripherical vascular diseases. PLC is a naturally occurring derivative of carnitine that plays an important role in the metabolism of both carbohydrates and lipids, leading to an increase of ATP generation. PLC is transported, into the cell, to the mitochondria, where it is transformed into free carnitine and propionyl-CoA. The latter is converted into succinyl-CoA and finally to succinate, which is involved in the citric acid cycle. PLC is also a potent antiradical agent and thus may protect tissues from oxidative damage. PLC has been demonstrated to exert a protective effect in different models of both cardiac and endothelial dysfunction, to prevent the progression of atherosclerosis, and, more recently, to improve some of the cardiometabolic alterations that frequently accompany insulin resistance (Mingorance et al., 2011). PLC is a novel carnitine molecule known in the dietary supplements sector as GPLC. Both PLC and GPLC have been reported to improve the physical condition, with increased nitric oxide metabolites (Bloomer et al., 2007, 2009).

CONCLUSIONS

L-carnitine is an amino acid derivative, available in several forms, which possesses multiple physiological properties. The main known functions of L-carnitine are the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix, the modulation of the acyl-CoA/CoA ratio, storage of energy as acetyl-carnitine, the modulation of the toxic effects of poorly metabolized acyl groups, and its antioxidant activity. Several studies have demonstrated the antioxidant properties for L-carnitine in different pathologies such as diabetes, hypertension, renal and liver diseases, and also in neurodegenerative conditions. L-carnitine, as a nutritional supplement, has been considered a promising candidate for the prevention and treatment of oxidative alterations in many metabolic diseases. Concerning the optimal dosage and route of administration, additional, well-controlled studies are still needed to clarify safe, practical, and therapeutic guidelines.

Acetyl L-carnitine and propionyl L-carnitine, the main esterified forms of L-carnitine, have been studied in terms of its role in enhancing cognitive function, exercise recovery, and in the heart and peripheral vascular system. L-carnitine and/or its esterified forms seem to play an important role in the metabolism of the human body when it can be used as a therapeutic agent. L-carnitine supplementation may be useful not only to prevent tissue deficiency, but also to avoid oxidative damage, secondary to an increased production of ROS. Considering the ability of L-carnitine to easily cross the blood–brain barrier, L-carnitine supplementation may also be beneficial in preventing neurological damage derived from oxidative injury. However, further studies are required to better explore this potential role.

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Chapter 2.6

L-Cysteine

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STRUCTURE AND BIOCHEMISTRY OF L-CYSTEINE

Knowledge of the chemical properties of the common amino acids is essential for understanding the biochemistry of proteins. In addition to the 20 commonly occurring amino acids, there are many other less common ones. All the commonly occurring amino acids are α-amino acids, containing a carboxyl group and amine group bonded to α-carbon. The general structure of amino acids is given in Fig. 2.6.1A and is common to all 20 amino acids except the cyclic proline amino acid (Fig. 2.6.1B). The amino acids vary from each other in their R group or side chain, which has great impact on their solubility in water, due to their variation in size, structure, polarity, and charge. The other carbons are labeled β, δ, γ, and so on proceeding outward from the α-carbon. Based on their R group amino acids are classified into five groups.

Cysteine (Fig. 2.6.2) is one of the "biogenic" amino acids, structurally it belongs sulfur-containing amino acids, the sulfur atom in the side chain is involved in the formation of a reactive sulfhydryl (–SH) group. Cysteine differs from methionine (also a sulfur-containing amino acid) in its methyl ($–CH_3$) group, which bonded to the sulfur atom instead of the hydrogen, as shown in Fig. 2.6.3. The methyl group makes it more hydrophobic, less reactive, and sterically hindered. Cysteine belongs to the group of amino acids which contain serine, threonine, cysteine, asparagine, and glutamine, with a polar and an uncharged R group. The R groups in these amino acids are more hydrophilic than the analogous amino acids bearing a nonpolar side chain. The polarity of cysteine is attributed to the sulfhydryl moiety, in serine and threonine hydroxyl (OH) groups contribute to H bonding whereas, in asparagine and glutamine it is the amide group, which forms the H bond. The sulfhydryl group is a highly reactive reducing moiety, has a high impact on reducing the potential of many proteins like oligopeptides (GHS), and can be the active site for many proteins and enzymes, for example, thiol proteinases. The antioxidative property of GSH is attributed to the availability of cysteine making it the most crucial amino acid. In the presence of cysteine, cells can synthesize GHS; low levels of cysteine can impair the body's immune system. Three amino acids namely glutamate, glycine, and cysteine are important precursors for the synthesis of GHS in the body. Orally GHS cannot be effectively used because of its high metabolism in the digestive tract which limits the increase in intracellular GHS levels in the most important detoxification organs, such as the liver, kidneys, and lungs (Lu, 2009).

Cysteine is prone to undergo oxidation at the sulfhydryl group, which has the tendency to react with free radicals or other groups to form covalent bonds. One such example is the formation of a dimer called cysteine linked by a disulfur bridge (Kim et al., 2014). This feature is quite important in determining the primary structure, perturbations in the secondary structure, and stabilization of the tertiary and quaternary structures. The sulfur bridge is weaker than the peptide bond but stronger than the salt bridge, hydrogen bonds, and hydrophobic and Van der Waals interactions. The propensity of the formation of covalent bonds depends on factors like the redox potential of the environment and pH. Acidic conditions favor the equilibrium to be shifted toward a reduced (-SH) form whereas a more basic pH tends to be in oxidized (-SR) form, whilst the R could be any group except a hydrogen atom.

BIOAVAILABILITY OF CYSTEINE

Cysteine is available as a supplement labeled as "L-cysteine" in health food stores and pharmacies. It is mainly given in the form of a 500-mg pill, tablet, or capsule. The bioavailability of L-cysteine is low because it is prone to undergo oxidation in the digestive tract. Furthermore, oxidation in the blood stream leads to the production of toxins and free radicals like hydroxyl (–OH) (Ott, 2010). The toxicity of cysteine will be discussed in the next section.

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00007-2

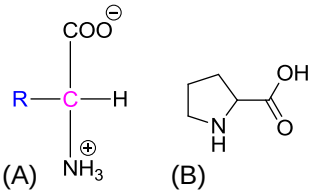

FIG. 2.6.1 (A) General structure of an amino acid: α-carbon is depicted in light gray *(pink in the web version)*. The R group or side chain bonded to the α–carbon, shown in dark gray *(blue in the web version)*, differs for each amino acid. (B) The structure of the cyclic amino acid proline.

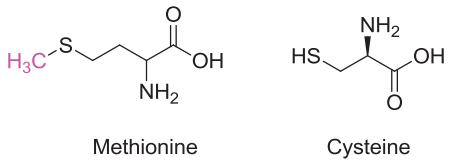

FIG. 2.6.2 Structure of methionine and cysteine.

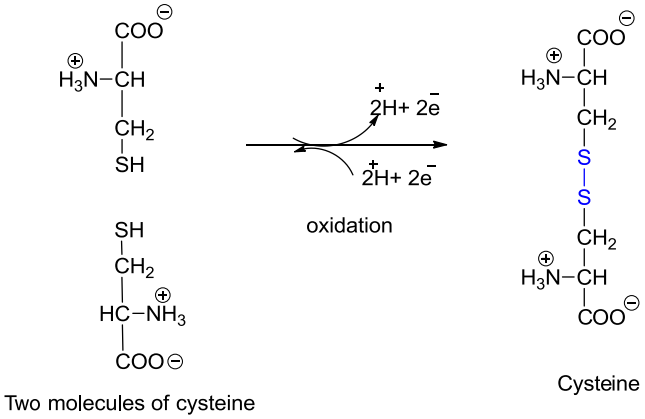

FIG. 2.6.3 Reversible disulfide bond formation by oxidation between cysteine molecules, the disulfide bond is shown in gray *(blue in the web version)* **in cysteine.**

NAC stands for N-acetyl-cysteine also known as N-acetyl-L-cysteine, and is the synthetic precursor form of cysteine and hence is used to overcome the problem of cysteine's low bioavailability. The insertion of an acetyl moiety enables cysteine to survive the digestive tract thereby increasing its half-life. L-cysteine supplements are used for several purposes such as antioxidation, heart health, and as an immune system booster to detoxify toxins and to produce glutathione, taurine, and T cells. NAC is an active component of inhalers and for decades it has been used as an effective drug used to break up mucus in diseases like asthma, emphysema, chronic bronchitis, and cystic fibrosis. NAC is given intravenously to spike GHS levels in patients with an acetaminophen overdose. In human and animal trials, NAC has shown positive effects in a wide range of diseases exemplified by oxidative stress and low levels of GHS. NAC has also been used in research on human immunodeficiency virus (HIV) patients; as such patients are severely GHS deficient. GHS plays a key role in detoxification, strong immune system health, antiaging, muscle strength, as the body's primary cellular antioxidant, and in overall disease prevention. Oral GHS supplements have gained popularity over the past few years because their convenience is considered as important as their end result. Only small amounts of premanufactured glutathione reaches the bloodstream, since most of it is metabolized in the digestive tract and cannot effectively raise the intracellular GHS levels in the most imperative detoxification organs like the liver, kidneys, and lungs. GHS is also prescribed in the form of its precursor, NAC, as described earlier. The two main drawbacks of NAC either given as a supplement and/or in clinics in emergency cases are: toxicity and a rapid spike in GHS levels followed by a sharp decline (lower than the normal levels) within hours. In order to maintain the normal levels of GHS it is imperative that NAC be taken quite often throughout the day, which is a relatively difficult task.

SOURCES OF L-CYSTEINE

L-cysteine can be obtained synthetically or from natural sources. Synthetically, L-cysteine is made either by using enzymes which foster the biochemical process or chemicals in a microbial fermentation. Synthetic L-cysteine is quite expensive,

for this reason natural L-cysteine has been widely preferred to date. Cysteine is the structural component of the protein keratin—hair, skin, and nails are made of. It is derived from natural sources like human hair and duck and chick feathers. Mostly cysteine is supplied from the Asian subcontinent where hair is collected from barbers and hairdressers. The process of cysteine production from natural sources is fairly coarse. Hair is either boiled in hydrochloric acid (HCl) or other chemicals with the end product being cysteine. An increased concern, coming from different religious groups, about the use of human hair has meant that hair is now commonly replaced by feathers. However, cheap hair continues to be a source of cysteine. The US Food and Drug Administration (FDA) regulations recommend that the source of cysteine should not be mentioned on labels of products if it is used as a flavoring.

The human body produces cysteine from essential sulfur-containing amino acid known as methionine and the protein is abundant in natural products like ricotta, cottage cheese, yogurt, sausage meat, chicken, turkey, duck, luncheon meats, wheat germ, granola, and oat flakes.

POSSIBLE INTERACTIONS OF L-CYSTEINE WITH OTHER SUPPLEMENTS, DRUGS, OR FOODS

L-cysteine is used as a supplement for various purposes, for example, to promote skin and hair health, to boost the immune system, and to combat inflammatory related problems and osteoporosis. L-cysteine induces the synthesis of GHS, which is a powerful natural antioxidant. It plays a key role in the elimination of toxins in the body and builds and maintains cell membranes and myelin sheaths, which protect neurons in brain. However, it is important to know about the possible side effects before considering taking it as a supplement especially if an individual has existing health concerns. All drugs and supplements have some level of adverse effect or toxicity to consider. Cysteine has been reported to cause unwanted effects due to the interaction with several drug classes described below.

1. Enzyme inhibitors: Angiotensin-converting enzyme (ACE) inhibitors are used to treat patients with high blood pressure and the drugs in this class include captopril (Capoten), enalapril (Vasotec), lisinopril (Prinivil), and benazepril (Lotensin). NAC, a precursor of cysteine may increase the effects of ACE inhibitors (Barrios et al., 2002).
2. Anticancer drugs: In an in vitro study using HepG2 cells, NAC demonstrated protective effects on cisplatin inducted oxidative stress and DNA damage. However, more studies are needed to clarify if this also applies to patients (Wang et al., 2014).
3. Immunosuppressive medications: NAC may induce the effectiveness of immunosuppressive drugs like azathioprine (Imuran), cyclophosphamide (Cytoxan), and prednisone (Deltasone). However, further research is needed to investigate the underlying findings and facts in this regard (Behr et al., 1997).
4. Nitroglycerin and isosorbide: These drugs are generally used to treat angina pectoris or chest pain. NAC may enhance the effectiveness of both drugs, which may possibly lead to an escalation of their side effects, such as severe headache and abnormal decline in blood pressure (Horowitz et al., 1988).
5. Antifungal drugs: Oxiconazole (Oxistat) is an antifungal medication used for athlete's foot. Topical use of NAC may increase the effectiveness of the drug (Yu and Van Scott, 2003).
6. Activated charcoal: Suppresses the effectiveness of cysteine (Renzi et al., 1985).

Cysteine toxicity can cause multiple disorders ranging from heart to renal (kidney) diseases. L-cysteine is recommended to be taken under strict supervision by a physician, especially if the patient has any other health concerns. This is because of its potential toxicity, hyperhomocysteine levels, and hyperhomocysteinemia. Furthermore, it may intervene with body's innate GHS synthesis system making it dependent upon an external source.

HEALTH EFFECTS OF L-CYSTEINE

Cysteine is a proteinogenic amino acid; a building block for about 2% of proteins, contributing to a multitude of functions in the body. The amino acid should be taken as a supplement to avoid its undersupply. It catalyzes many metabolic reactions in the body. Its typical structure, and the presence of a sulfur atom, underscores its significant contribution toward several biological processes and general well-being. An appropriate supplement of this amino acid might be beneficial to chronic conditions like cataracts or arthritis. In the case of intestinal problems, the body's cysteine requirement is increased because many nutrients cannot be absorbed and are simply lost as a result of a forfeited digestive system. Harsh environmental conditions or stress leads to an increased demand for cysteine.

Cysteine is given as a supplement in the form of its precursor, NAC, described earlier. According to recent study, cysteine not only slows down the process of aging but also helps prevent neurodegenerative disorders like dementia and multiple sclerosis (Dröge, 2005; Kondratov et al., 2009; Shahripour et al., 2014). NAC can protect cells from the side effects of

drugs and toxic chemicals. It plays a vital role in the breakdown of mucus in respiratory disorders. Moreover, it seems to be beneficial in treating respiratory disorders such as bronchitis and chronic obstructive pulmonary disease (COPD).

The role of cysteine is briefly summarized below:

1. Boosting the immune system: Apart from maintaining the communication between immune cells, GHS stimulates the production of leukotriene which is important in regulating the body's defense system by supporting macrophages—key elements in the immune system. GHS inhibits inflammatory reactions and strengthens the immune system (Zafarullah et al., 2003). A shortfall in cysteine may make the body prone to infectious diseases due to a decreased count of natural killer (NK) cells. In severe conditions, such a decline would make the immune system very fragile, which in turn may lead to cancer (Ghezzi, 2011). The antioxidative effects of NAC are associated with the production of GHS, which can palliate inflammatory effects. L-cysteine detoxifies the body's excess sulfuric acid (H_2SO_4) and oxidized vitamin C and E.
2. Lipid biosynthesis and metabolism: Cysteine plays a central role in building essential fatty acids and hence enables the synthesis of cell membranes and nerve myelin sheaths—usually around axon endings. Cysteine has the potential to prevent major neurodegenerative diseases, like Parkinson's or Alzheimer's, by protecting axons from oxidative stress and environmental stress (Hasanbasic et al., 2016; Pocernich and Butterfield, 2012).
3. Building blocks: Cysteine is a structural unit of connective tissue. Taurine, a multifunctional nutrient and an imperative factor in conducting electrical nerve impulses in the digestive and vascular systems, is synthesized from cysteine (Penttilä, 1990).
4. Reproductive system: Cysteine, when given in the form of NAC as supplement, has elicited a decrease in the level of reactive oxygen species (ROS) in semen with increase in sperm count, viscosity, and motility (Ciftci et al., 2009).
5. Osteoporosis: A decrease in bone tissue might enhance loss of bone density and ultimately lead to fractures among osteoporotic elderly women. Loss of bone density is often correlated with low concentrations of L-cysteine. Studies have shown that supplementation of cysteine remarkably decreased osteoporosis. Furthermore, L-cysteine increases the synthesis of collagen and reduces the activity of the osteoclasts (Ji et al., 2011).
6. Overdose of acetaminophen or acetaminophen poisoning: NAC is often given intravenously to people who are overdosed with acetaminophen to prevent kidney and liver damage. Acetaminophen is used in medical emergencies and may, therefore, be accidentally overdosed (Heard, 2008).
7. Angina pectoris or chest pain: NAC when given along with nitroglycerin and N-acetylcysteine, a vasodilator drug, works more effectively in reducing pain and risk of heart attack than when either are taken alone (Horowitz et al., 1988).
8. Chronic bronchitis and COPD: NAC has been demonstrated to play a central function in the breakdown of mucus in respiratory disorders like COPD and chronic bronchitis, leading to fewer flare ups (Sanguinetti, 2016).
9. Influenza: NAC decreases the chances of flu and inflammation (Gillissen, 2011).
10. HIV/acquired immunodeficiency syndrome (AIDS): In a small-scale clinical study, NAC has been found to replenish GHS. However, further research is needed to find whether NAC has a beneficial effect on HIV patients (De Rosa et al., 2000).
11. Acute respiratory distress syndrome (ARDS): This is usually the result of a medical emergency and occurs after injury to the lungs. Some studies have shown that intravenous NAC boosts levels of GHS and may prevent lung damage as a result of ARDS (Aitio, 2006).
12. Autoimmune diseases: NAC has been shown to decreases the symptoms—a dry mouth and dry eyes—associated with Sjögren syndrome (Walters et al., 1986).
13. NAC is believed to reduce the symptoms of respiratory system related disorders like asthma, cystic fibrosis, and emphysema (Sadowska et al., 2006).
14. Cancer: NAC has displayed protective effects in esophageal cancer (Balansky et al., 2002). It also reduces lung cancer risk among smokers and protects cells from the harmful effects of substances released from nicotine or tobacco consumption (Balansky et al., 2010).
15. Schizophrenia: Psychiatric disorders like schizophrenia have a multifaceted etiology that involves the upregulation of inflammatory cascades and proapoptotic proteins, neuronal calcium dyshomeostasis, oxidative stress, elevated production of ROS, mitochondrial dysfunction, and irregular glutamatergic transmission. NAC plays a remarkable role in these processes and studies have displayed its protective effects in schizophrenia patients (Lavoie et al., 2007).
16. *Helicobacter pylori* (*H. pylori*) have the potential to evade a host's immune system and resist antibiotics due to biofilm formation. The biofilm slows down the metabolic rate of cells and induces the metabolism of antibiotics. Studies have demonstrated that NAC increases the effectiveness of antibiotic drugs in patients with *H. pylori* infection. However, further research is needed in this regard (Makipour and Friedenberg, 2011).
17. Diabetes: Studies suggest that L-cysteine-containing supplements can be used as an adjuvant therapy for controlling glucose metabolism by upregulating insulin-signaling cascade in patients with type 2 diabetes (Jain, 2012).

CONCLUSION

In conclusion, L-cysteine plays a multipurpose role in the biological system, from forming a structural component of both proteins and the precursor of the radical scavenger GHS, to playing a protective role against several diseases. Cysteine is the building block of about 2% of proteins, and plays a key role in the biosynthesis of lipids and cell membranes. It is also involved in the synthesis of taurine, which is an imperative factor in conducting electrical nerve impulses in the digestive and vascular systems. L-cysteine is commonly given intravenously to patients with acetaminophen poisoning to prevent kidney and liver damage. When given in its precursor form as NAC, cysteine has a protective role in several disorders like angina pectoris, chronic bronchitis and COPD, inflammation, asthma, cystic fibrosis, and emphysema, and in doing so by boosting the levels of GHS it may also prevent lung damage. It increases the effectiveness of antibacterial medications used against *H. pyroli* infections like nitroglycerin (vasodilator drug). It acts as an immune system booster; a shortfall of cysteine may make the body prone to infectious diseases due to a decreased count of NK cells. Studies have elicited that L-cystiene decreases ROS production in semen leading to an increase in sperm count, motility, and viscosity. Furthermore, L-cysteine increases the synthesis of collagen and reduces the activity of the osteoclasts in osteoporosis. Supplements containing L-cysteine may be used as an adjuvant therapy in type 2 diabetes. However, it is imperative to consider the interactions of cysteine with drugs, foods, and other supplements, especially in patients with underlying health concerns. Researchers have demonstrated that L-cysteine may palliate the symptoms of disorders like autoimmune, schizophrenia, type 2 diabetes, influenza, colon cancer, and lung cancer. More research is needed to investigate the diverse and protective effects of L-cysteine in critical diseases like neurodegenerative disorders, cancer, and HIV/AIDS.

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Chapter 2.7

Creatine in Skeletal Muscle Physiology

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INTRODUCTION

In 1992 Roger Harris and his colleagues discovered that exogenous creatine (Cr) administration enhances muscle Cr and phosphocreatine (PCr) content (Harris et al., 1992). Since then Cr has become the most popular dietary supplement in the field of sport and exercise physiology. In particular, Cr was used for the first time in the Olympic Games in Barcelona by successful sprinters and, subsequently, has been used to enhance the physical performance in healthy individuals and athletes. At present, a huge body of evidence, from more than 25 years of research in the field, corroborates the efficacy of Cr supplementation to promote physiological function in many types of exercise of varying duration and intensity, and to aid improvements in strength, skeletal muscle mass, and bone mineral density, in healthy individuals and those with neuromuscular diseases (Bazzucchi et al., 2009; Bemben et al., 2010; Bosco et al., 1997; Candow et al., 2015; Chilibeck et al., 2015; D'Antona et al., 2014; Devries and Phillips, 2014; Griffen et al., 2015; Grindstaff et al., 1997; Gualano et al., 2011, 2014; Hespel et al., 2001; Martone et al., 2015; Metzl et al., 2001; Mihic et al., 2000; Pearlman and Fielding, 2006; Phillips, 2015; Ramirez-Campillo et al., 2015; Volek et al., 2004; Wilkinson et al., 2016). Indeed, Cr has also been recently recognized as playing a role as an antioxidant, antinflammatory, and immunomodulatory compound (Riesberg et al., 2016), as well as having interesting physiological effects on thermoregulation and cognitive performance (Twycross-Lewis et al., 2016). In this chapter we will focus on the effects of Cr supplementation in skeletal muscle physiology with particular attention to the known effects in healthy athletes.

CREATINE BACKGROUND

The Biochemistry of Creatine

Creatine (N-aminoiminomethyl-N-methylglycine, Cr) is a guanidine compound, which is endogenously synthesized by the kidneys, pancreas, and liver, through a process that involves three amino acids as group donors: (1) a methyl group from methionine, (2) an acetic group and nitrogen atom from glycine, and (3) an amide group from arginine (Fig. 2.7.1). After its production, Cr is mainly stored by the cardiac and skeletal muscle and brain. To perform its physiological role, Cr is transformed into PCr by Cr kinase. The phosphate group is provided by adenosine triphosphate (ATP), which is converted into adenosine diphosphate (ADP). PCr is a high-energy reserve, available for the conversion of ADP to ATP. This process is essential during very intensive physical activity and relative high-energy request. Cr kinase catalyzes the reversible transfer of the N-phosphoryl group from phosphoryl Cr to ADP to regenerate ATP and restore Cr skeletal muscle content (Wyss et al., 2000).

The distribution of Cr kinase isoforms at a subcellular level has led to differing points of view regarding the function of the Cr system. Wallimann et al. (1992) gave a description of the role of the different isoforms of Cr kinase. Cr kinase isoforms are typically found in the cell cytoplasm and, in particular, at sites with a great ATP demand, e.g., plasma membranes, sarcoplasmic reticulum, and myofibrils (Wallimann et al., 1992). A mitochondrial Cr kinase is also found across mitochondrial membranes and, in the presence of Cr, it guarantees the conversion to PCr when the ATP obtained from oxidative phosphorylation is available. These and other observations have promoted the fundamental role of Cr and PCr in an energy shuttle system (ESS) of high-energy phosphates between the mitochondrial sites of ATP production and the cytosolic sites of ATP utilization (Wallimann et al., 1992). The degree of involvement of this ESS depends on

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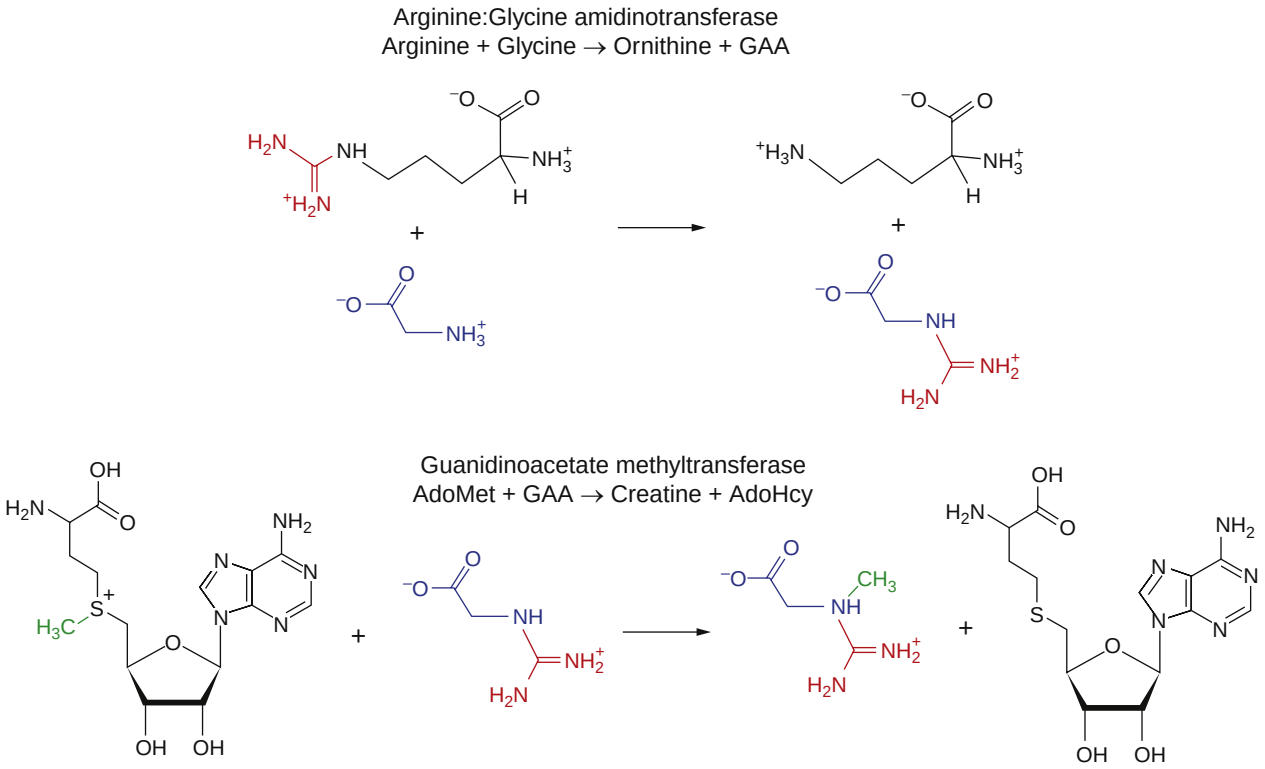

FIG. 2.7.1 Pathway of creatine synthesis. *GAA*, Guanidinoacetate; *AdoMet*, S-adenosyl-l-methionine; *AdoHcy*, S-adenosyl-l-homocysteine. *(From Brosnan, J.T., Brosnan, M.E., 2007. Creatine: Endogenous Metabolite, Dietary, and Therapeutic Supplement. Annu. Rev. Nutr. 27, 241-261).*

different physiological muscle fiber requirements. Thus, in fast-twitch muscle fibers, mainly anaerobic/glycolytic, the ATP production based on the efficacy of the ESS function predominates, whereas in slow-twitch, oxidative, the ESS function is less important. The Cr/PCr system has a number of additional functions (Wallimann et al., 1992). These include maintaining the cellular ATP/ADP ratio and buffering the products of ATP hydrolysis, which protects the thermodynamic efficiency of ATP splitting. ATP hydrolysis generates ADP, phosphate, and a hydrogen ion. When high-intensity exercise occurs, the ATP must be hydrolyzed very rapidly and it is essential that both ADP and the hydrogen ion are buffered so that: (1) prevention of a rise in [ADP] caused by the Cr/PCr system reduces the inhibition of ATPases; (2) local cellular hydrogen production, due to high rates of ATP utilization, is buffered by the Cr/PCr system when the Cr kinase reaction leads to ATP resynthesis (Brosnan and Brosnan, 2007). Fig. 2.7.2 illustrates the aspects of the Cr kinase system described above.

The Metabolism of Creatine: Loss, Replacement, and Tissue Transport Regulation

Cr and PCr elimination is primarily based on their break down to creatinine and its excretion through the urine. The rate of Cr loss in humans is about 1.7% of the total body pool per day (Wyss et al., 2000), considering that in a 70-kg man the total body Cr content is about 120 g, with a turnover of about 2 g/day (D'Antona et al., 2014). Since more than 90% of Cr and PCr is found in skeletal muscle, Cr losses (and creatinine excretion) vary in relation to gender and age. Creatinine excretion changes during life in an almost linear manner, reaching a maximum in 18–29 year olds, with mean rates of loss of about 24 mg/kg per 24 h, reducing to mean rates of about 13 mg/kg per 24 h in 70–79 year olds. Women have mean rates about 20% lower than men (Cockcroft and Gault, 1976).

Generally food provides 50% of our Cr daily requirement (approximately 1 g/day), with the body synthesizing the other 50% endogenously. Exogenous dietary sources of Cr include meat and fish, with Cr concentrations ranging from 4 to 5 g/kg in meat and from 4 to 10 g/kg in fish (D'Antona et al., 2014). No dietary Cr is available for vegans, and vegetarians have very few sources of Cr in foods. Both vegans and vegetarians essentially require de novo synthesis for all of their Cr stores, but studies have shown that Cr synthesis is insufficient in these subjects, with a decrease of serum/muscle Cr levels compared to omnivores (Delanghe et al., 1989; Lukaszuk et al., 2002). Cr synthesis rates change during ageing and

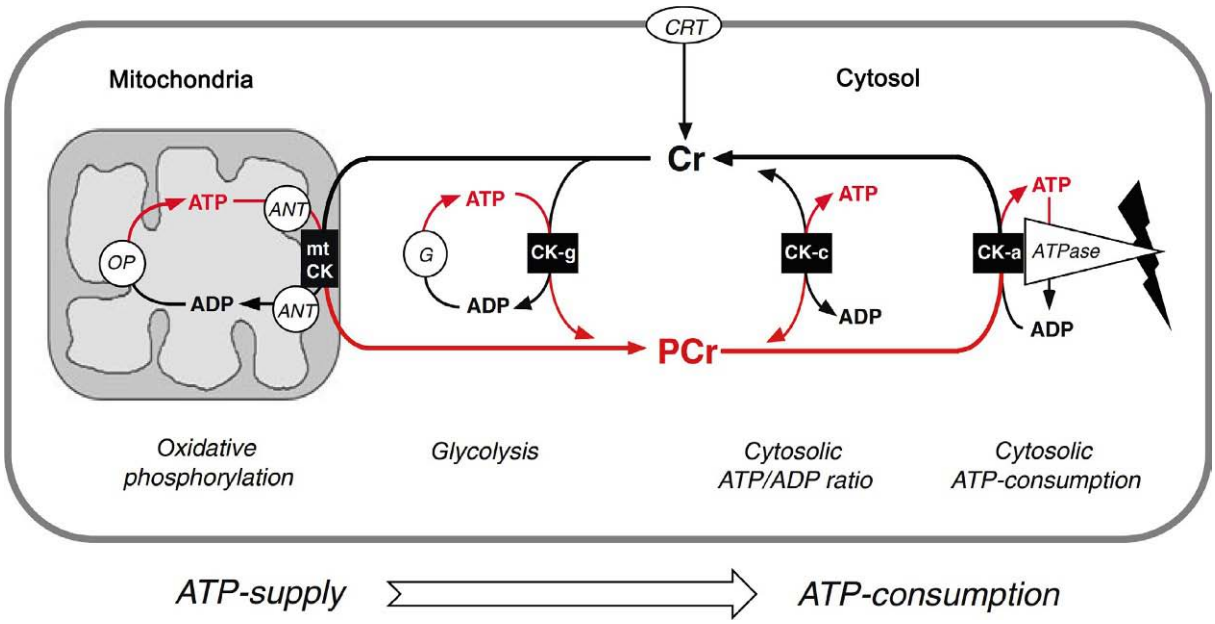

FIG. 2.7.2 The creatine kinase/phosphocreatine system. *ADP*, Adenosine diphosphate; *ATP*, adenosine triphosphate; *CK*, creatine kinase; *Cr*, creatine; *PCr*, phosphocreatine; *CRT*, Cr transporter; *mtCK*, mitochondrial CK isoforms; *ANT*, adenine nucleotide translocator; *OP*, oxidative phosphorylation; *G*, glycolytic enzymes; *CK-g,* glycolytic CK; *CK-c,* cytosolic CK; *CK-a*, ATPases CK (transporters, pumps, enzymes). *(Modified from Brosnan, J.T., Brosnan, M.E., 2007. Creatine: Endogenous Metabolite, Dietary, and Therapeutic Supplement. Annu. Rev. Nutr. 27, 241-261).*

in individuals with a typical Western diet, aged between 20–39, 40–59, and over 60, the estimated rates of Cr synthesis is about 7.7, 5.6, and 3.7 mmol/day, respectively. Women have mean rates of Cr synthesis of about 70%–80% of those observed in men (Brosnan and Brosnan, 2007).

Typically, the tissues containing the most Cr (e.g., skeletal and cardiac muscle) are essentially unable to synthesize it. A Cr transporter (CRT) mediates the uptake of Cr into different tissues such as the kidneys, cardiac muscle, brain, and primarily skeletal muscle. Cr uptake into tissues occurs against a high concentration gradient: blood Cr concentrations range from 50 to 100 μM while, for example, skeletal muscle Cr concentrations generally range from 5 to 10 mM. CRT is a Na^+-dependent neurotransmitter transporter (Gregor et al., 1995), enhanced by insulin. CRT activates Na^+/K^+-ATPase and presumably increases the driving force for Cr uptake (Snow and Murphy, 2001). Cr transport may be regulated through acute and chronic mechanisms. Acute regulation may be brought about by changes in the Cr concentration. Chronically, Cr transport may be regulated by gene expression, translation, or posttranslational modification of the CRT. Furthermore, an inverse relationship regulates Cr uptake and its intracellular concentration (Dodd et al., 1999). In fact, a high extracellular Cr concentration initially causes an increase in transport, which is followed by a down-regulation of CRT expression and a reduction in Cr transport (Loike et al., 1988).

CREATINE SUPPLEMENTATION: PROTOCOLS, PHARMACEUTICAL FORMS, AND THEIR SAFETY

The most popular Cr intake protocol found in the literature includes a loading phase of 20 g/day for 5 days (divided in four daily doses), followed by a 2–5 g/day maintenance dose. Generally, skeletal muscle significantly increases its Cr content over the first 2–3 days of supplementation. During this time, the osmotic effects of Cr uptake are assumed to be responsible for body water retention, leading to a reduction in urine output typically being observed (Ziegenfuss et al., 1998). Cr has frequently been taken together with carbohydrates and this combination seems to increase its uptake into the skeletal muscle, probably due to the effect of insulin. Different carbohydrate dosages were used in several trials, but the most effective dosage to significantly improve Cr uptake in muscle was 100 g per 5 g of supplemented Cr (Green et al., 1996).

Exercise is another potent stimulus for Cr uptake by skeletal muscle. This finding was demonstrated for the first time by Harris et al. (1992), in a typical one-leg study. Cr supplementation has been found to increase total muscle Cr (Cr plus PCr) by about 25% and when coupled with exercise, by an average of 37%, without affecting muscle ATP levels (Harris et al., 1992). Considerable interindividual variation exists in the degree of muscle loading after supplementation.

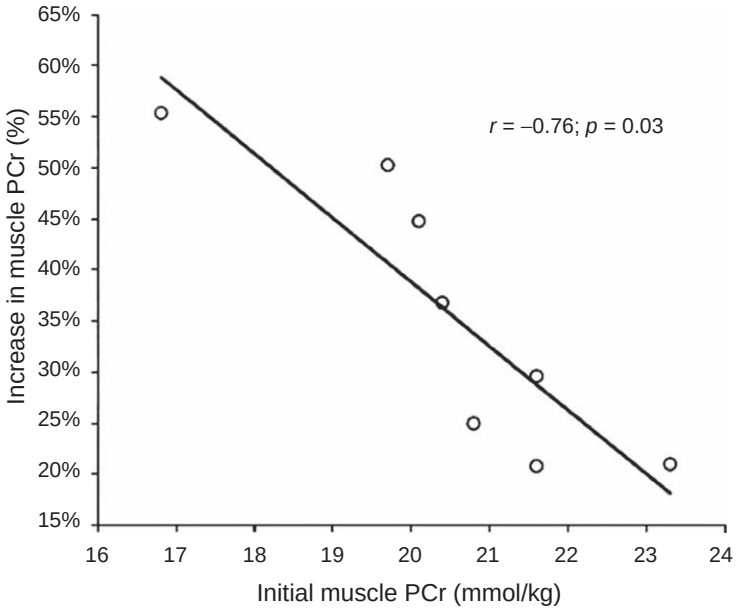

FIG. 2.7.3 The increase in muscle phosphocreatine (PCr) after creatine supplementation is inversely related to presupplementation PCr levels. *(From Brosnan, J.T., Brosnan, M.E., 2007. Creatine: Endogenous Metabolite, Dietary, and Therapeutic Supplement. Annu. Rev. Nutr. 27, 241-261).*

Although the reason for this is far from understood, it is clear that presupplement muscle Cr concentration is critical (Rawson et al., 2002). Indeed, an inverse relationship between the increase in muscle PCr following supplementation in the young (between 20 and 32 years of age) and their Cr presupplementation levels has been established (Fig. 2.7.3).

Cr monohydrate (CM) is the form most frequently cited in scientific literature and most commonly found in dietary supplement/food products (Jäger et al., 2011). Besides CM, other Cr forms (Table 2.7.1) were recently introduced into the market, with claims that they exhibit a higher physical performance improvement, bioavailability, effectiveness, and/or safety profile than CM (Andres et al., 2016). However, to support these marketing claims there is little to no evidence available in the literature (Jäger et al., 2011).

At present, there are no clinically significant side effects reported following CM supplementation at recommended doses, unlike weight gain—an attribute often required by many athletes and subjects affected by muscle diseases (Bender et al., 2008; Dalbo et al., 2008; Kreider et al., 2003a; Schilling et al., 2001).

CREATINE SUPPLEMENTATION AND PHYSICAL PERFORMANCE

Cr supplementation and its effects on strength and muscular performance have been widely studied. The International Society of Sports Nutrition's (ISSN) position claims Cr to be the most effective food supplement available so far for increasing high-intensity exercise and gaining lean muscle mass (Kreider et al., 2010). Typical studies indicate that Cr supplementation during training can increase one-repetition maximum (1RM) strength and power. Recently, a metaanalysis of 63 studies showed that Cr supplementation enhances 1RM leg press and 1RM squat by 3% and 8%, respectively (Lahners et al., 2015). In several experimental conditions the potential of Cr on anaerobic performance parameters such as total working capacity, peak power output, resistance capacity, total work performed, total strength expressed, and others, have been extensively demonstrated (Wilborn, 2015).

Strength, power, and muscle-building athletes are typically users of Cr supplements, although evidence suggests some benefits for endurance athletes could also be obtained. In particular, Cr supplementation has not been linked to functional improvement in runners involved in distance races longer than 5000 m, but performance increases could be obtained over shorter and/or intermittent training for distances under 2000 m (Earnest, 1997; Earnest et al., 1997). The Cr supplementation effectiveness on endurance capacity can be attributed to several mechanisms: (1) PCr aids ATP resynthesis, in a decreasing role in relation to duration and intensity of muscle work (Bangsbo et al., 1990); (2) Cr may help produce ATP aerobically, considering the ESS function of Cr between the mitochondria and muscle fibers (Wallimann et al., 1992); and (3) muscle Cr can support anaerobic glycolysis and this may lead to a reduced intramuscular lactate production (Earnest and Rasmussen, 2015). Furthermore, based on the osmotic effects of Cr uptake (Ziegenfuss et al., 1998), the use of Cr in endurance sports

TABLE 2.7.1 Different Forms of Creatine and Their Creatine Content Percentage (Jäger et al., 2011)

Form of creatine	Creatine content (%)	Difference in CM (%)
Creatine anhydrous	100.0	+13.8
CM	87.9	0
Creatine ethyl ester	82.4	–6.3
Creatine malate (3:1)	74.7	–15.0
Creatine methyl ester HC1	72.2	–17.9
Creatine citrate (3:1)	66	–24.9
Creatine malate (2:1)	66	–24.9
Creatine pyruvate	60	–31.7
Creatine a-amino butyrate	56.2	–36.0
Creatine a-ketoglutarate	53.8	–38.8
Sodium creatine phosphate	51.4	–41.5
Creatine taurinate	51.4	–41.6
Creatine pyroglutamate	50.6	–42.4
Creatine ketoisocaproate	50.4	–42.7
Creatine orotate (3:1)	45.8	–47.9
Carnitine creatinate	44.9	–49.0
Creatine decanoate	43.4	–50.7
Creatine gluconate	40.2	–54.3

CM, Creatine monohydrate.

(alone or in combination with other compounds) has been speculated as a possible strategy for preserving hydration and minimizing sweat loss (Beis et al., 2011; Easton et al., 2007; Francaux and Poortmans, 1999; Polyviou et al., 2012; Saab et al., 2002; Watson et al., 2006). However, despite the fact that some effects of Cr supplementation on thermoregulatory and cardiovascular responses have been reported, this does not seem to affect significantly muscle work capacity in hot environments (Beis et al., 2011; Easton et al., 2007; Kilduff et al., 2004; Polyviou et al., 2012). Other studies have failed to observe any effects of Cr on thermoregulation (Bennett et al., 2001; Branch et al., 2007; Oopik et al., 1998; Rosene et al., 2015; Terjung et al., 2000).

Creatine Supplementation and Muscle Hypertrophy

In several strength and power/speed sports an increase in muscle mass is often required to improve performance by athletes. Based on this statement, the hypertrophic effects of Cr supplementation have been investigated in different papers. During a typical Cr loading period (first 5–7 days), data indicate that individuals will experience approximately 0.6–2.0 kg gains in lean body mass (Earnest et al., 1995; Green et al., 1996; Kreider et al., 1998; Kreider, 2003). Furthermore, Cr supplementation during chronic resistance exercise studies (approximately 6–8 weeks) has shown to increase the lean body mass by about 3 kg (Earnest et al., 1995; Kreider et al., 1996; Stout et al., 1999). Other studies indicate that 4–8 weeks of strength training coupled with Cr supplementation, in combination with other substances (e.g., glucose), can stimulate greater gains in lean mass when compared to Cr supplementation alone (Kreider et al., 1998; Stout et al., 1997). These results are typically observed in men, but similar gains in lean muscle mass have been also reported in women.

Initial studies investigating the role of Cr supplementation suggested that additional water retention in the muscle primarily contributes to the gain in mass. However, subsequent research suggested that Cr supplementation increases body mass through greater muscle protein synthesis and therefore of muscle fiber hypertrophy. From this point of view the pioneering

contribution made by Volek et al. (1999) is very interesting, demonstrating that a Cr ingestion in resistance-trained males increased significantly the muscle fiber cross-sectional area in each of the muscle fiber types observed: type I (35% vs. 11%), type IIA (36% vs. 15%), and type IIX (35% vs. 6%). Based on these findings, Willoughby and Rosene further examined the effects of Cr supplementation on gene and myosin heavy-chain protein expression of contractile filaments (Willoughby and Rosene, 2001, 2003). The conclusion by the authors indicated that increases in lean body mass are not solely attributable to greater water retention in the muscle, but rather to regulation of protein synthesis through a different gene expression of myogenic regulatory factors induced by Cr. These myogenic regulatory factors (e.g., MRF-4, Myf-5, Myo-D, and myogenin) act to control gene expression by binding to DNA and subsequently promoting muscle-specific gene transcription of fundamental muscular proteins such as myosin heavy chain, myosin light chain, α-actinin, troponin I, and Cr kinase (Lowe et al., 1998). Furthermore, the influence of Cr supplementation on satellite cell (SC) function, based on myonuclear domain theory, was explored by Olsen et al. (2006) and Safdar et al. (2008).

Olsen et al. (2006) demonstrated that Cr ingestion (load: 24 g/day, 6 g/serving, 4 servings/day, 7 days; maintenance: 6 g/day, 1 serving/day, 15 weeks) during a resistance exercise promotes the proliferation of SCs and stimulates the myonuclei relative distribution in skeletal muscle. Safdar et al. (2008) referred that supplementation of Cr (load: 20 g/day, 10 g/serving, 2 servings/day, 3 days; maintenance: 5 g/day, 1 serving/day, 7 days) induces proliferation and differentiation of SCs thus activating genes of cytoskeletal remodeling.

Creatine Supplementation and Exercise-Induced Muscle Damage and Injuries

High-intensity muscle work leads to myofibrillar damage in athletes. Muscle recovery passes through structural reparation and Cr supplementation has been demonstrated to regulate at least four important mechanisms involved in the regeneration process (Kim J et al., 2015).

(1) The first mechanism concerns the inflammatory response induced by exercise-induced muscle damage. Santos et al. (2004) showed that 20 g/day of Cr, administrated to 34 male runners for 5 days before a competition, diminished prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), and lactate dehydrogenase (LDH) after a competition of 30 km. In agreement with these data, Bassit et al. (2008) described how 11 male triathletes who introduced 20 g/day of Cr for 5 days before a half-Ironman contest had reductions in postexercise levels of interferon-α (INF-α), TNF-α, PGE2, and interleukin-1β (IL-1β). Deminice et al. (2013) referred that 0.3 g/kg of Cr ingested for 7 days suppresses the rise in TNF-α after a repeated bout of anaerobic running tests.

(2) The second possible Cr mechanism is to reduce oxidative stress (Lawler et al., 2002; Rahimi, 2011). One of the first pieces of evidence for the antioxidant capacity of Cr was described by Lawler et al. (2002). Later a subsequent study (Deminice and Jordao, 2012) indicated that Cr ingestion during the 28 days before acute muscle activity diminishes lipid hydroperoxides and thiobarbituric acid-reactive substances (TBARS) with an increase of total antioxidant capacity and in particular glutathione (GSH) and glutathione disulfide (GSSG) ratio. In another clinical trial, for 7 days Rahimi (2011) administrated 20 g/day of Cr to individuals who went on to show decreased levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and malonyldialdehyde (MDA) in the blood after a resistance exercise session. Contrary to this, other studies reported that Cr supplementation does not decrease oxidative stress after exercise-induced muscle damage (Deminice et al., 2013; Silva et al., 2013). Although the role of Cr on exercise-related oxidative stress is very interesting, poor data are available at present and this mechanism needs to be investigated in more detail.

(3) The third mechanism of action of Cr possibly involves the regulation of muscle calcium trafficking. Muscle damage may increase calcium concentrations in the cytosol due to an impaired sarcoplasmic reticulum function, leading to further muscle damage (Beaton et al., 2002). Cr regulates the sarcoplasmic reticulum calcium pump function by phosphorylating ADP to ATP and decreasing cytosolic calcium levels (Cooke et al., 2009; Korge et al., 1993). From this point of view, Minajeva et al. (1996) proposed that an increase of muscle PCr, with Cr supplementation, promotes ATP regeneration leading to an attenuation of calcium-related damage. However, this hypothesis needs investigation.

(4) The last mechanism has been associated with SC activation and proliferation (Olsen et al., 2006; Safdar et al., 2008). SCs are known to play a key role during the regeneration process after muscle damage (Paulsen et al., 2012) and the role of Cr supplementation has already been shown in the previous paragraph.

CONCLUSION AND FUTURE DIRECTION

At present, there is an important core reference that underlines how Cr supplementation is safe and can lead to significant performance enhancement. The positive effect of Cr on muscle physiology is useful in several anaerobic/aerobic

exercise, resistance, and speed training programs, as well as intermittent exercise and muscle hypertrophy programs. Cr supplementation may also prevent exercise-induced muscle damage, facilitating recovery after training sessions and/or contests, and this can facilitate muscle sports-specific adaptation. However, a clear comprehension of how Cr can regulate the complex muscle subcellular signal network during exercise recovery needs to be better established with other well-designed studies. In addition, Cr promises to be effective on bone metabolism but available data on its effect on bone accretion are still inconsistent (and beyond the topic of the chapter). In conclusion, further accurate study must be designed with appropriate groups of subjects and longer duration treatments (>52 weeks) to understand the unclear mechanisms by which Cr supplementation can, in the long term, affect the physiology of the muscle.

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Chapter 2.8

Coenzyme Q_{10} and Embelin

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COENZYME Q_{10}

Chemical Structure and Distribution in the Body

Coenzyme Q (CoQ), also called ubiquinone for its structural similarity with fat-soluble vitamins K and E, is an organic molecule constituted by a benzoquinone ring with a long hydrophobic isoprenoid chain. The chemical nomenclature of CoQ is 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone where Q refers to the quinone chemical group (Bhagavan and Chopra, 2006). Coenzyme Q is endogenously synthesized starting from acetyl-CoA, through a multistep process requiring the presence of several enzymes derived from vitamins such as niacin, riboflavin, pyridoxine, and folic acid. The long isoprenoid side tail is synthesized by condensing farnesyl-PP (FPP) and then condensing the polyisoprenoid chain with hydroxybenzoate (derived from tyrosine or phenylalanine) (Dallner et al., 2003; Tran and Clarke, 2007). It was first identified in 1940 and isolated from the mitochondria of bovine hearts by F.L. Crane of the University of Wisconsin (Bliznakov et al., 2004). The structure was then characterized in 1958 by D.E. Wolf in the Merck labs and in 1961, P. Mitchel proposed CoQ as a component of the electron transport chain. Different types of CoQ can be distinguished depending on the number of isoprene units in the lateral chain. In mammals and primarily in humans, the most common type of CoQ is Q_{10} consisting of 10 isoprene units, but in other organisms also only 6 (CoQ_6) or 8 (CoQ_8) isoprene units can be present (Cluis et al., 2007). The long isoprenoid tail makes CoQ soluble in the lipid bilayer of membranes, where it can be found in both protein-bound and unbound form. The average of total pooled CoQ_{10} in a normal adult male is in the 0.5–1.5 g range (Bhagavan and Chopra, 2006), distributed in the membranes of the endoplasmic reticulum, peroxisome and lysosomes, in the vesicles, and inside the mitochondrial membrane, where it is mainly involved in aerobic energy production by accepting electrons from complexes I and II and transporting them to complex III. In human blood, the normal levels of CoQ_{10} vary from 0.7 to 1.0 μg/ml, where it is present in lymphocytes and platelets, whereas erythrocytes, which lack mitochondria, contain only a small percentage, mostly associated with membranes. Generally, the highest amount of CoQ_{10} is found in organs with high rates of metabolism such as the heart, kidneys, and liver, where it functions as an energy transfer molecule. In this contest, CoQ_{10}, depending on oxidation state, can be present in three forms: an oxidized form of ubiquinone, a semikinonic intermediate (QH), and a reduced form of ubiquinol (QH_2).

Antioxidant Activity

Ubiquinol has another important function—it is a powerful natural antioxidant that prevents and neutralizes the generation of reactive oxygen species (ROS). It also elevates the total antioxidant capacity of cells because it is able to regenerate ascorbate and recycle tocopheryl radicals back to tocopherol (Crane, 2001). Coenzyme Q_{10}, therefore, efficiently contributes to inhibit lipid peroxidation and protects against severe damage that an excess of ROS would cause to cellular structures (Al-Hasso, 2001). Conversely, an alteration in biosynthesis causes a decrease in the amount of CoQ_{10} in the body resulting in a decline in energy efficiency and antioxidant defense processes. This happens in some genetic diseases as well as other common pathologies. In these conditions, several biological and clinical studies have shown the positive effect of oral administration of CoQ_{10}, which rapidly restores physiological blood and tissue levels and often improves clinical symptomatology.

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Natural Sources and Food Supplementation

The daily average intake of CoQ_{10} is about 3–6 mg, with about half of the total amount being in reduced form. Human organism is also able to biosynthesize CoQ_{10}, but its levels in the tissues drop progressively with the increase of age (Ely and Krone, 2000). Coenzyme Q_{10} biosynthesis starts from tyrosine through a cascade of eight aromatic precursors, which also require the presence of eight vitamins (namely tetrahydrobiopterin, B2, niacin, vitamins B6, C, B12, folic acid, and pantothenic acid). Among the richest dietary sources of CoQ_{10} are: meat, fish, nuts, and some oils (10–50 mg/kg range), while reduced levels are found in vegetables, fruits, and cereals (1–10 mg/kg range) (Pravst et al., 2010). Animal hearts and livers represent one of the rich sources of the molecule with an indicative content ranging between 30 and 200 mg/kg, followed by oil ranging between 10 and 150 mg/kg. However, in some cases dietary intake may be inadequate to meet the body's needs. In order to improve the bioavailability of CoQ_{10} after an oral administration, several main fortification strategies have been adopted, such as the addition of CoQ_{10} to food during processing, its addition to the feed given to slaughter animals, or the genetic modification of plants (Pravst et al., 2010). Additionally, in cases where a significant supplement is needed, it is possible to use powder-filled capsules, an intravenous solution, or an intraoral spray. Generally, the most extreme cases of CoQ_{10} deficiency are highlighted in the elderly, in patients with coronary artery disease, and individuals with immune suppression or some neurodegenerative diseases (Weant and Smith, 2005; Yang et al., 2016).

Clinical Studies and Interactions of Coenzyme Q_{10} with Drugs

Several clinical aspects of CoQ_{10} have been reported in the literature (Kagan and Quinn, 2001). Case reports and both preclinical and clinical studies have analyzed its beneficial role in cardiovascular, neurodegenerative, and mitochondrial conditions, as well as in diabetes, periodontal disease, male infertility, and some other diseases. Coenzyme Q_{10} also decreases the onset of oxidative stress in the human skin and is helpful in the treatment of cancer patients. Itagaki et al. (2008) demonstrated that CoQ_{10} affects the transport activity of P-gp and that it is important to analyze the potential CoQ_{10} interactions with drugs in order to prevent undesirable and harmful effects. Although there are a limited number of studies, drug interactions have been reported between CoQ_{10} and warfarin, which has a structure similar to K vitamins (which may explain its interaction with the anticoagulant). Therefore, patients being treated with warfarin have to consider the possibility of its failure as a treatment when taking CoQ_{10} supplementation (Landbo and Almdal, 1998). Moreover cholesterol-lowering drugs (such as statins) decrease endogenous biosynthesis of CoQ_{10} (Levy and Kohlhaas, 2006)

EMBELIN

Embelin is a phytochemical, chemically known as 2,5-dihydroxy-3-undecyl-1,4-benzoquinone, which is a characteristic constituent of the fruits from the *Embelia ribes* Burm (Family: Myrsinaceae). Its specific features are the presence of a dihydroxyquinone core with a long hydrophobic tail (composed of eleven atoms of carbon) characteristic of the genus *Embelia*. The plant grows in the Indo-Malaysian area, and, in particular in India, Sri Lanka, China, Malaysia, and Singapore, where it is an essential element of folk and traditional medicine, utilized for the treatment of several chronic inflammatory disorders, heart and urinary conditions, snake and insect bites, and tumors (Radhakrishnan et al., 2012). Moreover, the dried fruits show astringent, anthelmintic, expectorant, depurative, digestive, antibacterial, stomachic, diuretic, carminative, and stimulant properties (Nadkarni, 1996; Venkateshwar et al., 2008).

Antioxidant Activity

The chemical structure of embelin makes it suitable for interaction with radical species. Embelin has shown: radical scavenging activity towards the diphenyl-picrylhydrazyl radical (with an IC50 of 23.3 ± 0.5 μM), hydroxyl radical induced deoxyribose degradation (with an IC50 of 102 ± 0.8 μM), a high Fe^{3+} reducing ability, an inhibition of lipid peroxidation, and a restoration of Mn-superoxide dismutase in rat liver mitochondria (Joshi et al., 2007; Sumino et al., 2002). Moreover, Singh et al. (2009) described the antioxidant activity of embelin against hepatotoxicity in rats (at a concentration of 25 mg/kg body weight), while Radhakrishnan et al. (2012) analyzed its antiperoxidation properties on lipid membranes and restoration of impaired superoxide dismutase in lymphocytes and fibroblasts treated with ultraviolet B radiation (UVB), resulting in an increase in antioxidant levels in irradiated cells treated with embelin. In the study of Singh et al. (2009) oral administration of embelin (25 mg/kg), from day 1 to day 15, during the treatment of rats with carbon tetrachloride (CCl_4), resulted in a significant decrease in lipid peroxidation in both liver and serum, along with concomitant elimination of radical species, and normalization of marker enzymes (such as transaminases, alkaline phosphatase, c-glutamyl transpeptidase, and lactate dehydrogenase), cytochrome P450, and total bilirubin and protein levels.

Antiinflammatory Activity

Embelin has been commonly utilized in traditional medicine for its potential antiinflammatory properties, especially relieving rheumatism and fever, and is a component of the *E. ribes* plant (Handa et al., 1992). Some of the first studies on embelin and its derivatives (such as 2,5-isobutylmine salts) have reported it to possess antiinflammatory properties in rat models (Chitra et al., 1994; Handa et al., 1992). The topical application of embelin in both acute and chronic models of psoriasis in mice treated with 12-*O*-tetradecanoylphorbol-13-acetate resulted in the decrease of skin thickness and weight, inflammatory cytokine release, and neutrophil-mediated myeloperoxidase activity, most probably due to a direct effect of this compound with the inhibition of proinflammatory cytokines IL-1β and TNF-α. Changes in substituents, and the condensation of embelin with various aromatics (especially primary amines), yielded the formation of new molecules with also remarkable antiinflammatory properties (Mahendran et al., 2011a,b). The oral administration of embelin (25 and 50 mg/kg) accelerated cutaneous wound healing in diabetic rats (Deshmukh and Gupta, 2013).

Modulation of Enzyme Activity

The activity of embelin can be attributed to its direct modulation of specific enzymes. The work of Hattori et al. (1993) showed that embelin is one of the major substances from *E. ribes* able to inhibit the activities of reverse transcriptase. Vijaya and Vasudevan (1993) described its potential as a noncompetitive and reversible inhibitor of trypsin. Moreover, embelin is a strong inhibitor of hepatitis C virus protease (IC50 = 21 μM) and acetylcholinesterase (Hussein et al., 1993; Vinutha et al., 2007).

Anticancer Activity

Quinone derivatives (such as embelin) are used as antitumour agents and their activity is known to be, in part, due to their redox potential and the creation of semiquinone radicals (Joshi et al., 2009). An in-depth and complete analysis of the anticancer potential of embelin has been performed by Poojari (2014). In general embelin can permeate the cell membrane and it induces apoptosis, activation of caspase 9, and a shift of the cellular redox balance toward oxidative stress and cell cycle block. In prostate cancer cells, for instance, it specifically binds to the XIAP BIR3 domain—a promising cancer therapeutic target—and shows synergistic effects with other drugs [as well as decreased androgen receptor (AR) and prostate-specific antigen expression (Chen et al., 2006; Nikolovska-Coleska et al., 2004)]. Moreover, the activity of embelin on several cell lines (such as inflammatory breast cancer cells, glioblastoma U251, LN229, and NCH89 cells, human myeloid HL-60 leukaemia cells, hepatocellular carcinoma HepG2, and Huh 7 cells, human multiple myeloma, prostate carcinoma, and head and neck squamous carcinoma cells) and mouse models has been reported in the literature (Poojari, 2014).

Metabolism and Toxicity

Embelin is rapidly metabolized in rats and reaches a peak in the plasm (9.0 μg/ml) half an hour after oral ingestion of potassium embelate (20 mg/kg). After absorption the compounds can be identified in the brain, liver, heart, lungs, and spleen. The kidneys are principally responsible for embelin excretion. Approximately 24 h after ingestion, over 60% of the compound is eliminated (Zutshi et al., 1990). The oral dose given to rats for 30 days, at 75 mg/kg per day, resulted in high levels of the compounds being found in the prostate, brain, heart, kidneys, liver, testes, spleen, and intestines, showing tissue accumulations with values ranging from 176 to 257 mg/g (Gupta and Kanwar, 1991). Embelin's safety and toxicity has been studied in rodents, but not in humans. It does not show, when administered orally in rodents, toxicity up to 3 g/kg after acute exposure and up to 10 mg/kg after repeated administration (Poojari, 2014). Short-term toxicity (6 weeks) in female rats, after 120 mg/kg of oral administration, showed an increase in enzyme activity (acid and alkaline phosphatase) in the kidneys and adrenal cells. Cells damage and necrotic events disappeared after ceasing embelin administration (Prakash, 1994).

Embelin Drug Interaction

Sandhay and Grampurohit (2004) described the interaction of embelin and iron in ayurvedic formulations, describing the direct action of embelin to form a complex with the metal, and as a consequence, decreased the amount of free phytochemicals available to perform its biological function. Embelin has also been shown to increase the sensitizing potential of human

prostate cancer models during treatment with ionizing radiation (Dai et al., 2011). The treatment suppressed prostate cancer PC-3 cells from proliferating with cell cycle blocks in the S and G2/M phases. This block resulted in caspase-independent apoptosis, but not autophagy. The same result has been obtained in vivo, where embelin treatment significantly improved tumor response to X-ray radiation in the PC-3 xenograft model. This therapy induced an enhancement of tumor growth delay and prolonged time of progression, with minimal systemic toxicity. Immunohistochemistry studies have revealed a significant inhibition of cell proliferation, induction of apoptosis, and decrease of microvessel density in tumors, suggesting the possible inhibition of tumor suppression and angiogenesis.

CONCLUSIONS

Coenzyme Q_{10} and embelin are two dihydroxyquinone substituted compounds with many biological functions and properties, which have been highlighted in many scientific and clinical studies. Moreover, their good tolerability by organisms, and their low interference with drugs, make them suitable elements for improving human health and preventing several pathological conditions.

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Chapter 2.9

Quercetin: A Flavonol With Versatile Therapeutic Applications and Its Interactions With Other Drugs

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INTRODUCTION

Polyphenols are secondary metabolites available in plants, generally they are related with guarding cells against prooxidants or pathogens. Epidemiological investigations and related metaexaminations unequivocally propose that long-term consumption of plants rich in polyphenols gives potential medical benefits through cell reinforcement, and offers security against the advancement of tumors, cardiovascular illnesses, diabetes, osteoporosis, and neurodegenerative maladies.

Despite various basic nutritious substances, plants also contain an array of polyphenolic substances an extensive and heterogeneous gathering of organically dynamic non supplements. Flavonoids are partitioned into numerous classes, including flavonols, flavones, catechins, proanthocyanidins, anthocyanidins, and isoflavonoids. Flavonols (quercetin; Fig. 2.9.1) are light yellow, ineffectively dissolvable substances found in not less than 80% of higher plant blossoms, leaves, and leafy foods. Quercetin is normally found as O-glycosides in food, being the most widely recognized sugar build-up. The favored restricting site for sugar build-up is the C3 position, and less commonly the C7 position.

STRUCTURAL FEATURES OF QUERCETIN

Quercetin is a polar auxin transport inhibitor found in nature. The name quercetin originates from quercetum (oak woodland), after *Quercus*, and has been used since 1857 (Fischer et al., 1997) (Fig. 2.9.1). Quercetin falls into the class of flavonoids (from flavus which implies yellow, which is their regular coloration), whose characteristics come from 2-phenylchromen-4-one (flavone). QE contain a double bond between positions 2 and 3 and an oxygen (i.e., a ketone group) in position 4 of the heterocyclic C ring with at least one O-glycosidic bond. Quercetin is a delegate of the flavonol family that has a 3-hydroxyflavone spine. Flavonols (with an "o") are not to be confused with flavanols (with an "a"), another subclass of flavonoids containing a 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton. Flavonoids were in the past alluded to as vitamin P, probably because of the impact they had on the penetrability of vascular vessels, however this term is rarely utilized now (Shiro, 1938) (Table 2.9.1).

DIETARY SOURCES

Flavonols exist in the edible parts of numerous plants: verdant green vegetables like spinach, horse feed, and brassica, tubers like sweet potato, globules, different natural products, herbs, flavorings, along with tea and wine—in all instances they are found as glycosides (Brown, 1980). The dietary group of quercetin-type flavonols comprises quercetin glycosides, a sort of conjugate in which quercetin is connected either with perhaps a couple of glucose deposits (quercetin glucosides) or with rutinose (quercetin rutinoside) as shown in Fig. 2.9.1. The measure of quercetin in food may fundamentally be impacted by the plant's developing conditions, for example, naturally developed tomatoes demonstrate higher quercetin aglycone content than routinely developed tomatoes (Day and Williamson, 1999). Numerous vegetables and organic products, especially onions, peppers, cranberries, blueberries, apples, and grapes contain flavonol at levels as high as 350

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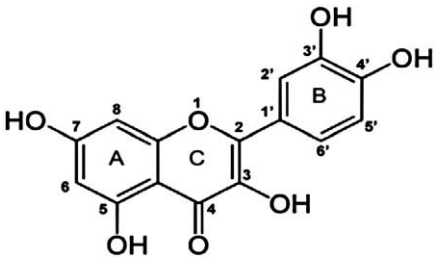

FIG. 2.9.1 Chemical structure of quercetin.

TABLE 2.9.1 Quercetin content in some of selected foods (USDA database)

S. No	Food source	Quercetin content (mg/100 g) in an edible portion
1	Capers, raw	233.84
2	Peppers, hot, yellow, and raw	50.73
3	Onions, red, and raw	39.21
4	Asparagus, cooked	15.16
5	Cranberries, raw	14.84
6	Peppers, hot, green, and raw	14.70
7	Lingon berries, and raw	13.30
8	Blueberries, raw	7.67
9	Lettuce, red leaf, and raw	7.61
10	Onions, white, and raw	6.17
11	Tomato, canned	4.12
12	Apples, Red Delicious, with skin	3.86
13	Apples, Gala, with skin	3.80
14	Apples, Golden Delicious, with skin	3.69
15	Broccoli, raw	3.26
16	Tea, green, and brewed	2.49
17	Cherries, sweet, and raw	2.29
18	Tea, black, and brewed	2.19
19	Grapes, black	2.08
20	Grapes, white	1.12
21	Wine, red, and table	1.04
22	Wine, white, and table	0.04

ppm (communicated as the aglycones) are the essential sources of everyday dietary quercetin (Hertog et al., 1993; Sampson et al., 2002). Prepared dark tea, and in addition red table wine and different organic product juices, additionally have been recognized as dietary sources of quercetin (Fang et al., 1986; Zeng et al., 2010).

Additionally, *Ageratina calophylla* (Del Santo and Guzzo, 2013) and C-glycosides are other kinds of quercetin subordinates, where the most regular site of C-glycosylation is the C6 carbon. One extremely uncommon quercetin subsidiary, quercetin-3-O-α-L-fucopyranoside, was discovered both in the red alga *Acanthophora spicifera* (Brown, 1980) and *Vitis vinifera* (Kühnau, 1976). In this situation quercetin is joined to the α-L-fucopyranosyl moiety at position C3 through a glycosidic linkage. No clinical examinations have been undertaken of these extremely uncommon quercetin subordinates.

In an ordinary balanced diet the day to day consumption of all flavonoids (i.e., flavanones, flavones, flavonols, anthocyanins, catechins, and biflavans) is considered to be around 1 g/day (expressed as quercitrin reciprocals, considering that one biflavan atom is equivalent to two particles of quercitrin). In combined states of which, contingent upon occasional changes (Jones and Hughes, 1982; Rimm et al., 1996; Hertog et al., 1995; Knekt et al., 1997) 160–175 mg/day is accounted for just by flavanones, flavones, and flavonols (Johannot and Somerset, 2006; Kimira et al., 1998). It is assessed that flavonol glycosides are consumed at levels of up to 100 mg/day (Jackson, 1997; Lin et al., 2006; Toutain and Bousquet-Melou, 2004). The national dietary records (i.e., from Australia, Croatia, Finland, Italy, Japan, The Netherlands, and the United States) for the consumption of quercetin for a standard balanced diet show mean utilization levels between 5 and 40 mg quercetin/day (Jones and Hughes, 1982; Lin et al., 2006). Nonetheless, daily consumption of quercetin may reach 200–500 mg by people buying and eating food harvested directly from the ground, especially in situations where the consumer eats the peel of quercetin-rich products, for example, tomatoes, apples, and onions (Jones and Hughes, 1982).

QUERCETIN BIOAVAILABILITY

Bioavailability is characterized as the proportion of the measure of an orally managed substance to the sum, which is consumed and thereby accessible for physiological movement or capacity (Jackson, 1997). Established on its pharmacokinetics evaluation, bioavailability could be dealt with as either supreme or relative (Toutain and Bousquet-Melou, 2004). Outright bioavailability is more exact, while relative bioavailability is less difficult to calculate, albeit less precise (Harnly et al., 2006). As officially revealed by Scholz and Williamson (2007), the variables that most impact quercetin ingestion are "the idea of the connected sugar, and also, the solvency as changed by ethanol, fat, and emulsifiers." The most dependable human quercetin research proposed to a great degree poor oral bioavailability after a lone oral estimations (∼2%) (Gugler et al., 1975). In this manner, the outright bioavailability of quercetin in people was evaluated at 44.8% when radio marked quercetin aglycone, dissolved in ethanol, was ingested before measuring the aggregate radioactivity of plasma (Walle et al., 2001). Additionally, to deliver a sufficient plasma reaction, of greater than 50 mg, quercetin aglycone or quercetin aglycone reciprocals were given, levels, which are higher than in a regular diet (i.e., 6–18 mg/day) (Zhang et al., 2010). In any case, in perspective of the potential clinical utilization of the atom, quercetin half-life and tissue appropriation give valuable data. Being the half-existence of the molecule and its metabolites in the scope of 11–28 h, this demonstrates a probable huge expanded plasma fixation resulting to constant supplementation (Boots et al., 2008; Manach et al., 2005). Regardless, until the point when the moment that nowadays investigate coordinated upon animal and individuals have enlightened an expansive cognizance about quercetin bioavailability.

QUERCETIN IN RELATION WITH TO HEALTH AND DISEASE

Epidemiological examinations have over and over again demonstrated a negative correlation between the poor human health and the utilization of a quercetin-rich eating regimen. The phenolic bunches in quercetin acknowledges an electron to frame generally stable phenoxyl radicals, thus disturbing the chain oxidation responses in parts of cells. It is well established that quercetin-rich nourishment may improve plasma cancer prevention. This extension in the antioxidative furthest reaches of plasma, following the use of quercetin-rich sustenance, may be illuminated either by the proximity of reducing phenols and their metabolites in the plasma, by their loads of other decreasing endogenous cell reinforcements. Utilization of quercetin has been linked to diminish levels of oxidative harm to lymphocytic DNA. Comparative views have been made, with polyphenol-rich food and drink, demonstrating the defensive impact of quercetin (Nabavi et al., 2012a). There is an increasing body of evidence that quercetin may secure cell constituents against oxidative harm and, hence, restrain the danger of different degenerative infections related to oxidative anxiety (Boots et al., 2008; Manach et al., 2005; Nabavi et al., 2012a).

CARDIOPROTECTIVE EFFECT

A significant amount of attention has been given to quercetin as a promising compound to be administered for coronary illness, anticipation, and treatment; quercetin has been linked to diminishing deaths from coronary illness and reducing the occurrence of strokes. Pashevin et al. (2011) revealed information linking its angioprotective properties to its proteasomal proteolysis. Specifically, by utilizing rabbits with cholesterol-initiated atherosclerosis they researched the capacity of quercetin to balance proteasomal movement. Unfortunately, other than their cancer prevention impact, flavonols like quercetin tend to affect biochemical-flagging pathways, and in doing so, have many physioneurotic side effects. There is solid confirmation that quercetin, and related flavonols, have a defensive impact, in vitro, on: nitric oxide and endothelial

capacity under oxidative anxiety, endothelium-autonomous vasodilation, and platelet aggregant impacts, restraint of LDL oxidation, lessening the effect of other inflammatory markers, and anticipation of neuronal oxidative and inflammatory harm (Perez-Vizcaino and Duarte, 2010). Moreover, as metainvestigations of epidemiological examinations report, quercetin produces undisputed hostile effects on the hypertensive and antiatherogenic impacts, forestalling endothelial breakdown and shielding the myocardium from ischemic harm (Larson et al., 2012). Quercetin had no reasonable impact on serum lipid profile and insulin resistance, yet despite the fact that there is yet no strong proof a considerable group of researchers propose that quercetin may keep at bay the most widely recognized types of cardiovascular sickness—adding to the defensive impacts that can be had by products which originated in the soil. Beneficial, late work utilizing hypertensive animals and people (N140 mmHg systolic and N90 mmHg diastolic) demonstrated a decrease in circulatory strain after quercetin supplementation (Yang et al., 2015). It is imperative to note two recent investigations which showed additional proof that quercetin ought to be respected as a recuperating operator against cardiovascular ailments (Gorlach et al., 2015; Hung et al., 2015).

ANTICANCER EFFECT

Everything considered, regardless of the way that mitochondria have all the earmarks of being engaged by quercetin starting apoptosis and the Cancer cell going in vitro (Dajas, 2012) until nowadays a strong quercetin intracellular target has not yet been found; obviously, the test is to recognize a qualified focus with a specific end goal to better define conceivable regular mixes to be included sustenance concentrates or pharmaceuticals. Surely, the antioxidative impacts and in addition the kinase and cell cycle restraint, and the actuated apoptosis are on the whole fundamental for the counter tumor properties appeared by quercetin. Specifically, the distinctive collaborations and exercises of quercetin that fine tunes the phosphorylation condition of particles and the quality articulation would follow up on the intracellular flagging balance, either repressing or strengthening survival signals. At any rate, these systems, which have been primarily seen in vitro considers, can't without much of a stretch clarify the counter tumor impacts saw in vivo on account of the generally low quercetin bioavailability in plasma and furthermore in light of the fact that the idea of the real dynamic particles is not unmistakably known (Bruning, 2013; Russo et al., 2014). In this way, to halfway clarify the atomic impact of quercetin on malignancy cells, among the diverse substrates suspected to be activated by quercetin an examination reports the capacity of quercetin to repress some protein kinases associated with deregulating the cell development in tumor cells. Besides, quercetin can apply its against tumor impact likewise repressing the mTOR action by numerous pathways (Maurya and Vinayak, 2015). Then again, in ascite cells of lymphoma-bearing mice, it is recommended that the growth preventive action of quercetin is proficient through the acceptance of apoptosis and balance of the protein kinase C (PKC) flagging which conveys to the lessening of oxidative anxiety (Sak, 2014). It has been demonstrated that the best quercetin activity is on blood, mind, lung, uterine, and salivary organ malignancy on a par with upon melanoma with a cytotoxic action substantially higher in the more forceful cells than in the moderate developing cells proposing that the most unsafe cells are the ones chiefly focused on (Jeong et al., 2012; Russo et al., 2014).

ANTIDIABETIC EFFECT

Recently, in animals exhibiting type 2 diabetes mellitus the hypoglycemic, hypolipidemic, and cell reinforcement impacts of dietary quercetin have been explored. In one investigation (Lai et al., 2012) one of the essential drivers of end-organized renal illness is diabetic nephropathy (DN). Many investigations have brought to attention that the changing development factor-β1 (TGF-β1) and the connective tissue development factor (CTGF) are both engaged with the DN pathophysiological components. Since quercetin has been proposed to ease DN, analysts have examined whether quercetin enhances renal capacity, likely influencing the effects of TGF-β1and CTGF in streptozotocin (STZ)-instigated diabetic Sprague Dawley rats (Chaudry et al., 1983). Also, aldose reductase, the protein that catalyzes the transformation of glucose to sorbitol, is especially important to the functioning of the eye and assumes a fundamental role in the development of diabetic mellitus. Quercetin significantly more potent than the previously known aldose reductase inhibitors. The inhibitory activity is of the noncompetitive type. In addition, quercitrin effectively blocks polyol accumulation in intact rat lenses incubated in medium containing high concentration of sugars. (Varma et al., 1975,1977). In individuals with type 1 and sort 2 diabetes and diabetic neuropathy, a lessening in the earnestness of deafness, jolting torment, and annoying was represented, and an adjustment in individual fulfillment measures with dynamic medications (Valensia et al., 2005).

As previously mentioned quercetin is the most plentiful flavonoid and it is thought to have defensive capacities against the pathogenesis of numerous ailments related to oxidative anxiety. In this setting, an important study 3T3-L1 cells researched

the subatomic components through which quercetin could impact adipogenesis and apoptosis (Ahn et al., 2008). The introduction of 3T3-L1 preadipocytes to quercetin brought about diminished articulation of adipogenesis-related elements and proteins and afterwards was found to constrict adipogenesis. In addition, the levels of phosphorylated adenosine monophosphate-actuated protein kinase (AMPK), and one of its substrates, acetyl-CoA carboxylase, were upregulated by quercetin; at the same time apoptosis was prompted and diminished extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) phosphorylation was observed. At the point when thought about together, this data shows that quercetin could apply its threatening vibe to the activity of adipogenesis by authorizing the AMPK signal pathway, however the quercetin-impelled apoptosis of create adipocytes is from every angle mediated by the aligning of the ERK and JNK pathways which expect fundamental parts in the midst of apoptosis.

ANTIOBESITY

As far as finding the nuclear frameworks influenced by quercetin on the physiological effects of hyperlipidemia, a couple of analysts have discovered that quercetin controls the nature of hepatic explanation related to lipid assimilation (Jung et al., 2013). Specifically, quercetin supplementation in mice fundamentally decreased the high fat-eating regimen (HFD), incited stoutness, diminished body weight, liver, and white fat tissue contrasted and the mice sustained just with HFD. It additionally profoundly diminished the HFD-initiated augments in serum lipids, including cholesterol, triglyceride, and thiobarbituric corrosive responsive substance. Steady with the diminished liver weight and white fat tissue weight, hepatic lipid aggregation and the extent of lipid beads, cushions in the epididymal fat were additionally decreased by quercetin supplementation. To additionally explore how quercetin may decrease weight, lipid digestion related qualities in the liver were likewise analyzed. For this situation, with respect to those in HFD control mice, the quercetin supplementation altered the articulation profiles of a few lipid digestion related qualities, including FNTA, PON1, PPARγ, ALDH1B1, APOA4, ABCG5, GPAM, ACACA, CD36, FDFT1, and FASN, The articulation examples of these qualities saw by quantitative switch transcriptase-polymerase chain response were affirmed by immunoblot examines. Together, these outcomes demonstrated that quercetin avoids HFD-initiated weight in C57B1/6 mice, and its hostile to stoutness impacts might be connected to the control of lipogenesis at the level of translation. All the more of late, a few important audits have been distributed on the part of dietary phytochemicals in stoutness, quercetin included (Siriwardhana et al., 2013). By the by, it is important to build up a spotless separation between the counter heftiness impacts of quercetin when it is distributed as unadulterated aglycone, from its putative capacities, and when it is available in polyphenolic removes, as portrayed in many works referred to in the previously mentioned last two audits (Kobori, 2014; Siriwardhana et al., 2013).

DRUG INTERACTION

Quercetin displays an in vivo inhibitory impact both on CYP3A4 (Choi et al., 2011; Pal and Mitra, 2006) and CYP1A2 though it increases CYP2A6, xanthine oxidase, and N-acetyltransferase action. In addition, quercetin in vivo hinders P-glycoprotein (Pgp), a medication efflux transporter that assumes a critical role in intestinal and biliary transport and disposal of many medications and their metabolites (Chan et al., 2009; Chen et al., 2009; Kim et al., 2009; Wang et al., 2004). Because of these interactions, quercetin may modify serum levels of all medications utilized by these proteins. Be that as it may, despite the fact that the everyday consumption of rutin (quercetin rutinoside) lessens the anticoagulant impact of racemic warfarin (Kale et al., 2010), dependable connections between quercetin and anticoagulants have not yet been explored. Associations amongst quercetin and distinctive medications have been, however, examined, particularly in light of its collaboration with CYP3A4 and the P-glycoprotein. In by far most of the connections quercetin lessens the level, or the effect of, the prescription by the P-glycoprotein (MDR1) efflux transporter; of course, particularly because of "Minor reality," quercetin reduces the effect(s) of the solution by pharmacodynamic restriction. Obviously, drug– supplement associations may moreover be affected by quercetin. Along these lines, an examination completed on pigs revealed a deadly association between digoxin—a substrate of P-glycoprotein with exceptionally limited benefits—and quercetin. In fact, the coorganization of quercetin (50 mg/kg) and digoxin (0.02 mg/kg) brought about the sudden deaths of two out of the three pigs within 30 min of consumption.

Surprisingly, in spite of the way that the coorganization with a lower dose of quercetin (40 mg/kg) viably lifted the Cmax (most outrageous concentration) of digoxin by 413% it didn't have savage effects (Moon and Morris, 2007). As can be envisioned quercetin may likewise modify the bioavailability of some dietary supplements; for instance, it seems to enhance the bioavailability of epigallocatechin gallate (Naidu et al., 2003) and potentially different flavonoids (Chen et al., 2010). Recently, preparatory proof demonstrated that quercetin may have synergistic impacts with a few medications and might play a role in multisedative resistance (Morino et al., 1982).

ANTIBIOTIC (QUINOLONE ANTIBIOTICS) INTERACTIONS WITH QUERCETIN

Using quercetin alongside some antiinfection agents may diminish their viability. A few researchers feel that quercetin may keep some antiinfection agents from eliminating microscopic organisms. However, it is too early to know whether this represents a major concern. Some of these antitoxins that may be connected with quercetin incorporate ciprofloxacin (Cipro), enoxacin (Penetrex), norfloxacin (Chibroxin, Noroxin), sparfloxacin (Zagam), trovafloxacin (Trovan), and grepafloxacin (Raxar).

CYCLOSPORIN INTERACTION WITH QUERCETIN

Cyclosporin (Neoral, Sandimmune) is altered and broken down in the liver. Quercetin may diminish rapidly the ability of the liver to break down cyclosporine. Taking quercetin may exacerbate the impacts and symptoms of this medicine. Before taking quercetin it is important the patient discusses these interactions with their doctor.

MEDICATIONS ALTERED BY THE LIVER

Cytochrome P450 2C8 (CYP2C8) Substrates Interactions With Quercetin

Quercetin may diminish rapidly the ability of the liver to break down solutions. Taking quercetin alongside these solutions may exacerbate the impacts and symptoms of a prescribed drug. Before taking quercetin it is important the patient discusses these possible interactions with their doctor. Drugs which are changed by the liver are: paclitaxel (Taxol), rosiglitazone (Avandia), amiodarone (Cordarone), docetaxel (Taxotere), repaglinide (Prandin), verapamil (Calan, Isoptin, and Verelan), among others.

Cytochrome P450 2C9 (CYP2C9) Substrate Interactions With Quercetin

A few prescription medications are altered and broken down by the liver. Quercetin may diminish rapidly the ability of the liver to break down solutions. Taking quercetin alongside these solutions may exacerbate the impacts and symptoms of a prescribed drug. Before taking quercetin it is important the patient discusses these possible interactions with their doctor. A few prescription drugs which are changed by the liver are: celecoxib (Celebrex), diclofenac (Voltaren), fluvastatin (Lescol), glipizide (Glucotrol), ibuprofen (Advil, Motrin), irbesartan (Avapro), losartan (Cozaar), phenytoin (Dilantin), piroxicam (Feldene), tamoxifen (Nolvadex), tolbutamide (Tolinase), torsemide (Demadex), and warfarin (Coumadin).

Cytochrome P450 2D6 (CYP2D6) Substrate Interactions With Quercetin

A few prescription medications are altered and broken down by the liver. Quercetin may diminish rapidly the ability of the liver to break down solutions. Taking quercetin alongside these solutions may exacerbate the impacts and symptoms of a prescribed drug. Before taking quercetin it is important the patient discusses these possible interactions with their doctor. A few prescription drugs which are changed by the liver are: amitriptyline (Elavil), codeine, flecainide (Tambocor), haloperidol (Haldol), imipramine (Tofranil), metoprolol (Lopressor, Toprol XL), ondansetron (Zofran), paroxetine (Paxil), risperidone (Risperdal), tramadol (Ultram), and venlafaxine (Effexor).

Cytochrome P450 3A4 (CYP3A4) Substrate Interactions With Quercetin

A few prescription medications are altered and broken down by the liver. Quercetin may diminish rapidly the ability of the liver to break down solutions. Taking quercetin alongside these solutions may exacerbate the impacts and symptoms of a prescribed drug. Before taking quercetin it is important the patient discusses these possible interactions with their doctor. A few prescription drugs which are changed by the liver are: lovastatin (Mevacor), clarithromycin (Biaxin), cyclosporine (Neoral, Sandimmune), diltiazem (Cardizem), estrogens, indinavir (Crixivan), triazolam (Halcion), verapamil (Calan, Isoptin, and Verelan), alfentanil (Alfenta), fentanyl (Sublimaze), losartan (Cozaar), fluoxetine (Prozac), midazolam (Versed), omeprazole (Prilosec), lansoprazole (Prevacid), ondansetron (Zofran), propranolol (Inderal), fexofenadine (Allegra), amitriptyline (Elavil), amiodarone (Cordarone), citalopram (Celexa), sertraline (Zoloft), ketoconazole (Nizoral), and itraconazole (Sporanox).

Pharmaceuticals which move directly through cells (P-glycoprotein substrates) interact with quercetin. Quercetin may make these pumps less dynamic and increment the amount of a few meds gets consumed by the body. This may cause

more symptoms from a few solutions. Drugs that are moved by these pumps include: iltiazem (Cardizem), verapamil (Calan, Isoptin, and Verelan), digoxin (Lanoxin) cyclosporine (Neoral, Sandimmune), saquinavir (Invirase), amprenavir (Agenerase), nelfinavir (Viracept), loperamide (Imodium), quinidine, paclitaxel (Taxol), vincristine, etoposide (VP16, VePesid), cimetidine (Tagamet), ranitidine (Zantac), fexofenadine (Allegra), ketoconazole (Nizoral), and itraconazole (Sporanox).

CONCLUSION

Quercetin has an unlimited extent of all around depicted pharmacological effects that fuse the upkeep of prosperity, the redesign of physical and mental development, and a couple of specific defensive effects. Clearly, bioavailability of quercetin is an imperative factor of its well-being impacts and for that, an unrivaled impression of the components managing quercetin digestion and bioavailability is foreseen to give its imminent part in controlling diverse afflictions. Despite the excess of research on quercetin and its subsidiaries, it is not yet plausible to discover dietary honors with reference to the sorts and amounts to be devoured. The inborn assortment of its subsidiaries comparable to structure, science, and regular dissemination of foodstuffs gives itself to blunders in detailing the sorts as well as sums expended, and additionally shortened discovery of requirements for impedance contemplates that plan to assess their efficacies in a clinical setting. In this manner, notwithstanding the scientific headway made over the previous decades, a few basic issues in the outline and revealing of studies keep on limiting advancement in utilizing quercetin look into results into considerable proposals for end clients. These issues predominantly incorporates: (1) inadequate/wrong utilization of investigative techniques, influencing assurance of nourishment to substance and dietary admission levels testing; (2) constrained information as well as portrayal of test materials utilized as a part of dietary mediation trials; and (3) challenges with the use of fitting strategies for evaluation of important bioavailability and metabolite development in organic tissues that can give enter experiences into sustenance and clinical markers/results.

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Chapter 2.10

Alpha-Lipoic Acid: A Dietary Supplement With Therapeutic Potential for Obesity and Related Metabolic Diseases

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ALPHA-LIPOIC ACID STRUCTURE

Alpha-lipoic acid (α-LA, thioctic acid, 5-(1,2-dithiolan-3-yl) pentanoic acid) is an organosulfur compound, which contains a single chiral center and asymmetric carbon, resulting in two possible optical isomers: *R*-lipoic acid and *S*-lipoic acid (Brookes et al., 1983). α-LA occurs naturally in mitochondria, where it acts as the coenzyme for several bioenergetic enzymes. Both α-LA and its reduced dithiol form, dihydrolipoic acid (DHLA), are powerful antioxidants (Moura et al., 2015).

ALPHA-LIPOIC ACID SOURCES, SAFETY, AND BIOAVAILABILITY

In small quantities the R α-LA isomer is naturally found in food. Thus, it is present in different foods such as vegetables (e.g., spinach, tomato, broccoli, brussels sprouts, and rice bran) and red meat and entrails (e.g., liver and kidney) in the form of lipoyllysine (α-LA with binding lysine residues), with the highest concentration of lipoyllysine detected in bovine kidney (Akiba et al., 1998). However, although this molecule is present in food, the dietary intake is unable to provide substantial amounts of α-LA in the bloodstream. For supplementation therapy α-LA is normally used as a racemic mixture of the *R* and *S* forms synthesized in laboratory (Ghibu et al., 2009; Shay et al., 2009). Interestingly, it has been reported that α-LA can be also endogenously produced by the enzyme lipoic acid synthase, and that this enzyme is directly responsible for important cellular functions such as maintenance of the antioxidant defense network and mitochondrial function or decreasing inflammation and insulin resistance (Padmalayam et al., 2009).

The safety and toxicity of α-LA have been widely tested. In fact, it has been successfully used as a therapeutic agent for about 60 years in the treatment of different diseases (e.g., diabetic neuropathy and various skin diseases) (Foster, 2007). In humans the greatest administered dose was 2.4 g/day over a 2-year period (Shay et al., 2009), with no reported significant adverse effects. Other authors observed mild side effects with the most common of these being gastrointestinal problems and itching sensations (Kim et al., 2016; Koh et al., 2011) at doses of 1200–1800 mg/day. These adverse effects were transient and patients spontaneously and successfully recovered.

Regarding bioavailability, α-LA is rapidly absorbed and has a mean plasma elimination half-life of 0.5 h (Teichert et al., 2003). It has been described that in healthy volunteers, after an oral single dose (200 mg) of the racemic mixture, α-LA has a mean bioavailability of 30% (Dörsam and Fahrer, 2016). This low bioavailability might be explained by the processes involved in the cellular uptake of α-LA, which occurs in a pH-dependent manner, mediated by two carrier proteins, the monocarboxylate transporter and the Na^+-dependent multivitamin transporter (SMVT) (Dörsam and Fahrer, 2016). Human SMVT is highly specific to the natural *R* α-LA form (Zehnpfennig et al., 2015) and the bioavailability of this *R* enantiomer has been found to be approximately two-fold higher compared to the *S* form, also present in the racemic mixture (Hermann

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et al., 2014). Moreover, it has been observed that administration with food may lower the bioavailability of both α-LA enantiomers (Gleiter et al., 1996).

ALPHA-LIPOIC ACID AND OBESITY-RELATED DISORDERS

α-LA has been suggested to be useful in several pathological states characterized by increased oxidative stress and inflammation, such as type 2 diabetes (T2D), rheumatoid arthritis, vascular disease, asthma, and multiple sclerosis; it has even been suggested as a cotreatment in patients with solid tumors (Fernández-Galilea et al., 2013; Moura et al., 2015). The efficacy of α-LA in the treatment for diabetic polyneuropathy has been also extensively evidenced (Papanas and Ziegler, 2014).

In this chapter, we will examine the current evidence regarding the potential efficacy of α-LA as a therapeutic agent for the treatment of obesity and associated comorbidities in humans.

Alpha-Lipoic Acid and Weight Loss

Table 2.10.1 summarizes the trials undertaken to evaluate the potential antiobesity effects of α-LA supplementation in overweight/obese subjects with or without associated disorders such as impaired glucose tolerance, dyslipidemia, or T2D.

Carbonelli et al. (2010) evaluated the effects of 0.8 g/day of α-LA taken orally over 4 months in a large population, finding that supplementation decreased body weight, fat mass, and waist circumference in overweight and obese Caucasian subjects, but not in individuals of normal weight. In addition, another trial performed with subjects having T2D, using a daily oral dose of α-LA (0.6 g/day) over 20 weeks, reported a decreased body weight in the supplemented group (Okanović et al., 2015). Moreover, in patients undergoing antipsychotic treatment (one side effect of which is an increase in body weight and associated metabolic complications) α-LA (1.2 g/day over 12 weeks) was found to decrease body weight and visceral fat and improved eating behavior (Kim et al., 2016).

There are few studies that have evaluated the additional effects of α-LA to a hypocaloric diet on anthropometric variables. The study from Koh et al. (2011), performed on Asian subjects with some features of metabolic syndrome following an energy-restricted diet, observed that α-LA (1.2 and 1.8 g/day) over 20 weeks promoted a reduction in body mass index (BMI), body weight, and waist circumference, but was only found to be significantly different compared with the placebo group at the highest tested dose. The trial of Huerta et al. (2015a) observed that α-LA supplementation (0.3 g/day), alone or in combination with eicosapentaenoic acid (EPA), over a 10-week period, was able to promote weight loss and fat mass reduction in healthy overweight/obese women following an energy-restricted balanced diet.

In contrast, other studies with a range of timescales, between 3 weeks and 1 year, performed in overweight/obese subjects with T2D or impaired glucose tolerance with a standard or a balanced diet, observed no significant effects of α-LA oral supplementation (0.3–1 g/day) on body weight and fat mass, or hip and waist circumference (Ansar et al., 2011; Derosa et al., 2016; Manning et al., 2012; McNeilly et al., 2011). Analogous outcomes in anthropometric

TABLE 2.10.1 Clinical Trials Evaluating the Effects of α-La Supplementation on Body Weight Loss and Anthropometric Parameters, as well as on Glucose and Lipid Metabolism and Inflammatory and Oxidative Stress Markers

References	Study design	α-LA treatment	Outcomes
Kamenova (2006)	Open-label, case-controlled study with T2D obese females and males, all treated with metformin (n = 24; mean age 52–53 years).	1.2 g/day (each tablet 600 mg/day, oral dose) of α-LA for 4 weeks (usual diet).	*Glucose metabolism*: ↔ FBG and FBI Improved glucose disposal rate and ISI
Carbonelli et al. (2010)	Open prospective clinical trial with healthy females and males classified as normal weight, preobese, and obese (n = 1127; age 18–60 years).	0.8 g/day of α-LA (Liponax, oral dose) for 4 months (diet not specified).	*Anthropometric parameters*: ↓ Body weight, fat mass, waist circumference, and appetite (in the preobese and obese group) *Inflammation and oxidative stress*: ↓ TNF-α and IL-6 (in the pre-obese and obese group) ↓ CRP (in the obese group) ↓ Erythrocyte sedimentation rate

TABLE 2.10.1 Clinical Trials Evaluating the Effects of α-La Supplementation on Body Weight Loss and Anthropometric Parameters, as well as on Glucose and Lipid Metabolism and Inflammatory and Oxidative Stress Markers—cont'd

References	Study design	α-LA treatment	Outcomes
Ansar et al. (2011)	Randomized, double-blind, placebo-controlled trial with parallel design in normal weight and overweight females and males with T2D (n = 57; age 50.4 ± 8.7 years).	0.3 g/day of α-LA (oral dose) for 2 months (diet not specified).	*Anthropometric parameters*: ↔ Body weight *Glucose metabolism*: ↓ FBG and HOMA-IR ↔ FBI *Oxidative stress*: ↔ GSH-Px
Koh et al. (2011)	Randomized, double-blind, placebo-controlled trial with parallel design in obese females and males with hypertension, T2D, or hypercholesterolemia (n = 228; age 18–65 years).	Either 1.2 g/day or 1.8 g/day of α-LA (oral dose) for 20 weeks (instructions for reducing the caloric intake to 600 kcal/day).	*Anthropometric parameters*: ↓ BMI, body weight, and waist circumference (with the greatest dose) *Lipid and glucose metabolism*: ↓ FFA ↔ TG, total-ch, HDL-ch, and LDL-ch ↓ Hb_{A1c} (diabetic subjects)
McNeilly et al. (2011)	Randomized, controlled trial with parallel design in obese females and males with impaired glucose tolerance (n = 24; age 54 ± 8 years).	A group with exercise (30 min/day) + α-LA (1 g/day, oral dose) and a group only with 1 g/day of α-LA (oral dose) for 12 weeks (usual diet).	*Anthropometric parameters*: ↔ BMI, body weight, fat mass, and waist circumference (α-LA alone) ↓ Fat mass and hip and waist circumferences (exercise + α-LA) *Lipid and glucose metabolism*: ↔ Total-ch, HDL-ch, LDL-ch and TG ↔ FBG and Hb_{A1c} *Inflammation and oxidative stress*: ↔ CRP ↑ Total antioxidant capacity ↑ oxidase-LDL (α-LA alone)
Zhang et al. (2011)	Double-blind, case-controlled study with T2D, overweight, or obese females and males (n = 32; age 52–53 years).	0.6 g/day administered intravenously for 2 weeks (normal equilibrated diet).	*Anthropometric parameters*: ↔ BMI and waist circumference *Lipid and glucose metabolism*: ↓ TG, FFA, total-ch, LDL-ch, VLDL-ch, and sdLDL-ch ↔ FBG and Hb_{A1c} ↓ Plasma glucose (120 min after load) and glucose disposal rate and ISI *Inflammation and oxidative stress*: ↓ TNF-α, IL-6 and adiponectin ↓ 8-iso-prostaglandin and MDA ↓ Oxidized LDL
Manning et al. (2012)	Randomized, double-blind, placebo-controlled trial with parallel design in overweight and obese females and males with MetS (n = 151; age 55–57 years).	0.6 g/day of α-LA (oral dose) with or without vitamin E (100 IU/day) for 1 year (usual diet).	*Anthropometric parameters*: ↔ BMI, body weight, and waist circumference *Glucose metabolism*: ↔ FBG, FBI, and HOMA-IR *Inflammation*: ↔ CRP, IL-6, TNF-α, and adiponectin
Yan et al. (2013)	Randomized, double-blind, crossover trial in overweight females and males with a borderline hypertension, dyslipidemia, or impaired fasting glucose without antidiabetic or antilipidemic drugs ($n \approx 92$; age 18–60 years).	1.2 mg/day of α-LA (oral dose) for 8 weeks with a 4-week washout period.	*Anthropometric parameters*: ↓ BMI, body weight, and waist circumference *Glucose metabolism*: ↔ HOMA-IR ↔ Oxidized LDL and 8-iso-prostaglandin F2α

(Continued)

TABLE 2.10.1 Clinical Trials Evaluating the Effects of α-La Supplementation on Body Weight Loss and Anthropometric Parameters, as well as on Glucose and Lipid Metabolism and Inflammatory and Oxidative Stress Markers—cont'd

References	Study design	α-LA treatment	Outcomes
Okanović et al. (2015)	Open-label trial with T2D females and males (overweight and obese), all treated with metformin and some with signs of peripheral neuropathy (*n* = 60; age 60–64 years).	0.6 g/day of α-LA (oral dose) for 20 weeks (diet not specified).	*Anthropometric parameters*: ↓ Body weight *Lipid and glucose metabolism*: ↔ Total-ch ↓ TG ↔ FBG
Derosa et al. (2016)	Randomized, double-blind, placebo-controlled trial with parallel design in overweight females and males with T2D (*n* = 102; age 18–74 years).	Oral intake of a supplement (LICA®) containing 600 mg of α-LA, 165 mg of L-carnosine, and 75 mg of zinc plus vitamins of group B for 3 months (controlled energy diet and dietary advice).	*Anthropometric parameters*: ↔ BMI and body weight *Lipid and glucose metabolism*: ↔ HDL-ch ↓ Total-ch, LDL-ch, and TG ↔ FBI ↓ FBG, PPG, Hb_{A1c}, and HOMA-IR *Inflammation and oxidative stress*: ↓ CRP ↑ SOD and GSH-Px ↓ MDA
Kim et al. (2016)	Randomized, double blind, placebo-controlled trial with parallel design in chronic schizophrenic patients receiving medication and with more than 10% weight gain after the beginning of psychotic treatment without any history of either medical illnesses or family history of obesity (*n* = 22; age 40.5 ± 6.6 years).	1.2 g/day (each tablet 200 mg/day, oral dose) of α-LA for 12 weeks. In some cases (<1 kg weight at week 4) the dose was increased to 1.8 g/day (diet not specified).	*Anthropometric parameters*: ↔ BMI, abdominal and subcutaneous fat ↓ Body weight and visceral fat *Lipid and glucose metabolism*: ↔ Total-ch, TG, and FFA ↔ FBG, FBI, and Hb_{A1c}
Huerta et al. (2017, 2016, 2015a,b)	Randomized, double-blind, placebo-controlled trial in overweight and obese healthy females (*n* = 77; age 20–50 years).	0.3 g/day (oral dose) of α-LA with or without EPA-rich capsules (1.3 g/day) for 10 weeks (energy-restricted diet <30%).	*Anthropometric parameters*: ↔ Waist circumference, WHR, lean mass, and android fat ↓ Body weight, hip circumference, fat mass, and gynoid fat *Lipid and glucose metabolism*: ↔ Total-ch, HDL-ch, LDL-ch, TG, and FFA ↔ FBG, FBI, HOMA-IR, glucose iAUC, and insulin iAUC ↑ Expression of genes involved in fat catabolism (SAAT) ↓ Expression of genes involved in deposition of lipids (SAAT) *Inflammation*: ↔ Neutrophils, lymphocytes, HMW adiponectin, total adiponectin, IL-6, chemerin, haptoglobin, and SAA ↓ Leukocyte count and CRP ↔ Platelet count, ADMA, and PAI-1 ↑ Apelin, ↔ Irisin

Abbreviations: ↔, No change; ↑, increase; ↓, decrease; *ADMA*, asymmetric dimethylarginine; *BMI*, body mass index; *ch*, cholesterol; *CRP*, C reactive protein; *FBG*, fasting blood glucose; *FBI*, fasting blood insulin; *FFA*, free fatty acids; *GSH-Px*, glutathione peroxidase; *HBA1c*, glycosylated hemoglobin; *HDL*, high-density lipoprotein; *HOMA-IR*, homeostasis model assessment of insulin resistance; *HMW*, high molecular weight; *iAUC*, incremental area under curve; *IL*, interleukin; *ISI*, insulin sensitivity index; *LDL*, low-density lipoprotein; *MDA*, malondialdehyde; *PAI*, plasminogen activator inhibitor; *PPG*, postprandial glucose; *sdLDL*, small density LDL; *SAA*, serum amyloid A; *SAAT*, subcutaneous abdominal adipose tissue; *SOD*, superoxide dismutase; *TNF*, tumor necrosis factor; *T2D*, type 2 diabetes; *TG*, triglycerides; *VLDL*, very-low density lipoprotein; *WHR*, waist-to-hip ratio.

variables have been found with 0.6 g/day of α-LA being administered intravenously over 2 weeks (Zhang et al., 2011) (Table 2.10.1).

Alpha-Lipoic Acid and Lipid Metabolism

It has been suggested that α-LA may be useful, either alone or in combination with other lipid-lowering agents, for treating severe hypertriglyceridemia (Pashaj et al., 2015). However, the evidence concerning the efficacy of α-LA on obesity-associated dyslipidemias is still limited. While some studies have reported that α-LA could decrease not only free fatty acids and triglyceride (TG) levels in the bloodstream (Okanović et al., 2015; Zhang et al., 2011), but also total and low-density lipoprotein (LDL) cholesterol (Derosa et al., 2016; Zhang et al., 2011), other investigations have reported no effect of this antioxidant on TG and cholesterol levels (Huerta et al., 2015a; Koh et al., 2011; Manning et al., 2012; McNeilly et al., 2011). Contrary to expectations, McNeilly et al. (2011) observed a greater LDL oxidation rate in subjects with impaired glucose tolerance supplemented with α-LA (Table 2.10.1). In this context, Pashaj et al. (2015) suggest that α-LA seems to be most efficacious as a lipid-lowering supplement when blood TG levels are elevated.

Alpha-Lipoic Acid and Glucose Metabolism

Some studies have described the beneficial effects of α-LA supplementation on insulin sensitivity and glucose metabolism biomarkers. Thus, the study of Ansar et al. (2011) reported that α-LA (0.3 g/day for 2 months) promoted a reduction in fasting blood glucose and HOMA-IR. More recently the study of Derosa et al. (2016) found that in patients with T2D α-LA (0.6 g/day), in combination with L-carnosin, zinc, and group B vitamins, improved fasting/postprandial blood glucose, HOMA-IR, and glycosylated hemoglobin (Hb_{A1c}). Importantly, the studies from Kamenova (2006) and Zhang et al. (2011), which evaluated insulin sensitivity using the hyperinsulinaemic euglycaemic clamp technique, found that supplementation with α-LA improved both glucose disposal rate and insulin sensitivity index in T2D subjects (Table 2.10.1). However, other trials in subjects with impaired glucose metabolisms have shown no effects of α-LA administered either orally or intravenously in glucose metabolism fasting parameters (Huerta et al., 2015a; Kamenova, 2006; Manning et al., 2012; McNeilly et al., 2011; Zhang et al., 2011). Therefore, whether α-LA is an effective therapeutic agent for improving glucose metabolism disorders in overweight/obese individuals is still an open question.

Alpha-Lipoic Acid, Inflammation, and Oxidative Stress

Although the alleviation of inflammation and oxidative stress are proposed mechanisms by which α-LA appears to reduce symptoms of diabetic polyneuropathy (Rochette et al., 2013), the modulation, by α-LA, of obesity-associated inflammation and oxidative stress has not been widely described (Table 2.10.1).

Carbonelli et al. (2010) showed that α-LA decreased the erythrocyte sedimentation rate, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) circulating levels in overweight and obese subjects while promoting a reduction of C reactive protein (CRP) only in the obese group. Also, Huerta et al. (2016) reported that α-LA in conjunction with an energy-restricted diet promoted a decrease in CRP levels and leukocyte count, which are considered important inflammatory factors implicated in insulin resistance and cardiovascular disease in overweight/obese women.

Zhang et al. (2011) observed that α-LA (0.6 g/day intravenously over 2 weeks) caused a drop in TNF-α, IL-6, and oxidative markers (8-iso-prostaglandin and malondialdehyde) in parallel with an increase in adiponectin in overweight/obese T2D individuals. Moreover, Derosa et al. (2016) described that after 3 months of administering a food supplement containing 0.6 g of α-LA, as its principal compound, patients showed a reduction in CRP and malondialdehyde together with an increase in antioxidant defenses. Additionally, McNeilly et al. (2011) observed that though α-LA (1 g/day over 12 weeks) did not induce changes in CRP, it did increase the total antioxidant capacity.

Contrary to these findings, Manning et al. (2012) showed that oral α-LA (0.6 g/day over 1 year) did not change any of the inflammatory markers measured. Moreover, another study performed in overweight/obese healthy individuals with a randomized, controlled crossover design with a 4-week washout period between an 8-week intervention, observed that 1.2 g/day of α-LA did not promote significant changes in oxidized LDL and 8-iso-prostaglandin F2α blood circulating levels (Yan et al., 2013). Finally, Ansar et al. (2011) observed that α-LA (0.3 g/day over 2 months) did not induce changes in glutathione peroxidase.

Taking these studies together, there is no clear evidence about the efficacy of α-LA to modulate circulating inflammatory and oxidative stress markers in obese subjects. Also, it is important to note that the effects of the treatment are possibly

affected not only by the baseline metabolic characteristics of the subjects, but also by the route of administration and the diet followed during treatment.

ALPHA-LIPOIC ACID MECHANISMS OF ACTION IN OBESITY AND ASSOCIATED DISORDERS

Several studies conducted in different experimental models of obesity have suggested that α-LA is able to promote body weight loss through different mechanisms including not only the decrease of food intake (Kim et al., 2004) and the inhibition of intestinal sugar absorption (Prieto-Hontoria et al., 2009), but also food efficiency decrease (Prieto-Hontoria et al., 2009) and stimulation of energy expenditure (Kim et al., 2004; Wang et al., 2010), mediated by suppression of hypothalamic AMP-activated protein kinase (AMPK), a key enzyme that integrates nutritional and hormonal signals and modulates food intake and energy homeostasis. However, the efficacy and required doses of α-LA to induce satiety in humans still remain unclear.

In addition to central actions, α-LA is also able to regulate the function of key metabolic peripheral organs such as the liver, muscles, and adipose tissues (Fig. 2.10.1) (Fernández-Galilea et al., 2013, 2015; Huerta et al., 2015b; Valdecantos et al., 2012). Regarding this issue, the study from Huerta et al. (2017) suggests that α-LA supplementation could modulate obesity and lipid metabolism by inducing the expression of genes involved in the catabolism of lipids, while decreasing the mRNA levels of those related with the deposition of lipids in subcutaneous abdominal adipose tissue, in overweight/obese healthy women.

CONCLUSIONS AND FUTURE PERSPECTIVES

Considering the current obesity epidemic and the high prevalence of obesity-associated disorders such as T2D, dyslipidemias, and metabolic syndrome, there is a need to identify new strategies that would be safe and effective as coadjuvants to

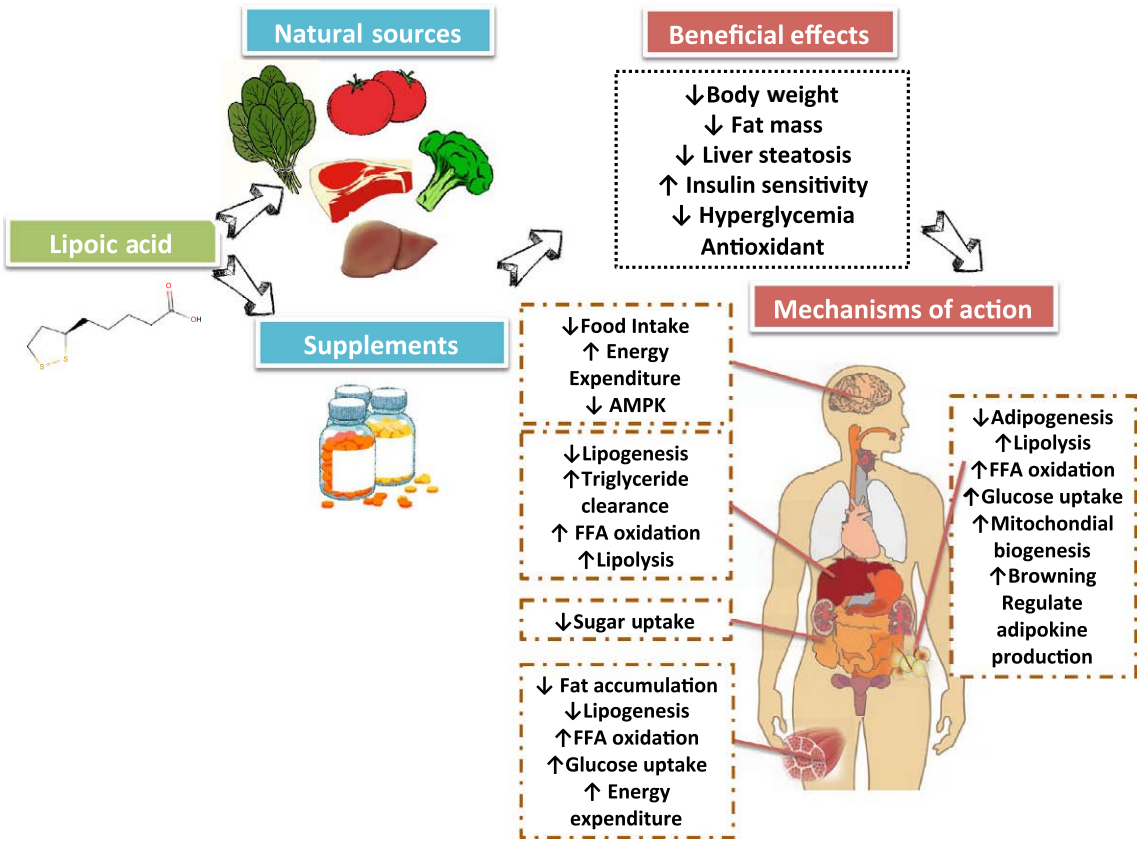

FIG. 2.10.1 Mechanisms potentially involved in the metabolic actions of α-lipoic acid in obesity and related disorders. *AMPK*, hypothalamic AMP-activated protein kinase; *FFA*, free fatty acids.

lifestyle modifications such as diet and exercise. In this context, the potential antiobesity effects of α-LA have gained attention, considering that this dietary supplement has been clinically shown to be safe and effective against symptoms of diabetic polyneuropathies and other diseases where oxidative stress is involved. Although promising, the data about the effectiveness and the proper doses of α-LA in humans for body weight–lowering effects and for the prevention/treatment of metabolic disorders associated to obesity are still unclear. This highlights the importance of performing longer randomized controlled trials to better characterize the relevance of this dietary supplement to manage obesity over long time periods and in subjects with metabolic disturbances. It is also important to find out whether α-LA might be a useful agent in the management or chemoprevention of obesity-related cancers and on obesity-associated inflammatory and neurodegenerative disorders in humans.

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Chapter 2.11

Methylsulfonylmethane (MSM)

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INTRODUCTION

Methylsulfonylmethane (MSM) (Fig. 2.11.1), a constituent of the natural global sulfur cycle, also known as dimethyl sulfone ($DMSO_2$), is the first metabolite of dimethylsulfoxide (DMSO) and widely found in plants, animals, and humans (Silva Ferreira et al., 2003). It is an odorless, stable, and colorless crystalline powder with a molecular weight of 94.13 g/mol, which is easily absorbed, distributed, and mostly excreted in the urine (Jones, 1987; Magnuson et al., 2007). The MSM presented in human body fluids is derived partly from food sources such as fruits, vegetables, and grains, as well as through bacterial metabolism or endogenous metabolism of human methanethiol (Engelke et al., 2005). It can also be easily synthetized from the chemical reaction of DMSO with hydrogen peroxide (Kim et al., 2006). The suggested oral therapeutic dose of the compound is about 4–6 g/day, while the optimum dosage has not been clearly defined. MSM can be effectively applied in the treatment of interstitial cystitis, allergic rhinitis, chemoprevention, autoimmune diseases, osteoarthritis, fibromyalgia, and scleroderma (Nakhostin-Roohi et al., 2013b; Silva Ferreira et al., 2003) due to its antiinflammatory, antiapoptotic, and indirect antioxidant properties (Beilke et al., 1987; DiSilvestro et al., 2008; Sousa-Lima et al., 2016). Some of these properties along with their suggested underlying mechanisms have been briefly discussed in this chapter.

ANTIINFLAMMATORY AND ANTIOXIDANT PROPERTIES

In Vitro

Murine macrophages, RAW264.7, were applied to investigate the antiinflammatory effects of MSM in a lipopolysaccharide (LPS)-induced inflammation model. Results clearly demonstrated that this compound not only intensely attenuated production of prostaglandin E_2 and nitric oxide through suppression of cyclooxygenase-2 enzyme activity and expression of nitric oxide synthase, respectively, but also dramatically restricted production of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) through blocking degradation of Iκ-Bα and nuclear translocation of nuclear factor kB (NF-kB) p65 in LPS-induced inflammation in RAW264.7 cell lines (Kim et al., 2009).

In another study, MSM was demonstrated to significantly inhibit activation of inflammasomes, a group of multiprotein complexes regulating the secretion of interleukin-1β and initiation of inflammatory responses, expression of pro-IL-1β and TNF-α, and production of proinflammatory cytokines and mitochondrial-related reactive oxygen species (Ahn et al., 2015; Baroja-Mazo et al., 2014).

In Vivo

Based on Amirshahrokhi et al., MSM could significantly alleviate paraquat (PQ)-induced acute liver and lung damage in mice, attributed to their antioxidant and antiinflammatory properties. MSM (500 mg/kg per day) was injected intraperitoneally 3 days before and 2 days after injection of PQ. Further histological examinations in lung and liver tissues demonstrated that MSM exerted its antiinflammatory and antioxidant effects both through reducing amounts of malondialdehyde (MDA), TNF-α, and myeloperoxidase (MPO), and enhancing levels of glutathione, superoxide dismutase (SOD), and catalase (Amirshahrokhi and Bohlooli, 2013).

Bohlooli et al. demonstrated that 7 days of pretreatment with MSM (100 mg/kg) could mostly attenuate acetaminophen-induced hepatic injury via its sulfur donating group and antioxidant effects. Resembling plasma-reduced

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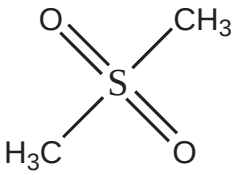

FIG. 2.11.1 Chemical structure of MSM. *MSM*, Methylsulfonylmethane.

glutathione (GSH), the prominent endogenous oxidative stress alleviating agent, MSM as a compound with free sulfur groups can mostly prevent GSH depletion and reduce MDA levels and MPO activity (Bohlooli et al., 2013). In addition, acetic acid–induced colitis was significantly ameliorated by oral administration of MSM (400 mg/kg/day) for 4 days in rats. In this study, administration of MSM was associated with a significant reduction in the levels of MDA, IL-1β, and MPO with an increase in GSH and catalase (CAT) amounts in the acetic acid–induced colitis group. Further, the ameliorating effect of the compound in improving colitis in the experimental model was also proved by scores of macroscopic and microscopic colonic damage (Amirshahrokhi et al., 2011).

In another study on osteoarthritis in animals, the safety and efficacy of an MSM-containing diet (0.06, 0.6, and 6 g/kg) on bone and knee joints was evaluated and the results demonstrated that an intake of MSM (13 weeks) could significantly decrease the degeneration of cartilage, dependent on dosage (Ezaki et al., 2013).

Pulmonary arterial hypertension (PAH) is a disease with a high morbidity and mortality rate and oxidative stress is the key player in its pathogenesis and development (Huggins et al., 2003). Mohammadi et al. showed that MSM could significantly attenuate monocrotaline (MCT)-induced PAH in rats by improving antioxidant capacity and attenuating induced oxidative stress. Further investigations revealed that CAT, SOD, and GSH-px activities were increased and GSH reservoirs were recovered by MSM in MCT-induced PAH groups (Mohammadi et al., 2012).

ANTINEOPLASM PROPERTIES

MSM has also shown cytotoxicity on multiple cancer cell lines such as gastrointestinal cells (IC_{50} between 21.07–28.04 mg/mL) (Jafari et al., 2012), breast cells (MDA-MB 231; IC50 300 mM) (Kang et al., 2016), and melanoma cells (Cloudman S-91) (Caron et al., 2010).

Jafari et al. reported that MSM exerts cytotoxic effects against multiple gastrointestinal cancer cells including HepG2, AGS, and KEYSE-30, dose dependently. They proposed that these antineoplastic effects mostly resulted from G2/M cell cycle arrest (Jafari et al., 2012). Additionally, in a recent study MSM was found to dramatically activate Bim, a proapoptotic molecule, and induce apoptosis in HCT-116 colon cancer cell lines regardless of p53 expression levels (Karabay et al., 2016).

Furthermore, proliferation of different liver cancer cell lines such as Huh7-Mock, HepG2, and Huh7-H-rasG12V were inhibited by the administration of 500 mmol/L of MSM. It has been proposed that activation of caspases and initiation of apoptosis in these cell lines by MSM is the underlying mechanism behind the observed antiproliferative activity. Moreover, oral administration of 100 μg/mL of MSM to H-ras12V transgenic mice for 3 months resulted in a significant improvement in liver function and activated apoptosis which resulted in the suppression of hepatic tumorigenesis (Kim et al., 2014).

In another study, it was shown that MSM displayed inhibitory effects on LPS/IFN-γ-activated macrophages in a dose-dependent manner. Lower concentrations (50–75 mM) modulated apoptosis via restricting the accumulation of p53, and the involved proteins, namely, Bax and Bcl-2. However, a higher concentration resulted in apoptosis and a reduction of cell viability (Karabay et al., 2014).

Lim et al. evaluated the antineoplastic effects of MSM on different breast cancer cell lines, including MDA-MB231, SK-BR3, MCF7, and T47D, and determined the IC50 to be approximately 300 mM. They reported a significant anticancer effect for MSM against breast cancer cell lines. In addition they demonstrated that MSM could significantly suppress MDA-MB231 human breast xenografts in Balb/c mice. Besides this, several other in vitro and in vivo reports, have demonstrated that MSM possesses regulatory effects on the signal transducer and activator of transcription 5b (STAT5b), the signal transducer and activator of transcription 3 (STAT3), insulin-like growth factor 1 (IGF-1), insulin-like growth factor 1 receptor (IGF1R), and vascular endothelial growth factor (VEGF) through which tumor initiation, growth, and metastasis is supressed (Lim et al., 2012). MSM can also reduce the expression of human epidermal growth factor receptor 2 (HER2) via decreasing the binding ability of STAT5b to the promoter of the HER2 gene (Kang et al., 2016). Alongside this, administration of MSM in combination with AG490, an inhibitor of Janus kinase 2 (Jak2), was conducive in reducing the expression of several signaling molecules including STAT5b, STAT3, VEGF, VEGF-R2, and IGF-1R in human bladder cancer which has been proved both in vitro and in vivo (Joung et al., 2014).

CLINICAL STUDIES

Single Ingredient

In a clinical study composed of 50 women and men suffering from osteoarthritis (OA) and pain in the knees who received 3 g of MSM or a placebo twice a day for 3 months, the MSM group showed a significant decrease in pain and physical function impairment (using the Western Ontario and McMaster University Osteoarthritis Index visual analog scale (WOMAC)) alongside an improvement performing activities involved with daily life, in comparison to the placebo-receiving group, observed using the SF-36 (indicative of state of health and quality of life) evaluation (Kim et al., 2006).

In another double-blind controlled clinical study on 49 patients with knee OA the efficacy of administration of 3.375 g/day of MSM for 3 months compared to a placebo was evaluated. Although the above mentioned regime could not improve significantly the WOMAC pain, stiffness and SF-36 total score compared to the placebo, WOMAC physical function and WOMAC total score in the test group were significantly improved compared to the placebo-receiving group. Nevertheless, these results propose there are beneficial effects to taking MSM, however, it is unclear whether they are of clinical significance (Debbi et al., 2011b).

In order to examine the effect of MSM supplements on oxidative stress in acute exercise (100 mg/kg, before running on treadmill for 45 min at 75% VO2max) and chronic exercise (50 mg/kg, 10 days before running a 14-km route) in 16 and 18 untrained healthy men respectively, Nakhostin-Roohi et al. conducted two double-blind, placebo-controlled trials. The oxidative stress markers, such as protein carbonyl (PC), bilirubin, and uric acid, were lowered and the total antioxidant capacity increased via MSM treatment compared to the placebo in the single-dose study. Levels of PC, MDA, and plasma oxidized glutathione (GSSG) reduced in comparison with the placebo and the plasma-reduced glutathione (GSH) level and the ratio of GSH/GSSG were elevated in the chronic study (Nakhostin-Roohi et al., 2011; Nakhostin-Roohi et al., 2013a).

The antioxidant and antiinflammatory properties of MSM after strenuous resistance exercise were also confirmed in other clinical studies (Barmaki et al., 2012; Kalman et al., 2012; van der Merwe and Bloomer, 2016). In a pilot study by Kalman et al., SM was given (doses of 1.5 and 3 g/day) for 30 days to 8 healthy moderately exercise-trained men (28 years old) displayed a significant reduction in muscle soreness and fatigue, and a significant elevation in glutathione and trolox equivalent antioxidant capacity (TEAC) (Kalman et al., 2012). The result of a placebo-controlled study showed that administration of MSM at doses of 3 g/day for 28 days in 40 physically active men, before performing 100 repetitions of eccentric knee extension exercises, successfully decreased the induction of IL-1β and IL-6 and levels of proinflammatory cytokines (van der Merwe and Bloomer, 2016). Also, in a double-blind, placebo-controlled study conducted by Barmaki et al., administration of 50 mg/kg of MSM before a 14-km run for 10 days in 18 healthy young men significantly increased the whole body's antioxidative potential and, secondary to that, reduced biomarkers of muscle damage compared to a placebo-receiving group (Barmaki et al., 2012).

Moreover, the effects of MSM on seasonal allergic rhinitis symptoms were also evaluated in an open-label clinical study on 55 subjects. Clinical respiratory symptoms and energy levels with plasma immunoglobulin E (IgE) and C-reactive protein, showing immune and inflammatory reactions, were calculated after 2.6 g/day of MSM supplementation for one month. It was found that there was a significant reduction in the upper and total respiratory symptoms at day 7 and improvement of lower respiratory symptoms by week 3. Nevertheless, the level of plasma IgE showed no change among the subjects (Barrager et al., 2002).

Multiingredients

In a randomized placebo-controlled trial, prescription of 500 mg of glucosamine (GS) with 500 mg of MSM 3 times/day for 3 months in 118 men and women with knee OA significantly improved the markers of OA, including pain, swelling, and Lequesne index compared to a placebo-receiving group (Usha and Naidu, 2004).

Results of a prospective randomized clinical trial, also confirmed more efficient effects of consumption of a combination of MSM (5 g) and boswellic acids (7.2 mg) compared to 1.5 g of GS per day for 3 months in 120 participants with knee OA. The outcomes of the study showed better value for the combination treatment in reducing the severity of pain, Lequesne index, and nonsteroidal antiinflammatory drugs (NSAIDS) and anticyclooxygenase-2 (COX-2) consumption. Nevertheless, no statistically significant difference between the two groups in terms of improvement in the maximum distance walked was observed (Notarnicola et al., 2016).

In 2015, a randomized clinical study was performed in Italy to evaluate the effectiveness of a combination of *Serenoa repens*, lycopene, bromoline extract, selenium, and MSM on the levofloxacin efficacy in 79 chronic bacterial prostatitis patients. Forty patients were treated with 500 mg/day of levofloxacin for 14 days plus the combination and 39 patients only

received 500 mg/day levofloxacin for 14 days. The patients were followed for 6 months and at the end of the first month, significant changes in the National Institutes of Health-chronic prostatitis symptom index and the international prostatic symptom score (IPSS) were observed (Cai et al., 2016).

MSM is also used in topical formulations in combination with other ingredients for its antiinflammatory and potent absorption-enhancing effects. In one controlled clinical study, the effects of an application of a gel medical device containing MSM, hyaluronic acid, and tea tree oil as its main ingredients, or a placebo, given twice daily for 14 days, were assessed in 36 patients with hemorrhoids. Pruritus, irritation, anal pain, pain during defecation, and visible bleeding, as the major symptoms of haemorrhoids, were significantly reduced compared to a placebo group. In topical administration, MSM not only prevents hyaluronic acid degradation but also helps with the reconstruction of damaged tissue via the formation of glycosaminoglycans as a source of organic sulfur (Joksimovic et al., 2012).

Another topical treatment based on silymarin and MSM displayed beneficial effects against rosacea, especially subtype 1 erythemato-telangiectatic phase, in 46 patients in a double-blind and placebo-controlled study. After 1 month of treatment, improvement of papules, skin redness, hydration, and itchy skin color were observed. MSM may act against rosacea through photoprotective action, with its antiinflammatory properties attenuating the release of IL-1α and inhibition of capillarogenesis by reducing the release of vascular endothelial growth factor from keratinocytes (Berardesca et al., 2008).

In order to search for an effective treatment for lower extremity pitting edema, which is the main problem linked to chronic venous insufficiency, a lotion containing MSM and ethylene diamine tetra acetic acid (EDTA) was evaluated in a two-phase, double-blind, placebo-controlled clinical study. Results demonstrated that MSM could significantly improve the penetration of EDTA to the cell membrane and the lotion could reduce the circumference of the ankle, calf, and foot for both legs. Thus lower extremity pitting edema can efficaciously be treated with EDTA plus MSM through reduction in oxidative stress (Tripathi et al., 2011).

SAFETY

Animal studies indicated very low toxicity in both topical and oral treatments with MSM (Takiyama et al., 2010). There are some mild side effects like gastrointestinal problems, headache, and fatigue which were reported in clinical trials due to MSM application (Debbi et al., 2011a). Administration of the compound (6 g/day) in OA for 26 weeks showed no side effects or abnormality in laboratory monitoring (Pagonis et al., 2014). A lethal dose of 50% (LD50) of the compound is in excess of 17.5 g/kg body weight (Horvath et al., 2002). In 2007 the United States Food and Drug Administration (FDA) introduced a specific brand of MSM as being generally recognized as safe (GRAS) (Butawan et al., 2017).

CONCLUSIONS

MSM is a prevalent dietary supplement, which can be used either alone or in combination with other compounds for the treatment of various pathological conditions such as OA, seasonal allergic rhinitis, hemorrhoids, rosacea, and bacterial prostatitis, although there is lack of evidence to support the use of MSM. Currently different oral and topical formulations of MSM are used in inflammatory diseases. Although, no serious side effects are reported in the published clinical trials of MSM, and the compound is considered as "possibly safe," there is still insufficient available data regarding the effects of its long-term use in humans, and further research is needed to evaluate its long-term safety.

ABBREVIATIONS

CAT	Catalase
CPS	Chronic prostatitis symptom
$DMSO_2$	Dimethyl sulfone
DMSO	Dimethylsulfoxide
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
GRAS	Generally recognized as safe
GSH	Plasma-reduced glutathione
GS	Glucosamine
GSSG	Plasma oxidized glutathione
HER2	Human epidermal growth factor receptor 2
IgE	Immunoglobulin E
IGF-1	Insulin-like growth factor 1

IGF-1R Insulin-like growth factor 1 receptor
IL-6 Interleukin-6
IPSS International prostatic symptom score
Jak2 Janus kinase 2
LPS Lipopolysaccharide
MCT Monocrotaline
MDA Malondialdehyde
MPO Myeloperoxidase
MSM Methylsulfonylmethane
NF-kB Nuclear factor kB
OA Osteoarthritis
PAH Pulmonary arterial hypertension
PC Protein carbonyl
PQ Paraquat
SASQ Seasonal allergy symptom questionnaire
STAT3 Signal transducer and activator of transcription 3
STAT5b Signal transducer and activator of transcription 5b
TEAC Trolox equivalent antioxidant capacity
TNF-α Tumor necrosis factor-α
VEGF Vascular endothelial growth factor
WOMAC Western Ontario and McMaster University Osteoarthritis Index visual analogue scale

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Chapter 2.12

Melatonin

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INTRODUCTION

Endogenous melatonin (5-methoxy-*N*-acetyltryptamine) is mainly derived from the pineal gland, which is synthesized from tryptophan via serotonin. In addition, different tissues are involved in melatonin production such as lymphocyte, retina, salivary gland, platelet, skin, and developing brain. Regarding biochemical aspects, melatonin is categorized as an indoleamine due to its substituted indolic ring, which has an amino group. In the pineal gland, its synthesis is controlled by a circadian signal from the suprachiasmatic nucleus, through retinohypothalamic pathways associated to the photoperiod. Melatonin is a highly lipophilic molecule which also possesses hydrophilic properties. Melatonin acts as a clock and calendar in the mammalian body and has a significant role in the regulation of the circadian as well as circannual rhythms including seasonal reproduction. This substance is also a biological modulator of mood, sleep, and sexual behavior (Acuña-Castroviejo et al., 2014; Claustrat et al., 2005; Macchi and Bruce, 2004). Both circadian production of melatonin as well as its intracellular and extracellular release, clarify the chronobiotic influence on vital functions, including endocrine, paracrine, and autocrine rhythms. Melatonin has been detected in several extrapineal organs and tissues such as the retina, lens, liver, kidney, thyroid, pancreas, thymus, Harderian gland, airway ducts, skin, reproductive tissue, gastrointestinal tract, spleen, carotid body, as well as endothelial tissues (Acuña-Castroviejo et al., 2014)

The biological functions of melatonin in the human organs are performed through two major signaling cascades comprising receptor-mediated and nonreceptor-mediated activities. G protein-coupled transmembrane receptors are the main receptors of melatonin which have two subtypes (MT1 and MT2). Membrane receptors of melatonin are expressed in several organs and cell types. Modification of functions of adenylate cyclate, guanylate cyclase, phospholipase C, as well as calcium and potassium channels is involved in the cellular signaling of melatonin receptors. Activation of MT1 receptors results in several biologic effects, that are mediated by the suppression of cyclic adenosine monophosphate (cAMP) as well as enhancement of cytosolic calcium through Gq11, and stimulation of MT2 receptors can cause cAMP and cyclic guanosine monophosphate (cGMP) inhibition (Pandi-Perumal et al., 2008; Yonei et al., 2010). A third subtype of melatonin receptor is a quinone reductase II, which has been found to be associated with the xenobiotic metabolism of the cell. Melatonin binding sites in the nucleus are also involved in the biologic function of this substance, which are mediated by orphan receptors from the retinoid orphan receptor -α and retinoid Z receptor family (Pandi-Perumal et al., 2008; Slominski et al., 2012). Mounting evidence showed that MT1 receptors are involved in metabolic functions, vasoconstriction, and reproductive activities; in contrast MT2 are associated with regulation of circadian rhythms as well as dopamine release (Acuña-Castroviejo et al., 2014; Claustrat et al., 2005).

Antioxidant and antioxidative stress function are frequently reported for this indoleamine agent which is mediated by diminishing the redox status of cells and generating reactive oxygen species in tissues as well as stimulating the expression and function of catalase, glutathione peroxidase, and superoxide dismutase, which possess strong antioxidant functions. Melatonin also decreases electron leakage from the mitochondrial electron chain and modulates mitochondrial homeostasis through enhancing the production of glutathione (Galano et al., 2013; Reiter et al., 2003). It has been found that melatonin has a well-established antiinflammatory function through suppressing proinflammatory cytokines and their producing cells (leukocytes), as well as regulating the relevant transcription signaling pathways like nuclear factor (NF)-kB and PI3K/Akt (Maldonado et al., 2010; Mayo et al., 2005). The antioxidant and antiinflammatory activates of this indoleamine are important in protecting neural cells against age-related neurodegeneration as well as oxidative damage pathologies.

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BIOAVAILABILITY

Melatonin is known to act to regulate circadian rhythm and its level in serum varies during the light–dark cycle, which is day and night, and is suppressed by bright light. The oscillatory cycle of melatonin levels as well as secretion depend on age, gradually declining as we get older. Mostly, older adults might face disorders related to a disrupted circadian rhythm, requiring exogenous administration of melatonin. Several doses of melatonin have been administered orally, sublingually, and intravenously to patients of various ages. The optimal dosage and the pharmacokinetic properties of melatonin are not completely clear and still needs to be established further for eliciting the pharmacological effects. Inconsistent results and interindividual differences for the pharmacokinetics of oral and intravenous administration of melatonin has been reported. This could be due to the fact that the pharmacokinetic properties of melatonin, including bioavailability, absorption, distribution, metabolism, and elimination, depend on various parameters such as dosage, patient age, route of administration, type of formulation, method of assessment, etc. The oral absorption of melatonin was found to be rapid and followed the first-order kinetic at doses up to 80 mg (Waldhauser et al., 1984). The absorption of melatonin is site-dependent, with the highest absorption rate in the rectum (Al-Omary, 2013). Maximal plasma concentrations could be achieved after approximately 30–45 min (t_{max}) and 30–60 min for intravenous and oral melatonin, respectively (Andersen et al., 2016b; Harpsøe et al., 2015; Markantonis et al., 2008). Slow-release oral formulations exhibited a prolonged t_{max} up to 167 min (Harpsøe et al., 2015). The t_{max} for intravenous administration of melatonin with doses up to 100 mg is similar to its oral administration with a maximum concentration achieved after 45 min (Andersen et al., 2016c). Similarly, the elimination of melatonin in the human body exhibits linear pharmacokinetics, while the elimination is both dose- and route-independent. Due to the high solubility of melatonin in water and lipid, it can penetrate easily into cell membranes and access various fluids, tissues, and cellular compartments (Al-Omary, 2013). Studies have reported that its elimination half-life ($t_{1/2\ elimination}$) is 45 min for an intravenous administration of up to 100 mg of melatonin (Harpsøe et al., 2015). It has been also established that $t_{1/2\ elimination}$ is in the range of 28–61 min for intravenous application dosages from 0.005 to 2 mg (DeMuro et al., 2000; Mallo et al., 1990) and 46–65 min in oral doses from 0.5 to 6 mg (Andersen et al., 2016b). Melatonin possesses unstable absorption from the gastrointestinal tract and extensive first-pass hepatic metabolism (Al-Omary, 2013). It has been established that only 10%–15% of the orally administered melatonin reaches systemic circulation, with 85%–90% of melatonin hydroxylated in the liver (by cytochrome P 450 (CYP) 1A2) to 6-hydroxymelatonin, conjugated with sulfate or glucuronic acid, and excreted in the urine (DeMuro et al., 2000; Di et al., 1997). It is worth noting that elimination of melatonin in children occurs more rapidly than adults (Cavallo and Ritschel, 1996). The low bioavailability of melatonin has been proven in published studies which reported various values for oral bioavailability of melatonin (ranging between 0.3% and 33% (Harpsøe et al., 2015), 10% and 56% (Di et al., 1997), 3% and 76% (Lane and Moss, 1985), 0.7% and 12.7% (Andersen et al., 2016b), etc.). The lack of bioavailability of melatonin could be attributed to low absorption from the gastrointestinal canal or considerable first-pass metabolism in the liver and high degree of metabolization (high ratio of metabolite/melatonin) (Di et al., 1997). Various values have been reported for the maximum plasma level of melatonin, C_{max}, and the area under the curve from time zero to infinity (AUC0-inf), corresponding to the oral dosage of melatonin. Andersen et al. (2016b, c) reported C_{max} in broad range from 1105 to 58,900 pg/mL for oral administration of 10 mg of melatonin. This variability could be due to different individual pharmacokinetic characteristics of the drug. Similar to other parameters, the heterogeneous data have been reported for systemic plasma clearance (CL) and volume of distribution (Vd); however, CL and Vd values are approximately in the range 0.015–0.030 L/min.kg (Andersen et al., 2016a) and 1.6–2.0 L/kg (Andersen et al., 2016a; Bellapart et al., 2016), respectively.

Due to the rapid first-pass metabolism of oral and intravenous doses of melatonin and poor bioavailability, some alternative routes of administration (intranasal, transdermal, oral transmucosal, and subcutaneous injection) have been proposed in the literature (Flo et al., 2017; Zetner et al., 2016). Intranasal administration of melatonin causes an increased bioavailability and t_{max} compared to oral administration of melatonin. Transdermal and transmucosal doses of melatonin exhibit a slower absorption rate and the higher C_{max}, respectively (Zetner et al., 2016).

THERAPEUTIC USES BASED ON CLINICAL EVIDENCE

Neural Disorders

Melatonin is considered as a chronobiotic and also as a chronobiological regulator agent. Low levels of melatonin and disturbance in secretion of nocturnal melatonin is involved in several neurological diseases, including insomnia (Wade and Downie, 2008; Wade et al., 2010), stroke (Gonzales-Portillo et al., 2015), depression (Cardinali et al., 2012), Alzheimer's disease (Savaskan et al., 2002), Parkinson's disease (Bolitho et al., 2014), as well as migraine and headache (Claustrat et al., 1989; Peres et al., 2006).

Sleep Disorders

Melatonin is considered a therapeutic agent for different forms of insomnia; however, there is still little evidence of its efficacy and safety from large-scale and long-term clinical trials. In healthy individuals, this substance significantly decreases the time to onset of sleep and also enhances sleep duration. Clinical evidence has showed that melatonin has higher efficacy in insomnia associated with circadian rhythm disturbances as well as age-related sleep disorders (Cajochen et al., 2003; Wade and Downie, 2008; Wade et al., 2010; Zhdanova et al., 2001). The variable effects of melatonin on insomnia may be due to its dose-dependent response and also time of administration, given that intake during the day has been extensively reported to induce soporific and hypothermic properties (Rogers et al., 2003).

Alzheimer's Disease

Reduced levels of melatonin in cerebrospinal fluid and serum have been reported in patients with dementia and Alzheimer's disease. During the development of dementia the levels of melatonin in the cerebrospinal fluid decreases. Age-related decreases in melatonin levels are associated with production of senile plaque and neurofibrillary tangles leading to neuronal loss in Alzheimer's disease (Zhou et al., 2003).

Migraine

The beneficial effect of melatonin for migraine is associated with the seasonal nature of headaches or migraine with the circadian pattern. Clinical evidence has revealed that melatonin serum concentration is reduced in migraine and other different types of headaches (Claustrat et al., 1984; Peres et al., 2006). In a cross-sectional study, patients with Parkinson's disease showed disturbance in their circadian rhythms of melatonin secretion compared with healthy people in which the 24-hour AUC, for circulating melatonin levels, was significantly lower ($P < .001$) (Videnovic et al., 2014).

Depression

Disturbance of sleep and circadian rhythms has a key role in the pathogenesis of depression. Human investigations demonstrated that a decrease in melatonin level occurs in depressed patients, in comparison with healthy subjects. Bipolar patients showed higher secretion of melatonin during their manic periods. Also, disorders in circannual rhythms and sleep are symptoms of depression (Cardinali et al., 2012; Claustrat et al., 1984).

Malignancies

There is evidence that the administration of melatonin in combination with chemotherapy and/or supportive care in patients with malignant melanoma, advanced lung cancer, breast cancer, and other advanced solid tumors, plays a significant role in the improvement of outcomes for tumor regression and patient survival (Gonzalez et al., 1990; Sookprasert et al., 2014). Melatonin's antiangiogenic effect is considered one of the main mechanisms by which it alleviates the complications in advanced cancer patients (Lissoni et al., 2001). Melatonin also was found to diminish physical fatigue and other symptoms in patients with advanced cancers who were receiving palliative care. However, melatonin did not affect appetite, weight, or quality of life in cachectic patients with advanced cancers (Del Fabbro et al., 2013; Lund Rasmussen et al., 2015).

Reproductive Effects

Melatonin has a regulatory role in human reproduction as well as local steroidogenesis. An increase in nighttime melatonin levels in pregnant women significantly enhanced parturition time and regulated placental hormones (Tamura et al., 2014). Melatonin is also associated with promoting Human chorionic gonadotropin (HCG) secretion and the growth and maturity of oocytes (Lanoix et al., 2008; Sakaguchi et al., 2013). However, these data require confirmation, and well-designed clinical trials are mandatory in order to evaluate the reproductive effects of melatonin.

Cardiovascular Diseases

In clinical studies it was established that melatonin plays a vital role in various coronary heart diseases and other cardiovascular diseases. It was found that melatonin monotherapy and combined treatment caused antihypertensive, antiischemic, and antianginal effects in patients (Zaslavskaia et al., 2010). It caused a decrease in nocturnal hypertension, a reduction in pulsatility index in the internal carotid artery, an inhibition of drug-induced myocardial injury, and a decrease in platelet aggregation (Dominguez-Rodriguez et al., 2010; Pandi-Perumal et al., 2017; Yang et al., 2014). The antiatherosclerosis activity and protective potential

of melatonin against the oxidative stress and its consequent cardiac conditions has been proven in many studies (Dominguez-Rodriguez et al., 2012; Rezzani et al., 2006; Tomás-Zapico and Coto-Montes, 2005). Melatonin has also exhibited the potential to preserve the microstructure of the cardiomyocytes and decrease myocardial ischaemia–reperfusion injury (Halladin et al., 2014; Yang et al., 2014). In addition to the beneficial effect of melatonin in cardiovascular disorders, its effect on metabolic abnormalities and cardiovascular risk factors, such as obesity and other metabolic disorders, has been identified in clinical trials. It was found effective in controlling carbohydrate, lipid metabolism, and other metabolic parameters (Shatilo et al., 2013). Melatonin monotherapy and combined treatments with metformin significantly improved levels of fasting and postprandial glycemia in type 2 diabetes mellitus patients (Hussain et al., 2006). Melatonin also exhibited antihypertensive effects in patients suffering from type 1 diabetes, by amplifying the decline of diastolic blood pressure (Cavallo et al., 2004).

SAFETY AND TOLERABILITY

There are limited clinical studies concerning the risk of long-term and short-term administration of exogenous melatonin and its moderate and severe adverse effects. In studies on preterm infants, children, and adolescents, intravenous and oral administration of melatonin for treatment of different disorders caused no significant side effects, even at high doses and with repeated administrations (Gitto et al., 2012; Gitto et al., 2004a,b; Gitto et al., 2005); it was found that it could affect the sexual maturation of patients in these groups (Andersen et al., 2016d). Oral and intravenous administration of melatonin also caused no signs of serious adverse effects on adults while mild symptoms, such as transient episodes of numbness, headaches, dizziness, paresthesia of the mouth, arms, or legs, and worsening dyspnea, have been documented for the oral administration of melatonin (de Matos Cavalcante et al., 2012; Hansen et al., 2014). In contrast, in a clinical study conducted by Nagtegaal et al. (1996) the possible adverse effects of melatonin on 97 subjects suffering from circadian rhythm disorders were studied. In this study, fever (on the first day of melatonin treatment), hyperkinesia, menorrhagia, headache, and abdominal reactions were observed following treatment with melatonin.

At this moment in time, no clinical study has been devoted to investigating the possible adverse effects of melatonin on pregnant women, so administration should not be recommended during the pregnancy (Andersen et al., 2016d). Administration of melatonin in breast-feeding women causes sedation and daytime sleepiness for infants (Merchant et al., 2013). In addition, the elderly are known to be major consumers of melatonin for treatment of sleep and anxiety disorders, therefore, they will often be faced with side effects such as daytime sleepiness (Gooneratne et al., 2012).

INTERACTIONS OF MELATONIN WITH OTHER SUPPLEMENTS, DRUGS, AND FOODS

Due to the liver metabolizing melatonin by P 450 (CYP) 1A2 (DeMuro et al., 2000), its levels in the plasma can be increased or decreased by any medication which slows down or facilitates this pathway (Culebras, 1999; Papagiannidou et al., 2014). Coadministration of 5-methoxypsoralen, as one of the CYP(1A2) enzyme inhibitors, with melatonin can cause clinical interactions and disturb the process of 6-melatonin hydroxylation (Papagiannidou et al., 2014). Administration of fluvoxamine, as another CYP(1A2) inhibitor, and cimetidine, has also been found to cause an increase the melatonin levels found in the plasma (Papagiannidou et al., 2014; Skene et al., 1994). Moreover, concurrent administration of omeprazole, lansoprazole, and citalopram with endogenous melatonin was found to increase the urinary excretion of 6-sulphatoxymelatonin (Huuhka et al., 2006). It was reported that smoking cigarettes suppresses melatonin plasma levels especially at the time that the levels of the hormone are high (Ursing et al., 2005). On the other hand melatonin can potentiate the effects of other agents such as vasointestinal peptides and norepinephrine (Culebras, 1999).

CONCLUSION

Exogenous melatonin possesses widespread applications and is recommended as a treatment for various diseases especially several neural disorders, some types of cancer, cardiovascular diseases, etc. There have been no reports implying severe adverse effects due to melatonin and it can, therefore, be considered as therapeutically safe, even at high doses. Despite the presence of numerous reports on the pharmacokinetic characteristics of melatonin, their inconsistent results reveal a requirement for further clinical investigations.

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Chapter 2.13

Resveratrol

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INTRODUCTION

Resveratrol (RV, *trans*-3,5,4′-trihydroxystilbene), the most well characterized stilbene, is produced in grapes by the enzyme stilbene synthase in response to injuries, such as infection or mechanical damage (Burns et al., 2002). Its common structure consists of two aromatic rings linked by a methylene double bond, which are part of a resorcinol structure from which RV takes its name (Catalgol et al., 2012). Besides being found in grapes, RV is produced naturally by several plant species including berries, peanuts, pines, plums, legumes, and several herbs. RV is also presents in a large variety of flowers and leaves such as *Gnetum*, butterfly orchid tree, white hellebore, corn lily, eucalyptus, spruce, etc. (Burns et al., 2002).

CHEMICAL STRUCTURE

In nature, RV is present as *cis* and *trans* isomeric forms (with different biological activities) and also as β-glycosylated derivatives, named piceids (Ficarra et al., 2016). *Trans*-RV is the predominant and more stable steric form of RV in which isomerization is facilitated by exposure to ultraviolet (UV) radiation. Its biological activity is dependent mainly on the number and position of hydroxyl groups. Its molecular structure reveals an extensive hydrogen bond network; hydrogen bonds are alternately formed and broken with each of the neighboring phenolic oxygen atoms. The dynamic behavior of the three hydroxyl groups which results in the mobility of three hydrogen atoms per resveratrol molecule correlates well with the antioxidant biological activity of RV. In fact, hydrogen atoms may be easily transferred to reactive oxidants that are rich in electron density (Rizvi and Pandey, 2010). Several studies have indicated the antioxidant properties of this compound, and the research around RV continues to investigate a variety of biological and pharmacological effects, including therapeutic uses.

METABOLISM

The three hydroxyl groups contained in the molecule drive the RV to be rapidly converted into glucuronides, sulfates, and five distinct metabolites found in the urine: RV monosulfate, *trans*- and *cis*-RV monoglucuronide, dihydroresveratrol monosulfate, and dihydroresveratrol monoglucuronide. Studies show that the nature and quantity of metabolites may differ among subjects, owing to variability between individuals, and that the main organs where the drug accumulates are the liver, heart, and kidneys (Cottart et al., 2010). Following oral administration, RV is absorbed by passive diffusion at the intestinal level and reaches its peak in plasma within 30–60 min. Once absorbed RV is rapidly transformed into its metabolic derivatives, primarily glucuronide and sulfate conjugates in the liver and intestines. In the bloodstream, RV can be found principally in three different forms: RV glucuronide, RV sulfate, or as free RV. The majority of the free RV form is bound to human plasma lipoproteins and/or albumin which facilitate its entry into cells (Walle et al., 2004). Several studies have shown that the biological activity of the major metabolites varies greatly; in general RV glucuronide has a smaller effect compared to RV sulfate, which acts as a free-radical scavenger, inhibits cyclooxygenase-1, and cytotoxicity (Hoshino et al., 2010). Overall, the metabolites are less active than RV, but may form an in vivo reservoir of available RV (Walle, 2011).

BIOAVAILABILITY

Despite the lipophilic characteristics that facilitate its absorption, RV exhibits a low bioavailability that, by reducing its effectiveness in vivo, can be a barrier for progress in new therapies (Walle, 2011; Gambini et al., 2015). Estimates of the

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plasmatic concentrations of RV metabolites, following oral administration, are significantly higher than free RV, indicating a very fast rate of RV metabolism (a half-life 0.13 h) (Walle et al., 2004), reaching plasmatic levels between 0.3 and 2.4 μM (Cottart et al., 2010). Moreover, experimental studies have suggested that the role of the matrix (e.g., in alcohol, natural sources, or vehicles), to supply resveratrol in humans, resulting in remarkable variability. This, ultimately leads to the delivery of small amounts of free RV in the plasma and, therefore, in the tissues. The biological activities of the conjugated metabolites and the conversion of both sulfates and/or glucuronides to RV, in specific organs such as the liver, might facilitate drug efficacy and bioavailability (Lu et al., 2013; Sergides et al., 2016). However, the content of RV in the human diet is almost negligible, and for this reason manufacturers have tried to capitalize on the drug's beneficial properties by selling RV in supplementary pills and liquids, in which it is sometimes combined with vitamins and/or other ingredients (Tomé-Carneiro et al., 2013).

RESVERATROL AND HEALTH

Numerous in vitro studies describe a multiplicity of RV biological effects, mainly caused by the abundance and diversity of the molecular targets of this compound. The main impacts of RV are antioxidative, antiinflammatory, and estrogenic, as well as having anticancer and neuroprotective effects (Csiszar, 2011; Galtieri et al., 2010; Tellone et al., 2014, 2015, 2016; Valdecantos et al., 2010). Like many other polyphenols, RV is an antioxidant, but it exerts a dual effect: it can neutralize free radicals and it can increase the activity of antioxidant enzymes like glutathione-peroxidase, S-transferase and S-reductase, superoxide dismutase, and catalase (Valdecantos et al., 2010). The antioxidant properties of RV, derived from its potential to increase nitric oxide synthesis when concentrations are low, which cause vasodilatation, may contribute to a decrease in oxidative damage and may protect the cardiovascular system (Hattori et al., 2002). It has been shown that RV interacts with the polar groups of the lipid bilayer preventing lipid peroxidation; this inhibition reduces and counteracts atherosclerotic lesions (Selvaraj et al., 2008). Atherosclerosis represents a serious risk leading to coronary damage and RV has been found to positively modify cardiovascular risk factors while also improving the cholesterol efflux mediated by apolipoprotein A1, up-regulating the ATP transporter and decreasing cholesterol influx (Berrougui et al., 2009). All these effects are significant for the protection and treatment of some neurodegenerative diseases because they may contribute to diminishing neurodegeneration plaque formation. In this context, several studies demonstrated the beneficial effects of RV in preventing structural alterations in central nervous system (CNS) in conditions such as Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (Tellone et al., 2015, 2016). Vingtdeux et al. (2010) demonstrated the antiamyloidogenic effect of RV through the activation of the AMP protein kinase and by potentiating SIRT1 activity which controls Aß metabolism and production (Qin et al., 2006). RV potentiating SIRT1 activity in reality counteracts not only neurodegenerative diseases but also promotes many beneficial metabolic changes, such as increases in fatty acid metabolic oxidation and mitochondrial respiration, and decreases in reactive oxygen species (ROS) production, inflammation, and genome instability. Moreover, RV through SIRT 1 activation reduces NF-kB-dependent activity and exerts an anticancer effect inhibiting the proliferation of tumors (Rocha-González et al., 2008; Han et al., 2015). In this regard, it should be recalled that RV is able to inhibit cyclooxygenase-1 and cyclooxygenase-2, which are usually overexpressed in cancer. Recent data have shown the potential of resveratrol supplementation (75 mg twice daily for 30 days) to induce physiological changes similar to the effect of calorie restriction in the treatment of obesity (Timmers et al., 2011).

SAFETY AND DRUG INTERACTION OF RESVERATROL

Organisms normally tolerate RV well at levels found in natural sources, but supplementation (1.0 g/day or above) can bring some advantages (Detampel et al., 2012). In particular resveratrol has been reported to act as an inhibitor of various cytochrome P450 metabolic enzymes (CYPs) and to reduce their transcription through nuclear aryl hydrocarbon receptor antagonism. CYPs are one of the fundamental enzyme involved in the phase I oxidative metabolism of many xenobiotic substances. These effects may modify the pharmacokinetics (i.e., absorption, modification, and distribution) of coadministered drugs, resulting in safety problems as far as the dosage and effectiveness of these compounds are concerned. Clinical trials show modification of buspirone, nicardipine, and diltiazem pharmacokinetics when coadministered with resveratrol (Choi et al., 2009; Chow et al., 2010; Hong et al., 2008).

CONCLUSION AND FUTURE TRENDS

Resveratrol is a phytoalexin well known for its health-promoting properties, having antioxidant, antimicrobial, and antiinflammatory properties along with demonstrating anticancer activities. However, its low bioavailability and persistence in

an organism limits its utilization. Future studies should be designed and performed to enhance our knowledge based on the complex potentialities of resveratrol at the level of cellular and molecular targets and to point out the roles of its metabolites and the fractions of which bind to proteins. Moreover, the distribution of the resveratrol and its metabolites within tissues and organs must be clarified along with the evaluation of their in vivo functions.

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Chapter 2.14

Rutin

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INTRODUCTION

Rutin was discovered in 1842 and it has been used in medicine to treat vascular disorders related to capillary permeability and fragility. It is one of the most common quercetin glycosides found in a number of plant sources. The concentrations of rutin reported in grapes and buckwheat are the highest among the other plant species. This compound is mainly found in different parts of the plants such as the fruit skins, leaves, flowers, and roots. Among the flavonoids, rutin belongs to the flavonols and it is the glycoside form of quercetin. As with other flavonoids, due to their chemical structures, rutin has significant antioxidant, chelating, and antimicrobial properties, and therefore many beneficial health effects. Because of its abundance and healthy properties, it is a suitable beneficial compound to be incorporated as part of the diet. Extracts and ingredients from different vegetable sources with high rutin content, such as buckwheat, can be incorporated into functional foods or used in nutraceuticals and medicinal products. Different strategies have been developed for the stabilization and bioavailability improvement of rutin through encapsulation. When rutin reaches the colon it is metabolized by probiotic bacteria, leading to the formation of quercetin and rutin metabolites that are responsible for beneficial antioxidant and antiinflammatory effects. In this context, these antioxidant and antiinflammatory effects of rutin have been extensively studied in neurodegenerative disorders and brain pathologies in which rutin has demonstrated a potential neuroprotective role.

SOURCES

Rutin is a flavonol widely present in a variety of fruits and vegetables (Marín et al., 2002). It was first reported in *Ruta graveolens* L. which gave its name to this compound (Chen et al., 2001). Grapes and buckwheat are the most important sources among fruits, vegetables, and grain crops. However, rutin is not found in cereals and pseudocereals (Kreft et al., 2006). The main sources of rutin are presented in Table 2.14.1. Rutin has been found in the extracts obtained from the skin of different grape (*Vitis vinifera* L.) varieties (Lacopini et al., 2008). The amounts found are highly variable within the different varieties, with values of 1.592 mg/100 g dry weight in Montepulciano and Sangiovese and 89.3 mg/100 g in Merlot and Cavernet Sauvignon varieties. Buckwheat is one of the most important sources of rutin. This genus comprises 15 annual and perennial species, and three of them are of interest for cultivation: *Fagopyrum esculentum* Moench., Tatary buckwheat (*Fagopyrum tataricum* L. Gaertn.), and perennial buckwheat (*Fagopyrum cymosum* Meisn.). Content is found to vary within this species, being affected by growing conditions. In addition, its content varies within different parts of the plant, being highest in concentration in common buckwheat in the hulls of the plant (3.250 mg/100 g dry weight), with lower concentrations in the green parts the plant (157 mg/100 g dry weight) (Kocevar Glavac et al., 2017). Tatary buckwheat is the variety that presents higher rutin content in its seeds compared to those of common buckwheat (Fabjan et al., 2003). Some environmental factors such as ultraviolet (UV) radiation may have also an impact on rutin content (Kreft and Skrabanja, 2002; Motoki et al., 2012). The contents of rutin in the genus *Amaranthus* have been also reported (Kalinova and Dadakova, 2009). The species has a marked influence on rutin content, with *Amaranthus* hybrids and *Amaranthus cruentus* the best sources. The different parts of the plant also have different rutin contents ranging from 8 mg/100 g dry weight in the seeds to 2.450 mg/100 g dry weight in the leaves. Cultivation conditions of asparagus are important as they can lead to higher rutin content if open cultivation conditions are achieved, with respect to hydroponic conditions. Rutin is also present in high amounts in the discarded cladophyls of the asparagus. Rutin can be also found in Gubeish (*Guiera senegalensis*), that is, an African plant used in folk medicine (Perwez et al., 2017). As presented in Table 2.14.1 rutin is also present in the different parts of capers, onions, green asparagus, and sea buckthorn, among others. The content in leaves of St. John's wort and capers is high and ranges from 2.400 mg/100 g to 2.750 mg/100g, respectively.

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TABLE 2.14.1 Rutin Content in Different Plant Sources

Plant name	Plant part	Content (mg/100 g d.w.)	Reference
Rue (*Ruta graveolens* L.)	Leaves	3.100	Proestos et al. (2006)
Common buckwheat (*Fagopyrum sculentum* Moench.)	Hulls Dried sprouts Seeds	3.250 157 10	Kocevar Glavac et al. (2017)
Tartary buckwheat (*Fagopyrum tataricum* Gaertn.)	Seeds	1250	Fabjan et al. (2003)
Grapes (*Vitis vinifera* L.) cv. Montepulciano, cv. Sangiovese, cv. Merlot, and Cavernet Sauvignon		1.592 89	Lacopini et al. (2008)
Amaranthus hybrid (*Amaranthus cruentus*)	Seeds Leaves	8 2.450	Kalinova and Dadakova (2009)
Gubeish (*Guiera senegalensis*)	Leaves	242	Perwez et al. (2017)
Capers (*Capparis spinosa* L.)	Buds Leaves Fruits	1.800 2.760 280	Musallam et al. (2012)
Onions (*Allium cepa* L.)	Skins (red onion)	6	Shi et al. (2016)
Sea buckthorn (*Hippophae rhamnoides* L.)	Leaves	300	Zu et al. (2006)
Asparagus (*Asparagus officinalis* L.)	Green spears Top Bottom Cladophylls	670 190 2.900	Motoki et al. (2012)
Bauhinia fortificata L.	Leaves	377	Toloza-Zambrano et al. (2015)
St. John's wort (*Hypericum perforatum* L.)	Leaves	2.400	Wach et al. (2007)
Apple (*Malus pumila* L.)	Peel	800	Wach et al. (2007)

CHEMISTRY

Flavonoids are polyphenolic compounds, mainly O-glycosylated, that can be found in fruits and vegetables (Marín et al., 2002). Rutin (quercetin-3-O-rutinoside) is a flavonol glycoside (Fig. 2.14.1) that is also known as vitamin P. It is synthesized in higher plants as a protectant against UV radiation and disease (Rozema et al., 2002). The phenolic part of the molecule is linked to sugar (this is the hydrophilic part of the molecule), increasing the solubility of the molecule in water. The hydrolysis of rutin by glucosidase results in quercetin and rutinose, this enzymatic reaction can be carried out by gut micloflora. Therefore, rutin usually appears with quercetin (Manach et al., 1997; Shen et al., 2002). Rutin and almost all flavonoid groups, have antioxidant activity, acting as free-radical scavengers (Marín et al., 2002; Tapas et al., 2008). This characteristic is associated with the presence in their structures of hydroxyl groups bound to aromatic rings (Rice-Evans et al., 1996). Rutin also exhibits metal chelating properties, inhibiting metal-ion-induced peroxidations (Lue et al., 2010; Marín et al., 2002). Quantitative analysis of rutin can be made by chromatographic methods, mainly high-performance, thin-layer chromatography (Perwez et al., 2017) and high-performance liquid chromatography, (Kocevar Glavac et al., 2017; Lacopini et al., 2008).

DEVELOPMENT OF RUTIN-BASED FOODS AND FOOD SUPPLEMENTS

The low water solubility of rutin (0.125 g/L) represents a limitation to its incorporation into functional foods and food supplements. The stability of rutin to processing conditions, and its possible interactions with other food components, is also important. Rutin and anthocyanins have been reported to have high stabilities in model juices due copigmentation

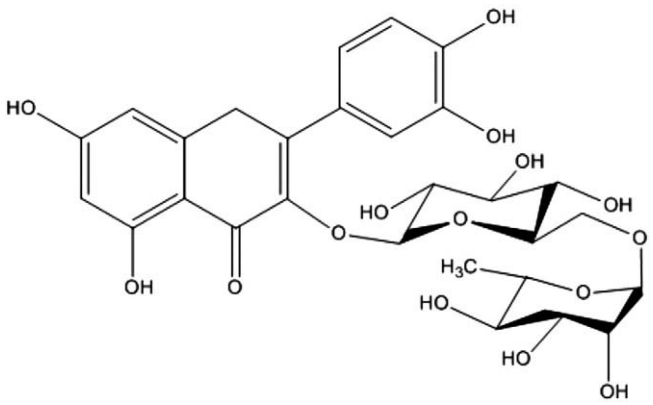

FIG. 2.14.1 Chemical structure of rutin (quercetin-3-O-rutinoside).

reactions, improving the stability of colour during storage (Hernandez-Herrero and Frutos, 2015). Rutin has antioxidant in vitro properties (Yang et al., 2008). Regarding heat processing, rutin is in generally stable at 100°C (Murakami et al., 2004). Heating buckwheat up to 150°C leads to the increase in rutin content, while steaming triggers high losses (Dietrych-Szostak and Oleszek, 1999). In model solutions Makris and Rossiter (2000) reported a higher stability of rutin with respect to quercetin and that oxygen accelerates the degradation of both flavonols. Processing conditions, like the chopping of asparagus, leads to a decrease (18.5%) in rutin content (Makris and Rossiter, 2001). Buckwheat has been used as a source of rutin for the production of functional food products such as bread. Rutin content decreases due to the bread-making process with values in dough and bread being ca. 0.24 mg/g dry weight, and values in buckwheat flour being ca. 12.3 mg/g dry weight (Kocevar Glavac et al. 2017; Vogrinci et al., 2010). Kreft et al. (2006), reported a content of 78 mg/kg dry weight in noodles, while the content in dark buckwheat flour used for their production was of 218 mg/kg dry weight. The content reported in groats was of 230 mg/kg decreasing to 88 mg/kg dry weight when cooked. The rutin content in other buckwheat-derived products such as beer and vinegar is negligible. The concentration of rutin reported for buckwheat leaf flour is about 2700 mg/kg dry weight and, therefore, this could be a suitable ingredient for the development of functional foods. The steaming of buckwheat seeds after mechanical cleaning results in a reduction in rutin content from a concentration of 14.1 mg/g dry weight to 8.8 mg/g dry weight (Kocevar Glavac et al., 2017). Rutin can also be enzymatically degraded to quercetin (Vogrinci et al., 2010; Yasuda and Nakagawa, 1994), for example, 85% of rutin is transformed into quercetin after the addition of water and yeast in the preparation of tartary buckwheat dough (Vogrinci et al., 2010). However, the application of rutin to the development of functional foods has a disadvantage associated with its low solubility in water, affecting its bioavailability and absorption. Therefore, nowadays, different techniques have been investigated that allow the development of functional foods with rutin and at the same time improve their delivery in the intestinal tract and therapeutic efficacy, such as the encapsulation of rutin by nanostructured lipid carriers designed for food applications or with phosphatidylcholine to form phytosomes that overcome the low water solubility of rutin (Babazadeh et al., 2016, 2017). Rutin extracts can also be used as part of medicinal preparations and are important flavonoids in the pharmaceutical industry. There are different extraction methods for plant-based rutin, using different techniques such as ultrasound, microwave, mechanochemical, infrared, and pressure-assisted methods. There is also a high variability in the efficiency of rutin extraction due to different factors such as the raw material, extraction temperature, process duration, and solvent-to-sample ratio (Chua, 2013). The solubility of rutin can be enhanced using different techniques, which can also result in its increased release and permeability (Sharma et al., 2015). Rutin can be incorporated into corn starch edible active films through zein–rutin composite nanorparticles to act as natural antioxidants, providing a controlled release of rutin and a higher antioxidant capacity (Zhang and Zhao, 2017). The aqueous solubility of rutin can be improved through the preparation of β-cyclodextrin inclusion complexes together with quercetin (Vijaya Sri et al., 2007). The poor solubility of rutin can be also improved through encapsulation in rutin-loaded nanoemulsions (Macedo et al., 2014) or tocopheryl polyethylene glycol 1000 succinate (TPGS) nanoemulsions.

SAFETY OF RUTIN AND ITS POSSIBLE INTERACTIONS WITH DRUGS OR FOOD SUPPLEMENTS

Rutin can interact with other polyphenols and/or metal ions such as anthocyanins and Mg(II) and Fe(III). It has been demonstrated that copigmentation reactions of rutin occur with the anthocyanin cyanidin-3-diglucoside-5-glucoside, leading

to changes in the antioxidant capacity of the complexes showing a synergistic effect (Qian et al., 2017). Rutin autoxidation in weakly alkaline aqueous solution is increased by Mg(II) and Ca(II) ions (Zivanovic et al., 2016). The combination of rutin with curcumin can promote curcumin interaction with the human serum albumin, improving its bioavailability (Liu et al., 2016) Rutin and other flavonoids can also interact with drugs such as tricagrelor, an antiplatelet drug, competing for the binding site to the human serum albumin, a process which could lead to an increase of free trigagrelor concentration in the plasma (Liu et al., 2015).

FUNCTIONAL PROPERTIES AND MECHANISMS OF ACTION

The role of flavonoids as potential therapeutic molecules has acquired much significance in the past few years due to increasing evidence for their beneficial and protective effects regarding different diseases. Rutin is a dietary flavonoid which has many therapeutic properties, mainly attributed to its potent antioxidant and antiinflammatory activities (Shen et al., 2002). Many studies have demonstrated these excellent health properties to prevent neurodegenerative disorders, cardiovascular diseases, and skin cancer, among others. However the health benefits of rutin can be influenced by its quantity and its bioavailability for absorption. Rutin concentration varies between both the plant species and specific parts of the plant, as well as showing variation linked to geographical origin. Normally, the daily intake of rutin varies between 1.5 and 70 mg/kg depending on the nutritional habit of an individual (Babazadeh et al., 2017; Chua, 2013; Tagliazucchi et al., 2010). Rutin is hardly absorbed by intestinal membranes due to its glycosylated structure with a disaccharide. Rutin is extensively metabolized at the large intestine where it delivers quercetin (Bang et al., 2015), suggesting that quercetin or rutin metabolites in the colon are the responsible for quercetin-mediated pharmacological effects. The molecular mechanism involves the quercetin-mediated inhibition of the infammatory signal tumor necrosis factor alpha (TNF-alpha)-induced nuclear factor kappa B (NFkB) activation (Kim et al., 2005). Therefore, rutin reaches the colon and is hydrolysed by some probiotic bacteria to produce quercetin and rutinose and other smaller metabolites that are absorbed in the large intestine (Chua, 2013; Hosseinzadeh and Nassiri-Asl, 2014). An earlier study in which rutin and quercetin were administered orally and parenterally to female Wistar rats, showed that rutin administration, but not that of quercetin, had antiinflamatory activity in experimental ileitis and colitis in rats. However, these effects are related to the release of quercetin through the metabolism of rutin by bacterial enzymes, resulting in a prolonged exposure of the mucosa to quercetin (Mascaraque et al., 2015). On the other hand, a recent in vivo study with Wistar rats, given powdered food supplemented with rutin (100 mg rutin/kg per day) showed that rutin improves cardiovascular detriments (function and structure) and chronic kidney associated diseases (Diwan et al., 2017). Furthermore, rutin also showed potential antimicrobial activity through its biofilm inhibitory capacity of foodborne pathogens (*Escherichia coli* and *Staphylococcus aureus*) (Al-Shabib et al., 2017). Morover, rutin at a dose of 200 mg/kg has been found to act against gastric injury caused by ethanol in mice due to its high antioxidant and protective properties, which increase the activity of the antioxidant enzyme GSH px and reduce lipoperoxide levels (La Casa et al., 2000). Recently, it has also been shown that rutin administration (8 mg/kg) is a potential therapeutic option for preventing systemic insulin resistance in obese rats, improving obesity and polycystic ovary syndrome through brown adipose tissue activation in Sprague–Dawley rats (Hu et al., 2017; Wang et al., 2015). In a mureine xenograft model rutin induces in vivo and in vitro apoptosis in mureine leukemia WEHI-3, and treatment with rutin or vinblastine (120 mg/kg and 120 μg/kg of body weight, respectively) inhibits leukemia tumor growth. Therefore, rutin can be used as a preventive treatment for leukemia (Lin et al., 2012). Moreover, rutin (watercress extract and rutin alone) has an effect against osteoporosis, stimulating bone formation due to the proliferation and differentiation of osteoblastic MG-63 cells (Hyun et al., 2014). Another health effect associated with rutin is the hepatoprotective effect investigated through the attenuation of hepatic injury related to cholestasis in rats (Pan et al., 2014). Therefore, rutin is a phytochemical dietary flavonoid abundantly distributed in food plants with enormous functional properties and health effects.

NEUROPROTECTIVE EFFECT OF RUTIN

Among the previously mentioned health properties of rutin, the neuroprotective effect of this flavonoid is one of the most studied because of the increasing prevalence of neurological disorders and brain pathologies in the world's population. The brain is very susceptible to oxidative damage and its low levels of antioxidant protection convert flavonoids, such as rutin, into potential pharmacological agents due to their antioxidant properties. Moreover, inflammation and glial cell activation have been related with the onset of several neurodegenerative disorders and rutin has also important antiinflammatory effects. The neuroprotective potential of rutin in terms of its previously mentioned properties has been evaluated using different animal models and cell types. In several Alzheimer's disease models, it has been demonstrated that rutin can prevent cognitive impairment by reducing neuroinflammation (Javed et al., 2012) and also inhibiting β-amyloid aggregation

and cytotoxicity by attenuation of oxidative stress (Wang et al., 2012; Xu et al., 2014), reduction of TNF-α and IL-1β production in microglia (Wang et al., 2012), and by inhibition of β-secretase enzyme activity (Jiménez-Aliaga et al., 2011). Furthermore, the property of inhibiting neuroinflammation has also been demonstrated in brain injuries like a subarachnoid hemorrhage, in which the neuroprotective effect of rutin is based on a suppression of the RAGE-NFkB signaling pathway (Hao et al., 2016), involved in the development of many neurological diseases (Ramasamy et al., 2009). Finally, some studies have demonstrated the role of rutin in preventing neuronal death, which is the ultimate state of neurodegenerative and injury-related brain disorders. In two different models of cerebral ischemia in rats, rutin attenuated ischemic neural apoptosis (Khan et al., 2009; Pu et al., 2007). Moreover, in a 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease model rutin also slowed dopaminergic neuron death by protecting them from the deleterious effects of 6-OHDA (Khan et al., 2012), suggesting the protective effect of rutin against this neurological disorder.

FUTURE PERSPECTIVES

The successful application of different sources of rutin, such as buckwheat, for the enrichment of functional foods has good potential for the development of preventive nutrition in terms of different diseases. The discarded parts of some edible plants have a high content of rutin, thus representing a potential source for the production of rutin extracts and ingredients which could be used in the development of functional foods, drugs, and food supplements. Rutin shows good stability to thermal processing, while interactions with oxygen and metallic ions should be avoided as they can accelerate rutin oxidation. The use of stabilized rutin as part of food packaging is also promising. Research into different techniques to improve the low solubility of rutin is very important, as it would result in increased rutin release and permeability. Different techniques can be used for the stabilization of this flavonoid in order to protect it from environmental and processing conditions. This stabilization will allow the presence of rutin in the colon and thus its fermentation by the intestinal microflora, which leads to the formation of quercetin that can be readily absorbed reaching different target tissues to exert its beneficial effects. There have been reported in the literature some "in vitro" drug interactions with flavonoids, particularly with rutin, meaning that more "in vivo" research is needed in order to test the effect when flavonoid enriched food or dietary supplements are concurrently administered or consumed.

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Chapter 2.15

Curcumin-Based Food Supplements: Challenges and Future Prospects

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INTRODUCTION

Plants are a rich source for high-efficacy drug molecules. *Curcuma longa* L. (turmeric) is one of the native perennial plants in India. Curcumin, a medicinal and bioactive constituent, is derived from its rhizome. Though turmeric is a popular spice and widely used to enhance food color and flavor, it has also been known for its medicinal value and it has served as a main component in Indian Ayurveda medicine since 1900 BC.

Recent in vitro and in vivo studies have shown that its constituent curcumin has a variety of medicinal and biological properties: wound healing (Li et al., 2012a), anticancer (Ammon et al., 1992, 1993; Banerjee et al., 2003; Fujiwara et al., 1992; Joe and Lokesh, 1997, 2000; Jones et al., 2000; Lantz et al., 2005; Reddy and Lokesh, 1994; Srivastava, 1989; Xu et al., 1997), antiinflammatory (Mukhopadhyay et al., 1982; Wu, 2003), antioxidative (Sharma, 1976), hepatoprotective (Park et al., 2000), neuroprotective (Hanif et al., 1997; Jiang et al., 2007), and antimicrobial (Thaloor and Sidhu, 1998). It also plays an important role in the cure and prevention of cardiovascular disease, acute and chronic lung disease, and gastrointestinal problems (Kawamori et al., 1999; Reddy and Rao, 2002). It is also known to have a hand in the prevention (and possible treatment) of Alzheimer's disease (Adlerz et al., 2003; Baum and Ng, 2004; Calabrese et al., 2003; Cole et al., 2004, 2005; Frautschy et al., 2001; Grundman and Delaney, 2002; Kim et al., 2001, 2005; Lim et al., 2001; Ono et al., 2004; Park and Kim, 2002; Ringman et al., 2005; Yang et al., 2005) and contributes in weight loss to a certain extent (Pierro et al., 2015). Research explains that these effects are due to the regulation of biomolecules including enzymes, growth factors, inflammatory mediators, and transcription factors. It has a well-characterized ability to block the proinflammatory transcription factor, nuclear factor-kappaB (NF-κB) (Chan, 1995; Cheng et al., 2001; Gulcubuk et al., 2006; Janq et al., 2001; Limtrakul et al., 1997; Matsuda et al., 2004), inhibit activation of signal transducers (Bharti et al., 2003; Shehzad and Lee, 2013), and downregulate adipokines (Ejaz et al., 2009; Gonzales and Orlando, 2008). It can target molecular pathways without any associated resistance (Chan, 1995; Gulcubuk et al., 2006; Janq et al., 2001; Matsuda et al., 2004) and has also been shown to overcome insulin resistance (Nishizono et al., 2000; Suryanarayana et al., 2005). Curcumin has been listed as a third generation cancer chemopreventive agent by the Institute of Cancer Chemoprevention, National Cancer Institute, National Institutes of Health of the United States (Aggarwal et al., 2007; Li and Lin-Shia, 2001). Curcumin is also seen to elicit responses such as apoptosis and cell cycle arrest (Chen et al., 1999; Han et al., 1999; Jiang et al., 1996; Kuo et al., 1996; Sharma et al., 2005; Yoshino et al., 2004). It has been proved that curcumin affords a curative role against nephrotoxicity (Manikandan et al., 2011). Due to its antiinflammatory and antioxidant properties, curcumin has now been widely exploited to prevent the onset of cancer (Aggarwal et al., 2007). Studies have been performed to understand the possible anticataract mechanism of curcumin. Experiments in rodents have shown the ability of curcumin to greatly reduce and prevent the occurrence of cataractogenesis (Manikandan et al., 2010a,b). The pharmacological safety and high efficacy of curcumin gives it immense potential for the treatment and prevention of a variety of diseases. However, one of the major (perhaps the only) drawbacks in curcumin usage is its bioavailability (Anand et al., 2007; Ammon and Wahl, 1991; Sharma et al., 2001), for which a variety of strategies are being developed to address this important issue.

SOURCES AND BIOAVAILABILITY OF CURCUMIN

Curcuma longa is an herbaceous perennial plant widely grown in tropical South and Southeast Asia. The bright yellow colour of turmeric comes from the fat-soluble polyphenolic compounds called curcuminoids (Araujo and Leon, 2001).

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Curcumin (Curcumin I) is the principal curcuminoid found in turmeric and it was first described in 1910, others being demethoxy curcumin (Curcumin II) and bisdemethoxy curcumin (Curcumin III) (Sharma et al., 2001). Curcumin is a bis-α, β-unsaturated diketone, commonly called as diferuloylmethane. The hydroxyl groups present contribute to the reactive oxygen species (ROS—play an important role in oxidative stress biology) (Atsumi et al., 2005; Priyadarsini, 2013; Yoshino et al., 2004) scavenging activities and the unsaturated moieties make them reactive with biological moieties that are responsible for properties like anticancer activity. Noncovalent interactions of curcumin make reversible changes whereas covalent interactions with certain metals bring irreversible changes in its biological function. The extraction of curcumin from turmeric used to be time consuming using traditional methods like Soxhlet extraction, maceration, and hydrodistillation (Fujisawa and Kadoma, 2006). The yield was poor and the extraction also employed toxic solvents. Nowadays, these extraction issues have been overcome using different methods. This process can now be achieved by supercritical carbon dioxide extraction (Bagchi, 2012; Baumann et al., 2000), ultrasonic-assisted extraction, pressurized hot water extraction, enzyme-assisted extraction, and hot and cold percolation (Chassagnez-Mendez et al., 2000; Wakte et al., 2011). Recently, microwave-assisted extraction has also shown promising results, like faster heating of compounds, reduced thermal gradients, high extraction yields, and small equipment sizes (Kurmudle et al., 2013; Mandal et al., 2008; Setyaningsih et al., 2015; Veggi et al., 2013). This method was widely accepted as green technology due to its low consumption of organic solvents (Alupului et al., 2012; Hadi et al., 2015; Rezaei et al., 2016).

Besides having many beneficial effects, curcumin still faces an issue with its bioavailability. In 1978, for the first time Wahlstrom and Blennow reported that after oral administration of curcumin to Sprague–Dawley rats it was found in negligible amounts in the blood plasma, which was reasoned to be because of poor absorption from the gut (Sahne et al., 2016). Low intrinsic activity, instability at intestinal pH, high metabolic rate, and rapid clearance and elimination also contribute to poor bioavailability. Modern science has paved the way to overcome this. Novel drug delivery systems like liposomal encapsulation (Chen et al., 2012; Li et al., 2012b; Wahlstrom and Blennow, 1978), nanocurcumin emulsion formulation (Guzman-Villanueva et al., 2013; Kumar et al., 2012; Takahashi et al., 2009), and administration with piperine (major constituent of black pepper) (Sharma et al., 2010; Shoba et al., 1998; Suresh and Srinivasan, 2010; Zhongfa et al., 2012) and polylactic-co-glycolic acid (Anand et al., 2010; Khalil et al., 2013; Tsai et al., 2012; Yallapu et al., 2010) or cyclodextrin (Rachmawati et al., 2013; Yadav et al., 2010) encapsulations have been proved to increase absorption and uptake (Prasad et al., 2014).

CURCUMIN-BASED SUPPLEMENTS

The extraordinary chemopreventive and pharmacological effect of curcumin has made it an essential supplement for consumption in order to help prevent diseases. Curcumin supplementation comes in the form of capsules containing powder, fluid extract, or tincture. Its poor bioavailability means that it is logical to supplement it via substances that help overcome its bioavailability issue. An enzyme found in pineapple juice, bromelain, reduces swelling and inflammation. This is often combined with turmeric products to increase bioavailability and enhance antiinflammatory effects (Nagpal and Sood, 2012; Ravindranath and Chandrasekhara, 1980; Taussig and Batkin, 1998).

The most common curcumin supplements are Meriva®, Longvida®, BCM-95® (Biocurcumax™), Theracurmin®, Sabinsa's Curcumin C$^{3®}$, and MicroAactive Curcumin® (Fig. 2.15.1). *Meriva®* is composed of 20% curcumin with the rest of it being phospholipids. One ingredient, namely, phytosomes, non-genetically modified organism (GMO) soy lecithins, are plant extracts that are bound to phosphatidylcholine, which can be easily absorbed by our body hence increasing curcumin absorption (Cuomo et al., 2011). Meriva® releases the drug in a sustained pattern. Some nutrients have relatively short half-lives. To attain maximum efficacy frequent consumption is therefore required. To overcome this, sustained release of a drug can be designed. This provides a controlled release of nutrients, increases the bioavailability of nutrients, and reduces the need for frequent intake. The effectiveness of Meriva® was compared with ordinary curcumin and found to provide 5–20 times higher plasma levels. It is best used for inflammatory diseases like osteoarthritis, uveitis, rheumatoid arthritis, and inflammatory bowel disease. It also fights cancer and lowers the risk of heart disease.

Longvida® is also composed of 20% curcumin with its other constituents being primarily phospholipids (Frautschy et al., 2011). This is based on liposomal encapsulation technology and is known to work for brain diseases. It was formulated by University of California, Los Angeles neuroscientists and Verdure Sciences. Curcumin is encapsulated to protect it from hydrolysis. This technology is patented as Solid Lipid Curcumin Particle Technology (Fig. 2.15.2). Through this technology curcumin escapes from the stomach acid and dissolves in the target tissues. This formulation works in a way to cross the blood–brain barrier and is very effective in cases of dementia and Alzheimer's disease. It also takes advantage of curcumin's ability in aiding the regeneration of nerve cells. Brain-derived neurotrophic factor (BDNF) level decreases with an increase in age, which contributes to brain diseases. Curcumin has been proved to increase the levels of BDNF

FIG. 2.15.1 Examples of common and currently marketed curcumin supplements.

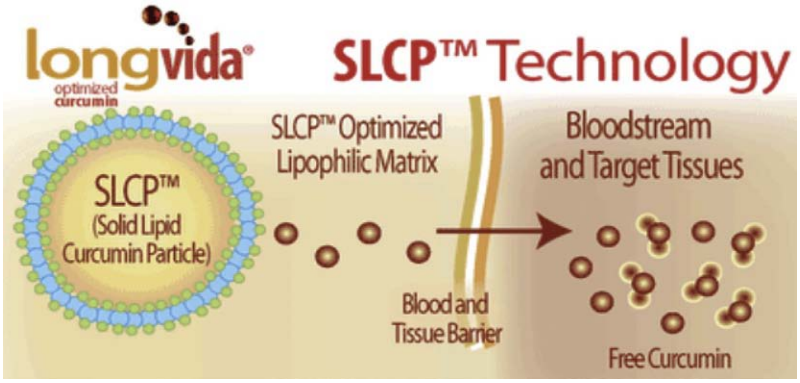

FIG. 2.15.2 Solid lipid curcumin particle technolology used in Longvida®.

thereby preventing the occurrence of such diseases. Alzheimer's disease is caused by amyloid B protein fragments which enhance oxidative stress and inflammation. Curcumin's antioxidant property plays an important role by binding with these fragments and preventing plaque formation. Longvida®, when compared to unformulated curcumin has been shown to be 65 times more bioavailable in the blood plasma (Kulkarni and Dhir, 2010; Gota et al., 2010).

BCM-95® is composed of 86% curcuminoids, including demethoxy-curcuminoid and bis-demethoxycurcuminoid along with curcumin, and 7%–9% essential oils (50% Ar-tumerone, α-tumerone, and β-tumerone and 50% Ar-curcumene, a-curcumene, zingeberene, β-sesuiphellandrine, ß-atlantone, and germacone) of turmeric. This seems to be unique in a way that it is purely derived from turmeric with no additional factors added. The essential oils are extracted by double steam distillation and play the role of phospholipids, increasing bioavailability. These oils have gastroprotective properties

and some studies have shown they give beneficial effects to patients suffering from type 2 diabetes (Funk et al., 2010; Liju et al., 2015; Negi et al., 1999). *Theracurmin*® is composed of 10% curcumin, 2% other curcuminoids (demethoxy curcuminoids), with the remainder being water (Imaizumi, 2015; Sasaki et al., 2011). This has been claimed to prevent aggregation and sedimentation even when a concentrated amount is dissolved in a solvent. *Sabinsa's Curcumin C3*® is comprised of curcuminoids and has been proved to show a reduction in serum triglycerides which indirectly reduce the risk of cardiovascular diseases, obesity, and Alzheimer's disease. This uses piperine to enhance bioavailability (Curcumin C3 complex). Piperine slows down hepatic and intestinal glucuronidation, decreasing the elimination of substances from the liver and gut. *MicroActive*® has a selfemulsifying system forming curcumin microparticles, thus making it water soluble and highly bioavailable. Absorption is further enhanced with carriers that assist its movement through the digestive tract and cell walls. This also ensures that the active nutrient is transported as such to the target site, producing a sustained release for 12 h (Madhavi and Kagan, 2014) and reducing intake requirements (http://products.mercola.com/curcumin-supplement/).

CLINICAL ASPECTS

The potential of curcumin and its supplements are mostly examined through preclinical studies, pilot studies, or epidemiological studies. Only limited clinical trials have been performed. A randomized, double-blind crossover human study involving nine people with an average age of 35 was completed by Cuomo et al. (2011). Each received either a low or high dose of Meriva® or unformulated curcuminoid. The curcuminoid absorption for Meriva® was 20 times higher than unformulated curcuminoid. The phospholipid formulation was shown to increase the absorption of demethoxylated curcuminoids, which was found to be a more potent analog in many in vitro antiinflammatory assays, than curcumin (Cuorno et al., 2011). A 3-month study on 50 osteoarthritis patients using Meriva® showed a decrease in joint pain and also an improvement in joint function (Belcaro et al., 2010a). The same was done for 100 osteoarthritis patients in order to study the long-term efficacy and safety of Meriva®. Through this study curcumin has been claimed to be safe when administered at 1.2 g/day (Belcaro et al., 2010b). Another study showed the beneficial effect of curcumin treatment on levels of protein oxidation, lipid peroxidation, and BDNF levels (Franco-Robles et al., 2014). In one single-blind, randomized trial 40 subjects of nondiabetic obese males of age 25–35 years with body mass indexes between 30 and 39.5 kg/m^2 were selected. They were randomly assigned to groups and were treated to a daily oral dosage of 500 ($n = 20$) and 750 ($n = 20$) mg of curcumin over a period of 12 weeks, without dietary restriction. BDNF levels in subjects treated with 500 mg were found to be higher in sera after 6 weeks of treatment, which after 12 weeks dropped to a basal level. For those treated with 750 mg of curcumin, BDNF was found to be similar throughout the duration of the study. Lipid peroxidation was found to increase in the group with the 500-mg treatment whereas no effect was found in the other group. Protein carbonyl levels in sera decreased in both dosages of curcumin. The study concluded that oxidation caused by obesity was decreased but the BDNF levels did not give promising results in human subjects (a study in diabetic mice showed that curcumin restored BDNF levels in the hippocampus and frontal cortex) (Franco-Robles et al., 2014). Furthermore, a 30-days placebo-controlled randomized trial revealed that a single capsule of Longvida® significantly improved markers associated with disease pathogenesis including plasma beta-amyloid and a range of inflammatory and oxidative markers (Frautschy et al., 2001). This group looked at beta-amyloid protein, which is involved in both aging and Alzheimer's disease. They found that although the decrease was only a little, it was still statistically significant. A clinical trial to test the vascular protective effects of curcumin in aging humans was done (Santos-Parker et al., 2017). The hypothesis, based on analyses using endothelium-dependent dilation (EDD) in middle-aged and older adults (45–74 years), was that curcumin significantly improves vascular endothelial function through enhancing the bioavailability of nitric oxide, which is an important vasodilatory and vascular protective gas. In this study, conduit and resistance artery EDD, was studied by both brachial artery flow mediated dilation (FMDba) and fore arm blood flow as a response to acetylcholine administration in increasing doses via the brachial artery (flow to brachial artery infusion of acetylcholine (FBFach)). This was done before and after 12 weeks of supplementation with curcumin (2000 mg/day of Longvida®; $n = 16$) or a placebo ($n = 13$). FMDba increased by 34% and FBFach increased by 44% following curcumin supplementation whereas no increase was found in the other group. This was mediated by an increase in NO bioavailability. Thus, curcumin supplementation was found to improve EDD in middle-aged or older adults and increase NO bioavailability (Santos-Parker et al., 2017). A pilot cross-over study, in human volunteers, was done to evaluate the oral bioavailability of BCM-95® (Antony et al., 2008). For this study 11 subjects with ages ranging 28–50 years were recruited. The subjects, divided into three groups, were treated with (1) BCM-95® ($n = 4$), (2) unformulated curcumin ($n = 4$), and (3) curcumin–lecithin–piperine ($n = 3$) for 2 weeks. The results showed that bioavailability was 6.93 times higher compared to normal curcumin and 6.3 times higher compared to the combination of curcumin–lecithin–piperine. The absorption of curcumin was observed to peak in the first hour and gradually drop, but residual curcumin remained in the blood even after 8 h for the group treated with BCM-95®. In case of the control curcumin, absorption was slower and it was observed to be removed from the blood after about 4.5 h (Antony et al., 2008).

A double-blind, three-way crossover study was performed to evaluate the efficiency of Theracurmin® (Sunagawa et al., 2015). The plasma curcumin level after administration of Theracurmin® was compared with that of BCM-95® and Meriva®. This study involved nine healthy subjects, divided into three groups for which each group had three types of curcumin capsule administered every 7 days. Blood specimens were obtained prior to taking the curcumin preparations and at 0.5, 1, 2, 4, 6, and 24 h after. The plasma curcumin concentration of Theracurmin® was 5.6 and 10.7 times higher than that seen with Meriva® and BCM-95®, respectively. The area under the blood concentration–time curve at 0–24 h was observed to be 4.6 times and 11 times higher with Theracurmin® when compared to both Meriva® and BCM-95®, thus demonstrating a higher absorption efficiency for Theracurmin. A randomized double-blind and placebo- controlled clinical trial with Sabinsa's Curcumin C^3 complex® was performed on dyslipidemia in obese patients (Mohammadi et al., 2013). This particular study analysed the potential of curcumin to lower lipid levels in individuals with obesity, in which 30 subjects were treated with 1 g/day. Serum profiles were analysed before and after treatment considering total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and C-reactive protein. Serum triglycerides were found to be significantly reduced after curcumin supplementation and confirmed the systemic effect of Curcumin C^3 complex®. A clinical study to compare dissolution and bioavailability of Microactive Curcumin® with unformulated curcumin was also performed. In this study 10 healthy male and female volunteers with ages ranging 21–66 years, who were not using turmeric powder or any curcumin supplements, were recruited. Blood was withdrawn after overnight fasting and stored. MicroActive Curcumin® was administered after a standard breakfast and blood samples were drawn at specific intervals. After 7 days washout period, the same was repeated with unformulated curcumin. Higher absorption at all times was seen with MicroActive Curcumin®, with T_{max} being observed at 4 h, followed by a sustained release. The plasma levels of curcumin remained high for 12 h. The area under the curve (represents the concentration of a drug in plasma against time) for MicroActive Curcumin® and unformulated curcumin was found to be highly significant. Presence of demethoxycurcumin and bisdemethoxycurcumin was found after consumption of a single dose of MicroActive Curcumin®, which was absent in unformulated curcumin (Madhavi and Kagan, 2014).

SAFETY AND TOXICITY

The use of herbs for medicinal purposes has been practiced from ancient times. However, certain herbs can trigger side effects and may interact with other supplements or medications. Blood-thinning medication like aspirin and heparin taken along with curcumin may enhance its effect resulting in increased risk of bleeding, as both inhibit platelet aggregation. Hence it is advisable for patients with such medications not to consume curcumin supplements (Choudhary and Sekhon, 2012). Piperine is used along with curcumin to enhance bioavailability by slowing down glucuronidation. However, glucuronidation is the body's natural process for eliminating toxins and metabolized drugs, thus inhibiting the adverse effects of toxins or drugs in the body. Using piperine for a long term may delay the elimination of harmful toxins in the body. People who are using other medications should avoid long-term piperine use since it can result in liver damage (Singh et al., 1986; Shoba et al., 1998; Bajad et al., 2001; Ononiwu et al., 2002; Han, 2011). People who are allergic to pineapple, wheat, or pollen might express an allergic reaction to bromelain. Curcumin supplements in high doses are known to interfere with intestinal iron absorption by binding to them (Baum and Ng, 2004; Ishihara and Sakagami, 2005; Jiao et al., 2009). An in vitro study suggested that curcumin, in high concentrations, is very capable of activating apoptotic machinery in healthy human T cells, similar to cancerous leukemia cells, suggesting that it may result in immunosuppressive side effects in theory. The possible mechanism proposed was the activation of the p53-independent apoptotic pathway (Garcea et al., 2005; Vareed et al., 2008; Ravindran et al., 2009; Korwek et al., 2013). Curcumin is also found to contract the gall bladder and thus is not recommended for gallstone patients (Rasyid et al., 2002). Limited in vitro studies show that higher doses of curcumin might lead to DNA damage (Tinq et al., 2015). In clinical trials, up to 8 g/day has been administered without any side effects except for a yellowing of the stools, which was found to stop upon supplement cessation (Cheng et al., 2001; Dhillon et al., 2008). This dose, however, cannot be said to be totally harmless to individuals who are not healthy. In the case of a clinical trial wherein cancer patients were given curcumin at doses ranging from 0.9 to 3.6 g/day for 1–4 months, the patients showed adverse side effects including nausea, diarrhoea, and an abnormal increase in the levels of serum alkaline phosphatase and lactate dehydrogenase (Sharma et al., 2004). A dose of between 500 mg/day and 12 g/day caused headaches and skin rashes (Cheng et al., 2001). Abdominal pain was noted with a dose of 8 g/day taken for 2 weeks (Carroll et al., 2011).

For a substance to be tagged safe it should carry a supportive study on long-term toxicity. Though curcumin and its supplements are claimed as nontoxic, a well-controlled, large clinical trial on its optimum dosage, without side effects, is a necessity. Nevertheless, curcumin supplements still carry many beneficial effects and they are safe if taken in recommended doses.

CONCLUSION

Curcumin proves to have extraordinary medicinal effects at appropriate doses. Recent developments have made it easy to access its benefits in the form of supplements. Consumption of plant-based supplements, namely curcumin-based supplements, under proper guidance, keeping in mind its interactions, might help us prevent, and possibly cure, many diseases. Further studies and clinical trials focusing on its side effects, interactions with other supplements, and long-term toxicity, if proved safe, may make possible the prevention and eradication of many life-threatening diseases.

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Part III

Plant and Algae Extracts

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Chapter 3.1

Açai or Brazilian Berry (*Euterpe oleracea*)

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INTRODUCTION

The açai palm tree (*Euterpe oleracea* Martius), native to the northern area of Brazil, produces a reddish-purple berry called açai, açai berry, or Amazon açai berry, which has been consumed since ancient times by native Brazilians of that region (Schauss, 2015). The name açai comes from Tupi Guarani (the native people's language) and means "fruit that cries," because during açai extraction its pulp flows slowly like tears. Nowadays, the consumption of açai as a functional food is widely used throughout the world and there is a growing market for the nutraceuticals and dietary supplements which contain açai (Portinho et al., 2012). The açai has been claimed to have a wide range of health-promoting and therapeutic benefits due to its extraordinary antioxidant and antiinflammatory properties compared to other fruits (Heinrich et al., 2011).

In this chapter, the biological effects of açai and açai-based supplements, including their antioxidant, antiinflammatory, cardioprotective, and neuroprotective properties as well as their ability to improve dyslipidemia and obesity, are highlighted by considering studies published in recent years in order to draw conclusions about their efficiency.

CHEMICAL CONSTITUENTS

Among the different chemical constituents in açai, we must note the presence of polyphenols: anthocyanins and flavonoids (Pacheco-Palencia et al., 2009). This composition is related to the beneficial effects attributed to the fruit and its subproducts. The significant content of phenolic compounds plays an important antioxidant role (Ulbricht et al., 2012). Indeed, açai has a high nutritional value and is high in energy content due to its high levels of fatty acids, carbohydrates, phytosterols, fiber, vitamins, and minerals (Portinho et al., 2012). The phytochemical and nutrient profile of freeze-dried açai was analyzed in detail by Schauss et al. (2006). The high degree of nutritional density in a 100-g sample of açai pulp is due to: total fat (32.5 g); saturated fat (8.1 g); cholesterol (13.5 mg); sodium (30.4 mg); total carbohydrate (52.2 g); dietary fiber (44.2 g), sugars (1.3 g); protein (8.1 g); vitamin A (1002 IU); vitamin C (<0.1 mg); calcium (260.0 mg), and iron (4.4 mg). Regarding polyphenols, which are important antioxidants, açai contains cyanidin 3-glucoside and cyanidin 3-rutinoside as its major antocyanins and, in minor quantities, peonidin 3-rutinoside, peonidin 3-glucoside, cyanidin 3-*O*-sambubioside, and pelargonidin 3-*O*-glucoside. Furthermore, the major flavonoids found in açai are quercetin, orientin, and its derivatives, as well as proanthocyanidins (Gordon et al., 2012).

BIOLOGICAL ACTIVITIES

There is great interest to find natural products with antioxidant capacities since there are several diseases related to oxidative stress. Mertens-Talcott et al. (2008) studied the effect of antioxidants in human volunteers submitted to an acute consumption of açai juice and pulp revealing a three times enhancement in plasma antioxidant capacity. In recent years, several studies have showed that açai modulates the antioxidant/prooxidant status (Barbosa et al., 2016; Peixoto et al., 2016; Yamaguchi et al., 2015). Indeed, açai polyphenolics are also involved in antiinflammatory activity with a downregulation of expression of proinflammatory genes and proteins investigated in human colon myofibroblastic CCD-18Co cells (Dias et al., 2015), lung inflammation induced by cigarette smoke in C57BL/6 mice (Moura et al., 2012), and human endothelial

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cells with LPS-induced inflammation (Noratto et al., 2011). Furthermore, açai possesses strong antiproliferative activity as well as having neuroprotective and anticonvulsivant effects (Hogan et al., 2010; Peixoto et al., 2016; Souza-Monteiro et al., 2015). Açai juice was found to prevent lipid peroxidation in the cerebral cortex, showing a potent direct reactive oxygen species scavenging property (Peixoto et al., 2016). These studies suggest that açai has a role in protein homeostasis and consequently attenuates neurotoxicity.

The preventive potential of açai polyphenols is also associated to lipidaemia and obesity. Martino et al. (2016) showed that açai polyphenols reduced the accumulation of intracellular lipids during adipocyte differentiation. Moreover, these polyphenols decreased the expression of proinflammatory cytokines with and without TNFα-challenge and reduced the generation of reactive oxygen species and cellular adhesion molecules. In addition, it was demonstrated that polyphenol-rich açai extract decreased food intake and body mass gain, and ameliorated both adiposity and hepatic steatosis in mice (Oliveira et al., 2015). It is known that flavonoid-rich açai meal is related to acute improvements in vascular function and cardiovascular benefits (Alqurashi et al., 2016). Furthermore, Zapata-Sudo et al. (2014) had already showed that açai extract prevented the development of exercise intolerance, cardiac hypertrophy, fibrosis, and dysfunction in myocardial infarction in rats. Açai pulp also was found to reduce the levels of selective markers of metabolic disease risk, and it was used safely for up to one month by healthy overweight subjects (Udani et al., 2011). Preclinical evidence of açai and its mechanisms of action on inflammation and weight loss are associated with its flavonoid content (Oliveira et al., 2015). In rats, açai pulp promoted the hypocholesterolaemic effect through the enhancement of expression of transporters as well as low-density lipoprotein receptor genes (De Souza et al., 2012). Açai and its subproducts present several phytochemical components especially flavonoids which could result in interactions with xenobiotics or endogenous substances in humans (Ma et al., 2014). Interactions with flavonoids and nonsteroidal antiinflammatory drugs (NSAID) on overall pharmacokinetic dispositions were evaluated in relation to the potentiated and prolonged PGE2-inhibitory antiinflammatory effect of NSAIDs by the cyclooxygenase-2 pathway, and stomach ulcer adverse effects (Fong et al., 2015). It is important to emphasize, that açai is widely available in powder and capsule form and that it is not listed on the US Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS) (Schauss, 2016). Although several scientific papers concerning the antioxidant and antiinflammatory properties of açai have been published, to date, there are no clinical reports that provide evidence of adverse effects or specific contra-indications regarding açai supplement consumption (Edwards et al., 2015).

CONCLUSION

This brief scientific chapter outlined the biological activities of açai pulp or fruit berry extract. The health benefits of açai are associated with its phytochemical composition which is rich in polyphenols. Although all cells have antioxidant systems for their protection oxidative damage, these systems are insufficient to prevent all possible damage. Therefore, it becomes important to improve our diet with a source of natural, nontoxic antioxidant. It is worth pointing out that the combination of nutritional and medicinal benefits of açai could classify it as a functional food. There is little reliable information about the safety of açai as a supplement, so recommendations are difficult to make. Thus, additional studies are required to evaluate the safety of its use.

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Chapter 3.2

Artichoke (*Cynara scolymus* L.)

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INTRODUCTION

Artichoke (*Cynara scolymus* L.) was a food very much appreciated by the ancient Greeks and Romans because of its medicinal properties and beneficial effects on health. Nowadays, there is a growing interest in the relationship between food and health. Therefore, consumers demand foods with high nutritional value and beneficial effects for the prevention of deficiencies and diseases such as: metabolic syndrome, cardiovascular diseases, and different types of cancer. Given the demand for healthy foods, the production and consumption of fruits and vegetables is increasing (El-Sohaimy, 2014; Guillen et al., 2017). The artichoke is not simply an excellent candidate for a healthy food, but it also has the added benefit that its by-products, derived from industrial processing which represents from 40% to 50% of the weight of the artichoke head, are a potential source of bioactive compounds for the elaboration of functional foods, reducing pollution and resulting in a beneficial environmental impact.

BOTANICAL DESCRIPTIONS AND CULTIVATION

The globe artichoke (*Cynara scolymus* L.), originated in Mediterranean Europe, and it is part of the Asteraceae family**.** It is an herbaceous perennial plant, robust, with vigorous growth, medium salt tolerance, marked resistance to pathogens and little insects, and highly adapted to the Mediterranean climate (Ceccarelli et al., 2010). The name "artichoke" refers to either the whole plant including the capitulum inflorescence or the edible floral head. Its botanical name derives from the Latin *cinis*, *cineris*, because of the tradition of using ash as a fertilizer, and from the Greek *skolymos*, that means "thistle" due to the thorns located in the bracts surrounding the inflorescence, as part of the edible part of the plant.

The culture of the artichoke is widely distributed throughout the world with a harvested area of 129,308 ha with a quantity of production of 1,573,363 ton in 2014. Apart from the Mediterranean region, where its cultivation is very relevant, it is also cultivated in northern Africa, southern America, the United States (mainly California), and Asian countries as China. The artichoke plays and important role in human nutrition, contributing significantly to the agricultural resources in the countries of the Mediterranean region, with Italy being the largest producer in the world with a harvested area of 46.440 ha and a total production of 451,461 ton in 2014, followed by Spain and France with productions of 234,091 ton and 38,354 ton in 2014, respectively (FAOSTAT, 2017).

CHEMICAL COMPOSITION

The artichoke capitulum contains from 15% to 20% dry matter, with a nutritional composition consisting mainly of 6.8% carbohydrates and 2.9% nitrogen compounds, with low caloric value and high fibre content. It is a source of some minerals such as potassium, calcium, and sodium, in addition to magnesium, phosphorus, iron, copper, and manganese. Moreover, it is also a source of vitamin C and folates and vitamins of the B complex (biotin, niacin, and pyridoxine) (Mataix et al., 2003; Moreiras et al., 2011).

In comparison with other vegetables, different artichoke varieties are particularly rich in inulin (19%–36% d.m.) (Lattanzio et al., 2009). The artichoke has a high antioxidant capacity (9000 μmol of trolox equivalent antioxidant capacity (TEAC)/100 g of artichoke fresh weight). Moreover, together with blueberries and soybean, the artichoke is one of the best sources of dietary antioxidant content, ranking 17th out of 50 foods in a list according to antioxidant content elaborated by Halvorsen et al. (2006), and 4th when expressed per serving. The artichoke is a natural source of phenolic

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acids (cynarine and chlorogenic acid), flavonoid derivatives (luteolin and apigenin), and xanthophylls (zeaxanthin) (Gouveia and Castilho, 2012). However, the nutritional and phytochemical compositional values can vary among due to agronomic practices, soil characteristics, climatic conditions during plant growth, artichoke species, ripeness stages, and technological processes (Tómas-Barberán and Espín, 2001; Ruiz-Cano et al., 2014).

USES OF THE ARTICHOKE

Artichoke is widely consumed either fresh or processed. There are several artichoke cultivars, with the green globe and purple globe artichoke being the most consumed, either fresh or under different preparations in the winter season or frozen throughout the year. The ratio of edible parts to the total weight of the head is 10%–18% in the lower part of the plant (receptacle), and about 40% for the core (receptacle and inner bracts) (Pandino et al., 2011). Artichoke by-products (stems, leaves, and external bracts) resulting from industrial processing (canned, fresh processed, and frozen), represent a huge amount of discarded material (45%–50%). Those by-products are rich in phenolic compounds, inulin and dietary fiber, having great potential for use in the agrofood industry. Recovery of by-products from the artichoke canning industry as a source of bioactives has been considered (Frutos et al., 2008; Ruiz-Cano et al., 2014; Ruiz-Cano et al., 2015a). The chemical composition and functional properties of artichoke by-products resulting from the canning industry have been described by Ruiz-Cano et al. (2014). Artichoke by-products have a distinct composition depending on when they are removed from industrial processing. The main factors affecting their composition are the temperature and duration of the thermal treatment they undergo (no thermal treatment, blanching at 96°C for 5 min, or boiling at 96°C for 25 min) and their origin (inner parts or external bracts of the head). The more functional fractions regarding the composition in inulin and phenolic compounds and antioxidant activity are the inner parts detached from the processed artichoke hearts. All the by-products present high dietary fiber content and low fat content. The thermal treatment increases functionality, with the boiled inner bracts presenting the highest inulin content. According to Ruiz-Cano et al. (2015a), artichoke flour made from artichoke by-products from the canning industry presents nutritional and technofunctional properties that make it suitable for the food industry as a source of minerals and biologically active chemical constituents such as fiber, inulin, and antioxidant phenolic compounds. Among the potential applications of the ingredients derived from artichoke by-products, the bakery industry could be one of the most interesting. Frutos et al. (2008) have incorporated artichoke flour from artichoke by-products, as a source of fiber (55%), into bread in concentrations (flour basis) up to 12%. The use of artichoke ingredients leads to the modification of the sensory and physicochemical properties of the bread. More significant modifications to the textural properties of bread are observed at a concentration of 9%, resulting in greater hardness and chewiness, and negatively affecting the color, taste, and overall appearance. The incorporation of 3% and 6% of artichoke flour results in a modification of the bread's sensory texture, but maintained within acceptable limits. Another product, where an ingredient rich in inulin from industrial artichoke by-products has been successfully applied, is traditional meat pies. The by-products were found to improve both nutritional and health properties by lowering energy content (15.4%) and reducing total fat, unsaturated fat, and salt (39%, 48%, and 45%, respectively), and decreasing, by 30%, the atherogenic, trombogenic, and hypercholesterolemic indexes with respect to the traditional product, while maintaining organoleptic properties (Ruiz-Cano et al., 2015b). Thus, artichoke by-products could be a source of potential food ingredients for the bakery industry, because of their content in inulin and antioxidant compounds which improve the nutritional and functional properties of bakery products. An alternative method to the direct consumption of artichoke in the diet is to process artichoke as a raw material for the extraction of bioactive carbohydrates such as inulin and inositol through microwave-assisted and pressurized-liquid extraction (PLE) (Ruiz-Aceituno et al., 2016). Processing temperature has a marked influence on PLE, with 75°C being the most suitable temperature. The extraction of inulin has also been optimized by the electromagnetic induction heating process (Terkmane et al., 2016). Inulin from artichoke agroindustrial waste has been produced through aqueous extraction procedures. López-Molina et al. (2005) have developed a process for the production of a high molecular weight fraction of inulin from artichoke. The frozen bracts are ground and after several physical steps (filtration, ultrafiltration, and ionic chromatography) in aqueous media, at 4°C, the extracts are lyophilized. Some physicochemical properties of inulin produced in this process are similar to chicory inulin but with higher average degree of polymerization, an inulin/oligofructose content of 99.5% dry weight, and a low solubility in water (5 g/L).

APPLICATIONS FOR HEALTH PROMOTION AND DISEASE PREVENTION

There are different studies in the scientific literature that report the beneficial effects to health derived from the consumption of artichoke. Inulin is a natural fructose oligomer present in artichoke with a polymerization degree that ranges between 2 and 60 U. This inulin is not digested and absorbed in the small intestine, thus it reaches the colon where it shows a prebiotic

effect due to its selective promotion of the growth of probiotic bacteria (Gibson and Roberfroid, 1995; López-Molina et al., 2005; Valero-Cases and Frutos, 2017a). Artichoke inulin can be used as a prebiotic source together with probiotic bacteria for the development of synbiotic foods. This prebiotic could improve the viability of probiotics during manufacturing, storage, and through the gastrointestinal period during in vitro digestion (Valero-Cases and Frutos, 2017a,b).

In a double-blind, placebo-controlled randomized clinical trial, the beneficial effect of artichoke leaf extract (ALE) supplementation (250 mg of standardized ALE, film-coated tablets for 8 weeks) was demonstrated with a decrease in total cholesterol and low-density lipoprotein (LDL)-cholesterol and an increase of high-density lipoprotein (HDL)-cholesterol in 92 overweight subjects with primary hypercholesterolemia (Rondanelli et al., 2013). In addition, supplementation with ALE (1800 mg ALE, four tablets per day for 12 weeks) also demonstrated the antioxidant response in metabolic syndrome (MetS) in 80 patients in a recent double-blind, placebo-controlled randomized clinical trial (Rezazadeh et al., 2018). The phenolic compounds present in ALE could make the main contribution to the antioxidant response in MetS. Previous "in vitro" studies showed a high correlation between the content of the main polyphenols in artichoke by-products (caffeoylquinic acids, luteonil 7-*O*-glucoside, leutenin 7-*O*-rutinoside, and apigenin-7-glucoside) and antioxidant capacity (Pandino et al., 2011; Ruiz-Cano et al., 2014). At the same time, polyphenol extracts from the edible parts of the artichoke (from to 2.5 to 60 μM after 10 days of treatment) have showed potential healthy in vitro effects inhibiting breast cancer cell lines, MDA-MB231, and thus acting like chemopreventive and anticancer dietary compounds. Treatment of up to 30 μM resulted in high inhibition, about 90%, of cell proliferation in viable cells (Mileo et al., 2015).

CONCLUSIONS

Artichokes are widely consumed fresh or processed, with the edible part only representing a small proportion of the artichoke head. Therefore, when considering its content in terms of bioactive compounds, mainly polyphenols, inulin, and dietary fibre, the artichoke, and its industrial by-products, represent a source of bioactive compounds with healthy properties, that can be used to develop ingredients and foods to be included as part of our diet, helping us maintain good health. However, due to limited research on the beneficial properties of the artichoke, with regards to the prevention of some degenerative diseases, further "in vitro" and "in vivo" studies are required to support the beneficial effects of the artichoke in relation to cardiovascular diseases, metabolic syndrome, and breast and colon cancers.

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Chapter 3.3

Agnus castus

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INTRODUCTION

Vitex agnus-castus L. (agnus castus) (VAC) is a shrub or small tree from belonging to the Verbenaceae family which has important medicinal properties and grows in tropical and temperate zones (Mediterranean region, Central Asia and southern Europe) (Kirmizibekmez and Demir, 2016; Pal et al., 2013).

The name "agnus castus" is originated from Latin and is derived from "castitas" (chastity) and "agnus" (lamb). This plant is also commonly known as the chaste tree, chaste berry, or monk's pepper (Daniele et al., 2005; Ahmad et al., 2010a).

The leaves are used as a spice. The fruits of VAC are popularly used as a phytomedicine in Europe for symptomatic treatment of premenstrual syndrome (PMS) and menopause, because of it is hormone-like effect (Kuruüzüm-Uz et al., 2003; Li et al., 2002; Liu et al., 2004; Stojkovic et al., 2011).

The fruits of VAC are traditionally used as an emmenagogue, sedative, anaphrodisiac, and galactagogue for relief of premenstrual complaints, menopausal symptoms, lactation disorders, and other female problems (Choudhary et al., 2009; Hajdu et al., 2007).

The fruits of VAC contain flavonoids, terpenoids, iridoid glycosides, and essential and fatty oils. The therapeutic effects of this plant have been attributed especially to its diterpenoids which have dopaminergic activity (Berger et al., 2000; Hoberg et al., 1999).

Many clinical trials have confirmed that its fruit extracts have positive effects on PMS-related psychic and somatic complaints.

VAC has been approved to be used by the German Commission E in phytopharmaceutical products for three indications: menstrual rhythm anomaly, mastodynia, and premenstrual disturbances (Daniele et al., 2005; Lauritzen et al., 1997).

GENERAL INFORMATION

Synonyms/Common Names

There are a great number of synonyms used for the common name agnus castus: abrahamsstraugh, agno-casto, agnolyt, agnus castus, angarf, banjankush, chaste berry, chaste tree berry, chaste tree, chaste tree, cyclamen, felfele barry, gattilier, hayit, hemp tree, hub-el-faked, jurema, kef-meriem, kerwa, keuschlamm, monchpfeffer, monk's pepper tree, monk's pepper, panj angosht, pepe falso, ranukabija ma, sauzatillo, sumbhalu-ke-bij albero del pepe, tree of chastity, *Vitex agnus-castus*, and *Vitex* (Dugoua et al., 2008; Heinrich, 2001; Choudhary et al., 2009; Stojkovic et al., 2011).

Botanical Description

VAC is a strongly aromatic shrub or low tree of the Verbenaceae family with densely short-puberulent branches. The leaves, 5–9 partite, are digitate and velvety. Leaflets, 5–7, are mostly unequal, the central one being the largest, the lowermost pair the smallest, the 3 largest petiolulate, the 2–4 smallest usually being sessile and narrow-elliptical in shape, the central one being 4.5–11.5 cm long and 9–21 mm wide, attenuate or acuminate at both ends, pulverulent or glabrate above. Petioles, 1.5–2.5 cm long, are densely puberulent, and are resinous–granular. Flowers are pale purple or violet, in interrupted spikes, forming several groups. Drupes are small, 4-celled, globose, and exceed the calyx (Heinrich, 2001).

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Traditional Uses

VAC is a plant native to the Mediterranean area and Asia and its ripened fruits have been used as a folk medicine since Greek antiquity for the relief of symptoms associated with obstetric and gynecological diseases including menstrual issues (amenorrhoea, dysmenorrhoea), PMS, corpus luteum insufficiency, infertility, acne, menopause, and lactation disorders (Ohyama et al., 2003; Lauritzen et al., 1997; Stojkovic et al., 2011; Loch et al., 2000).

It was indicated that Hippocrates, Dioscorides, and Theophrast mentioned this plant in their works (Daniele et al., 2005).

Traditionally, various parts of the plant have been used in different countries as an emmenagogue, sedative, anaphrodisiac, contraceptive, galactagogue, diuretic, carminative, vulnerary, carminative, anthelmintic, antiinflammatory, antispasmodic, tonic, antiflatulent, narcotic, digestive, and antifungal, being preparing in various decoctions, traditional tinctures, cider vinegar tinctures, syrups, and elixirs (Choudhary et al., 2009; Kirmizibekmez and Demir, 2016; Daniele et al., 2005; Heinrich, 2001; Kuruüzüm-Uz et al., 2003; Ahmad et al., 2010a).

It has also been reported that locally, this plant is used as an insectifuge and insecticide (Ahmad et al., 2010a).

Some commercial preparations of VAC include: Agnolyt®, Agnucaston®, Mastodynon®, Prefemin®, and Premens®.

PHYTOCHEMICAL CONSTITUENTS

Various phytochemical research has shown that different parts of VAC (fruits, leaves, and flowering stems) contain numerous second metabolites including iridoids, flavonoids, terpenoids, essential oils, and ketosteroids (Kuruüzüm-Uz et al., 2003; Kirmizibekmez and Demir, 2016).

It is known that the fruits of VAC contain flavonoids, iridoid glycosides, diterpenoids, essential and fatty oils, and a bitter component named castine (Hoberg et al., 1999; Chen et al., 2011; Daniele et al., 2005).

Ketosteroid hormones have been found in the flowers and leaves of VAC and flavonoidal compounds have been isolated from the root bark of this plant (Ahmad et al., 2010b).

It was reported that sabinene (17.8%) and 1,8-cineole (17.5%) were the main compounds in the oil of the unripe fruits of VAC, however, in the oil of ripe fruits the major compounds were 1,8-cineole (16.3%) and sabinene (13.4%). 1,8-cineole was found to comprise 22.0% of the oil from the plant's leaves (Stojkovic et al., 2011).

Some secondary metabolites are listed below, having been isolated from different parts of VAC:

- *Flavonoids*: eupatorin, casticin, penduletin, vitexin, orientin luteolin 6-*C*-(4"-methyl-6"-*O*-*trans*caffeoylglucoside), luteolin 6-*C*-(6"-*O*-*trans*caffeoylglucoside), luteolin 6-*C*-(2"-*O*-*trans*caffeoylglucoside), and luteolin 7-*O*-(6"-*p*-benzoylglucoside), together with four known ones, 4',5-dihydroxy-3,3',6,7-tetramethoxyflavone, luteolin, artemetin, isorhamnetin isoorientin, isovitexin, kaempferol 3-*O*-sophoroside, and luteolin 6-*C*-(2"-*O*-*trans*-caffeoyl) glucopyranoside (Hajdu et al., 2007; Hirobe et al., 1997; Kirmizibekmez and Demir, 2016).
- *Iridoids*: agnucastoside A, agnucastoside B, agnucastoside C, aucubin, agnuside, mussaenosidic acid, 6'-*O*-*p*-hydroxybenzoylmussaenosidic acid, agnusoside, *trans*-eurostoside (Kuruüzüm-Uz et al., 2003; Kirmizibekmez and Demir, 2016).
- *Diterpenoids*: vitetrifolin B, vitetrifolin C, rotundifuran, vitexilactone, 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene, vitexlactam A (novel labdane diterpene alkaloid), viteagnusin I, (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,16*S*)-6-acetoxy-9,13-epoxy-16-methoxy-labdan-15,16-olide, (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,16*S*)-6-acetoxy-9,13-epoxy-16-methoxy-labdan-15,16-olide, (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*)-6-acetoxy-9,13-epoxy-15-methoxy-labdan-16,15-olide, (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*)-6-acetoxy-9,13-epoxy-15-methoxy-labdan-16,15-olide, vitexilactone, viteagnusin C, 8-*epi*-sclareol, vitetrifolin D. vitexlactam, and 8-*epi*manoyl oxide (Hajdu et al., 2007; Hoberg et al., 1999; Li et al., 2002; Chen et al., 2011; Kirmizibekmez and Demir, 2016; Pal et al., 2013).
- *Others*: spathulenol (sesquiterpene), castusic acid (caffeoylquinic acid derivative), chlorogenic acid, isochlorogenic acid A (caffeoylquinic acid derivatives), 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid (phenolic acids), 2,3-dihydroxy-12-ursen-28-oic acid, 2α-hydroxyursolic acid, maslinic acid (triterpenoids), 4α,10α-dihydroxyaromadendrane (sesquiterpenoid), linoleic acid (fatty acid), castine pinnatasterone, and 17-OH-progesterone (Hajdu et al., 2007; Kirmizibekmez and Demir, 2016; Daniele et al., 2005; Ahmad et al., 2010a).

Some of these compounds are associated with different activities of VAC. For example, it has been reported that 6β, 7β-diacetoxy-13-hydroxy-labda-8,14-diene and rotundifuran have affinity to the dopamine-D2-receptor and it was also indicated that diterpenoids might carry out their activity, by way of increasing the β-endorphin plasma level besides binding to opioid receptors (Hoberg et al., 1999; Berger et al., 2000).

In a bioassay guided phytochemical study which was performed to isolate the ERβ-selective compounds in VAC, apigenin (flavonoid) was isolated and was determined to be the most active ERβ -selective ingredient. It is, therefore, thought

that the phytoestrogens in VAC are ERβ-selective (Jarry et al., 2003). Furthermore, it has also been reported that neuroactive flavonoids found in VAC extracts, may affect PMS-related mood disorders (Berger et al., 2000).

In another study, apigenin, 3-methylkaempferol, luteolin, and casticin were isolated from the fruits of VAC. It was shown that these compounds bind to delta and mu opioid receptors dose dependently (Chen et al., 2011).

6a,11a-dihydro-6H-[1] benzofuro [3,2-c][1,3]dioxolo[4,5-g]chromen-9-ol and vitexcarpan were identified in VAC. They have demonstrated urease and chymotrypsin inhibitory activity and also antiinflammatory activity in various in vitro tests (Ahmad et al., 2010a,b).

For the first time, a diterpenoid alkaloid (9α-Hydroxy-13(14)-labden-16,15-amide having an α,β-unsaturated-γ-lactam moiety) has also been isolated from VAC, and it has been determined that this diterpenoid alkaloid has potent cytotoxic activity (IC_{50} = 0.70 μg/mL) against K562 (human chronic myelogenous leukemia) cells (Pal et al., 2013).

PHARMACOLOGICAL PROPERTIES AND CLINICAL EVENTS

VAC has important biological activities and has been traditionally used to eliminate many female discomforts associated with hormonal changes such as PMS, menstrual disorders, menopause-related problems, hyperprolactinemia, sexual dysfunction, acne, etc. (Saberi et al., 2008; Kuruüzüm-Uz et al., 2003; Khalilzadeh et al., 2015).

It has been shown that different parts of VAC have estrogenic (Liu et al., 2004), antinociceptive (Khalilzadeh et al., 2015), opioidergic (Webster et al., 2011), cytotoxic, apoptosis-inducing (Ohyama et al., 2003), antiepileptic (Saberi et al., 2008), antioxidant (Hajdu et al., 2007), antifungal (Stojkovic et al., 2011), antibacterial, and phytotoxic (Ahmad et al., 2010b) activities as noted in various in vivo and in vitro experiments.

Crude methanolic extract, from above-ground parts of VAC, and fractions that were obtained from methanolic extraction by partition, were investigated in terms of hemagglutination, antibacterial, and phytotoxic activities using in vitro methods. Neither the crude extract nor the fractions have shown hemagglutination activity against human erythrocytes. However, it was determine that the $CHCl_3$ fraction has significant antibacterial activity against *Klebsiella pneumoniae* (81% with an MIC50 of 2.19 mg/mL) with *n*-hexane (69.56%) and butanol (69.56%) fractions showing significant activity (respectively, MIC_{50} of 1.8 and 1.7 mg/mL) against *Escherichia coli.* In addition good phytotoxic activity has been reported at higher concentrations of plant extracts (Ahmad et al., 2010b).

Essential oils obtained from different parts of VAC (unripe and ripe fruits and leaves of the plant) have been investigated in terms of their antimicrobial effects besides their chemical constituents. Investigation showed that 1,8-cineole (the predominant constituent of the oils) and α-pinene were found to be potent antimicrobial compounds (Stojkovic et al., 2011).

Some in vitro research revealed the cytotoxic effects of different VAC extracts against various cancer cells. An ethanolic VAC extract has been subjected to cytotoxicity tests against two noncancerous, and six cancerous, human cell lines: human uterine cervical canal fibroblast (HCF), human embryo fibroblast (HE-21), ovarian cancer (MCF-7), cervical carcinoma (SKG-3a), breast carcinoma (SKOV-3), gastric signet ring carcinoma (KATO-III), colon carcinoma (COLO 201), and small cell lung carcinoma (Lu-134-A-H) cells. As a result, it has been revealed that the cytotoxic activity of this extract changes according to the growth rate of the cells. Furthermore, death of cells with relatively low growth activity has been associated with VAC extract-induced apoptosis (Ohyama et al., 2003).

The antiproliferative effects of one VAC fruit extract were explored. The data showed that VAC fruit extract inhibits the proliferation, and induces apoptosis, in human prostate epithelial cell lines. Therefore, it has been reported that this extract may be beneficial for the prevention and/or treatment of human prostate cancer besides benign prostatic hyperplasia (Weisskopf et al., 2005).

Nowadays the fruit extract of the plant is used as a dietary supplement in the treatment of different conditions associated with estrogen hormone imbalance such as menstrual cycle irregularity and PMS, because of its hormone-like effect (Chen et al., 2011).

A bioactivity guided isolation study was performed to identify the estrogenic properties of a methanol extract obtained from the fruits of VAC using an in vitro ER-binding assay. It was found that the methanol extract showed its effect by binding to both ER-α and ER-β receptors (respectively, IC_{50}: 46 ± 3 μg/ml and 64 ± 4 μg/ml). As a result of this bioactivity guided study, linoleic acid was isolated as the active compound of the extract (Liu et al., 2004).

Extracts of VAC show their therapeutic effects through not only binding to estrogen receptors but also to opioid receptors, the histamine H_1 receptor and dopaminergic receptors. In addition the dopaminergic actions of extracts at the pituitary gland cause a decrease in prolactin secretion (Loch et al., 2000; Lauritzen et al., 1997).

Furthermore, the effectiveness of the extracts in the treatment of PMS and cycle irregularities was shown in some clinical studies including double-blind, placebo-controlled investigations (Kirmizibekmez and Demir, 2016; Hajdu et al., 2007).

VAC was tested against a placebo in a double-blind, randomized, placebo-controlled trial to alleviate symptoms associated with PMS. In this clinical trial, little difference was observed in the PMS-related symptoms treated with VAC compared to the placebo (Turner and Mills, 1993). Another study was performed to prove the efficacy and tolerability of VAC fruit (extract Ze 440) against a placebo in women with PMS. The results showed that symptoms were significantly improved in the active group which was given the extract compared with the placebo group ($P < 0.001$) (Schellenberg, 2001).

The efficacy and safety of one VAC extract (VACBNO1095, corresponding to 40 mg of the herbal drug) was investigated in a double-blind, placebo-controlled clinical trial in Chinese women with PMS-related complaints. This extract was found to be successful in relieving the symptoms of PMS. Furthermore, no serious side effects were observed (He et al., 2009).

In a randomized, controlled trial, the efficacy and tolerability of VAC was compared with that of pyridoxine in woman with premenstrual tension syndrome (PMTS). This study showed that VAC was more effective in relieving typical PMTS complaints (such as breast tenderness, oedema, inner tension, headache, constipation, and depression) comparison to pyridoxine. The study also demonstrated no serious adverse effects (Lauritzen et al., 1997).

In a placebo-controlled, randomized, double-blind study, the effect of one VAC extract containing solution (VACS) was investigated in patients with cyclical mastalgia, and was found to be effective in its treatment. However, there were side effects, but these were rare (Halaska et al., 1999).

Toxicity and Side Effects

No life-threatening side effects have been reported. Nausea, headache, gastrointestinal disturbances, menstrual disorders, acne, pruritus, and erythematous rashes were reported as the major side effects of the plant. Fatigue, hair loss, increased intraocular pressure, palpitations, polyurea, sweating, and vaginitis are the minor side effects of the plant (Daniele et al., 2005; WHO, 1999).

Drug Interactions

No interactions have been reported. However, theoretically, VAC might interfere with oral contraceptives, hormone replacement therapy, sex hormones, and dopamine agonists and antagonists because of its dopaminergic action (Daniele et al., 2005; WHO, 1999).

Contraindications

Fruits of VAC should not be used during pregnancy and breast feeding because of a lack of data about safety (Daniele et al., 2005; WHO, 1999).

CONCLUSIONS

VAC is a valuable medicinal plant and its fruits have been widely used in folk medicine for the treatment of various female reproductive system disorders. Fruits of VAC contain important compounds associated with its phytoestrogenic, dopaminergic, opioidergic, and analgesic effects. So, it is commonly used in the relief of psychic and somatic symptoms related to PMS and menopause. Beneficial effects of the plant on these symptoms have been confirmed by a significant amount of clinical research and it has been shown that VAC is also useful for the treatment of some male diseases such as prostate cancer, benign prostatic hyperplasia, and infertility. Furthermore, the fact that no life-threatening side effects have been reported so far during the use of VAC makes this plant even more striking. Therefore, VAC is a popular ingredient for a large number of pharmaceutical preparations and food supplements that are used in the symptomatic treatment of PMS and menopause. In conclusion, VAC is considered to be an effective, safe, and well-tolerated agent in the treatment of PMS. However, more extensive and longer term clinical trials may be helpful in understanding the different effects of the plant and the consequences of its long-term use.

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Chapter 3.4

Aloe vera

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ALOE VERA (*ALOE BARBADENSIS* MILLER)

Aloe vera, a member of the Liliaceae family, is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. The *Aloe vera* leaves are formed by a thick epidermis (skin) covered with cuticles surrounding the mesophyll, which can be differentiated into chlorenchyma cells and thinner walled cells forming the parenchyma (Femenia et al., 1999). The parenchyma makes up the majority of the leaf by volume containing the *Aloe vera* gel, synonymous with the inner leaf, inner leaf fillet, or *Aloe* fillet (Boudreau and Beland, 2006; Guo and Mei, 2016).

Aloe vera gel consists of about 98.5%–99.5% water with the remaining solids containing more than 200 different components, polysaccharides being the most abundant compounds (Femenia et al., 1999). Other interesting chemical compounds such as soluble sugars, glycoproteins, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, essential, and nonessential amino acids, sterols, saponins, and vitamins, have also been identified (Eshun and He, 2004; Rodríguez et al., 2010).

It is important to highlight that *Aloe vera* has enjoyed a long history of providing a myriad of health benefits, being one of the herbal remedies most frequently used in the treatment of different diseases, which have been associated mainly to polysaccharides and phenolic compounds, the main bioactive components present in *Aloe vera* (Guo and Mei, 2016; Minjares-Fuentes et al., 2016; Pothuraju et al., 2016). Nevertheless, the geographic location (including soil and climate), growth periods, horticultural conditions, and postharvest treatments might play a critical role determining the compositional and structural features of the main bioactive compounds from *Aloe vera*, which in turn might result in the modification of its beneficial effects (Ray and Aswatha, 2013; Rodríguez et al., 2010; Rodríguez-González et al., 2012; Yaron, 1993). Thus, this chapter summarizes not only the most relevant scientific information related to the main bioactive components from *Aloe vera* but also the current use of *Aloe vera* gel as a food supplement. Finally, the most relevant scientific evidence of the beneficial effects of *Aloe vera* on health has also been revised.

BIOACTIVE COMPOUNDS IN *ALOE VERA*

In the last two decades, several scientific reports have been published based on the different bioactive compounds present in *Aloe vera*. In most of these studies related to the beneficial properties attributed to *Aloe vera*, bioactive polysaccharides, in particular the storage polymer known as acemannan, and different phenolic compounds, seem to be the key components to explain most of the pharmacological properties attributed to the *Aloe vera* plant.

Acemannan

Acemannan, commercially known as Carrisyn, is the storage polysaccharide located within the protoplast of the parenchymatous cells of *Aloe vera* leaves (Femenia et al., 1999). According to the scientific literature, acemannan is mainly composed of a main single-chain backbone of partially acetylated mannose units (>60%) and glucose (∼20%) with side chains formed by galactose (<10%) units attached to C-6 of mannose (Chokboribal et al., 2015; Chow et al., 2005; Femenia et al., 1999; Kim, 2006; Rodríguez-González et al., 2011; Talmadge et al., 2004). Acetylation may occur at the C-2, C-3, and C-6 of mannose residues with an acetyl:mannose ratio of approximately 1:1 or even higher (Fogleman et al., 1992; Hamman, 2008; Manna and McAnalley, 1993; McAnalley, 1993; Talmadge et al., 2004; Simões et al., 2012). Structurally, these acetyl groups are the only nonsugar functional groups present in acemannan and seem to play a key role in the physicochemical properties and biological activity of *Aloe vera* (Campestrini et al., 2013; Chokboribal et al., 2015;

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Ni et al., 2004b). In general, the molecular weight (MW) of this polysaccharide might range from 30 to 45 kDa, although high MWs have also been reported (>200 kDa) (Chokboribal et al., 2015; Femenia et al., 2003; Im et al., 2005; Turner et al., 2004).

Pectic Polysaccharides

Apart from acemannan, pectins are the most abundant polysaccharide present in *Aloe vera* gel. Opposite to the storage acemannan polymer, pectins are an important component of the cell walls of the parenchyma (Femenia et al., 1999). *Aloe vera* pectins contain a very high percentage of galacturonic acid residues, usually higher than 95% with less than 5% of neutral sugars. This is indicative of a structure primarily comprised of long galacturonic acid blocks with very few neutral sugar branches (Femenia et al., 1999; Mandal and Das, 1980; McConaughy et al., 2008a,b; Ni et al., 2004a).

The MW of pectins from *Aloe vera* gel ranges from 200 to 523 kDa, although low MWs have also been observed (Gentilini et al., 2014; McConaughy et al., 2008a,b). In addition, *Aloe vera* pectins exhibit a low degree of methylesterification, ranging from 2% to 20% (Gentilini et al., 2014; McConaughy et al., 2008b).

Phenolic Compounds

Most of the phenolic compounds of *Aloe vera* are found in the exudate or latex, which is distributed within the vascular bundles located between the plants outer skin (rind) and the pulp. Usually, the exudate exhibits a yellow-brownish color and has a bitter taste (Guo and Mei, 2016). Approximately 80 chemical constituents have been isolated from the exudate, by liquid chromatography, with anthraquinone C-glycosides, anthrones, chromones, phenyl pyrones, and naphthalene derivatives being the most abundant phenolic compounds (Eshun and He, 2004; Guo and Mei, 2016; Lee et al., 2012; Park and Kwon, 2006; Wu et al., 2013).

The anthrone C-glycosides are considered typical constituents of the *Aloe* leaf exudate although they do not, in fact, occur in all species (Kanama et al., 2015). Where they do occur, they are usually the main component of the exudate and are mostly represented by aloin. This compound has been found in the majority of *Aloe* species at levels from 0.1% to 6.6% of the leaf dry weight, representing between 3% and 35% of the total exudate (Kanama et al., 2015; Reynolds, 1985). Aloin appears to be exclusive to the leaf exudate, which is also the bitter principle of most of the *Aloe vera*-based pharmaceutical products, and was characterized as the C-glycoside of aloe-emodin anthrone (Reynolds, 1985, 2004). On the other hand, most of the chromones so far described from *Aloe vera* leaf exudate are derivatives of the chemical compound 8-C-glucosyl-7-hydroxy-5-methyl-2-propyl-4-chromone (Chen et al., 2012; Chinchilla et al., 2013). Variation arises from the degree of oxidation in the propyl side chain, methylation of the hydroxyl group on C-7, and esterification of the glucose moiety (Chen et al., 2012; Kanama et al., 2015; Machado and Marques, 2010; Reynolds, 2004; Sun et al., 2016; Zhong et al., 2014). It is noteworthy that C-glycosylated compounds are found to represent a class of naturally occurring secondary metabolites that are known to be unique compounds of the *Aloe* genus, not having been reported in other plants (Hutter et al., 1996; Wu et al., 2013).

Although these components are highly valorized in the pharmaceutical industry, they are considered as contaminants in *Aloe vera*-based food products. In fact, in European countries, the aloin content regulation limit is 0.1 ppm in food and beverages, whereas the International *Aloe vera* Science Council (IASC) recommend an aloin concentration lower than 10 ppm in a 0.5% *Aloe vera* solids solution for oral consumption (Javed and Atta-Ur-Rahman, 2014).

ALOE VERA IN FOODS

With the recent resurgence of herbal products as part of "the green movement," *Aloe vera* has been witnessing a new renaissance, representing an opportunity to open up new ranges of products with significant added value and high acceptance by consumers demanding a healthier lifestyle (Guo and Mei, 2016; Javed and Atta-Ur-Rahman, 2014; Vega-Gálvez et al., 2011). This movement has increased the interest in *Aloe vera* worldwide as a valuable source of many functional ingredients, in particular those used for the preparation of healthy food drinks and other beverages (Christaki and Florou-Paneri, 2010).

For the preparation of *Aloe vera* extracts different processes are usually involved; in particular pasteurization, to obtain *Aloe vera* juice, and dehydration, to produce *Aloe vera* powders, are probably the most applied procedures by the *Aloe vera* industry (Ahlawat and Khatkar, 2011; He et al., 2005; Ramachandra and Rao, 2008). Although *Aloe vera* juice and powders can be consumed directly or mixed with fruit juices, they are commonly used as raw materials for manufacturing many food products based on *Aloe vera*.

The potential use of *Aloe vera* in the food industry is mainly due to its beneficial properties, such as improvement to the immune system, protection against some types of cancer, reduction of glycemia, lipidemia, and cholesterolemia in diabetics, among others (Sánchez-Machado et al., 2017). However, it should be taken into consideration that processing may affect the original structure of the different bioactive components present in *Aloe vera*; which in turn may lead to considerable changes, not only in their physicochemical characteristics but also in their physiological and pharmacological properties (Minjares-Fuentes and Femenia, 2016; Minjares-Fuentes et al., 2016).

BENEFICIAL EFFECTS OF *ALOE VERA* ON HUMAN HEALTH

In the last decades, several authors have associated most of the beneficial properties of *Aloe vera* gel to the acetylated polysaccharide acemannan present in the gel (Choi and Chung, 2003; Ni et al., 2004a; Pothuraju et al., 2016; Radha and Laxmipriya, 2015). However, numerous studies have also reported that other compounds, such as aloin and aloe-emodin, could also exert some benefits on health (Hutter et al., 1996; Lee et al., 2000; Lucini et al., 2015; Park et al., 2011; Sun et al., 2016; Zhong et al., 2013).

These premises have led to the publication of numerous in vitro and in vivo studies, as well as clinical trials, with the aim of gaining more insight into the potential effects of all these *Aloe vera* bioactive compounds. Thus, the most relevant scientific information about the main effects of the bioactive components, whether isolated or not, of *Aloe vera* as food supplements on the treatment of different disorders or pathologies have been summarized in this section.

Anticarcinogenic and Chemoprotective Effects

In recent years, several authors have investigated the effects of *Aloe vera* administration as a preventive treatment for different types of cancer (Im et al., 2016; Lin et al., 2011; Masaldan and Iyer, 2014; Pan et al., 2013). However, possible carcinogenic activity has been reported in connection with the administration of whole-leaf extracts from *Aloe vera* in rats, showing that long-term exposure of rats to the *Aloe vera* whole-leaf extract induced an injury to the intestinal tract and caused a progression in lesion types from goblet cell hyperplasia to mucosal hyperplasia to adenoma and carcinoma in the large intestine (Boudreau et al., 2013). On the contrary, a recent study, assessing the effect of processed *Aloe vera* gel (PAG) on colon carcinogenesis, by an azoxymethane (AOM)-initiated and dextran sodium sulfate (DSS)-promoted mouse colon carcinogenesis, showed that the oral administration of PAG suppressed colitis-related colon carcinogenesis in an AOM/DSS animal models (Im et al., 2016). In fact, the suppression of colitis-related colon carcinogenesis by the oral administration of PAG has been associated to the inhibition of chronic inflammation and cell cycle progression in the colon (Im et al., 2016).

Recently, the remarkable potential of aloin in terms of its therapeutic options against cancer have also been reported, showing chemoprotective effects against 1,2-dimethylhydrazine-induced preneoplastic lesions in the colon of Wistar rats (Boudreau et al., 2013; Hamiza et al., 2014). Particularly, it was observed that aloin was able to inhibit the secretion of vascular endothelial growth factor (VEGF) in cancer cells, causing the inhibition of proliferation and migration of endothelial cells. VEGF is one of the most important proangiogenic cytokines known and well characterized as an inducer of tumor neovascularization (Pan et al., 2013). On the other hand, aloe-emodin has also demonstrated inhibition of colon cancer cell migration by reducing the DNA binding activity of the nuclear factor K-light-chain-enhancer of activated B cells (Suboj et al., 2012), avoiding the proliferation of some types of cancer cells, such as lung, squamous, glioma, and neuroectodermal (Lin et al., 2011; Masaldan and Iyer, 2014).

Antibacterial and Antiviral Activity

Aloe vera polysaccharides have also been related to direct bacterial activity through phagocytic leukocyte stimulation, which destroy bacteria (Sánchez-Machado et al., 2017). Interestingly, in vitro studies testing multiresistant strains of *Helicobacter pylori* have showed that concentrations of *Aloe vera* gel no greater than 100 mg/mL promoted the inhibition of bacterium (Cellini et al., 2014; Wang et al., 1998). Particularly, the inhibition of *H. pylori* by *Aloe vera* has been associated, on the one hand, to the antiadhesive effect of *Aloe vera* (Cellini et al., 2014) and, on the other hand, to the inhibition of the *N*-acetyltransferase activity of *H. pylori* (Wang et al., 1998).

Further, *Aloe vera* gel has also showed potential antiviral activity against herpes simplex virus (HSV) type 2 strains (Zandi et al., 2007). In this case, *Aloe* crude extracts applied at a high concentration (700 μg/mL) demonstrated inhibition of the cytopathic effect caused by the HSV-2 replication in Vero cells. Particularly, it was observed that aloe-emodin was able to inhibit the influenza A virus, reducing the virus-induced cytopathic effect and avoiding the replication of influenza A (Li et al., 2014).

Antiinflammatory and Immunomodulatory Effect

Commonly, *Aloe vera* has been widely associated to wound healing and to the inhibition of inflammation. Particularly, Arosio et al. (2000) observed that aloe-emodin was able to abolish the transcription of the albumin gene in rats. Transcription levels of albumin and tumor necrosis factor-α genes are involved in the early phase of acute inflammatory response. Tumor necrosis factor-α was weakly detectable in protected livers after aloe-emodin administration. Further, histological analysis showed a reduced inflammatory infiltration on lymphocytes and Kuffer cells observed in rats treated with aloe-emodin.

Furthermore, several in vitro studies have showed that modified *Aloe vera* polysaccharides, with MWs ranging from 5 to 400 kDa, as well as aloin and aloe-emodin, were able to increase phagocytic and proliferative activity by inhibiting the cyclooxygenase pathways and reducing prostaglandin E2 production, which plays a key role in inflammation (Im et al., 2005; Park et al., 2009). Particularly, the acemannan polysaccharide has been considered as the main responsible of the immunomodulatory properties exhibited by *Aloe vera* for macrophages and monocytes, with a minimal systemic toxicity following intraperitoneal or intravenous administration (Chantarawaratit et al., 2014; Guo and Mei, 2016; Jettanacheawchankit et al., 2009; Karaca et al., 1995; Kumar and Tiku, 2016; Talmadge et al., 2004). The studies suggested that acetylated mannose units are responsible for the activation of the hematopoietic cells, resulting in the immunostimulatory activity of acemannan (Lee et al., 2001; Reynolds and Dweck, 1999). However, the exact mechanism of action of acemannan has not yet been elucidated (Kumar and Tiku, 2016).

Antioxidant Effect

Interestingly, whole-leaf *Aloe vera* extracts, mainly containing polysaccharides and flavonoids, have demonstrated a radical scavenging potential comparable to that of the synthetic antioxidant butylated hydroxytoluene (BHT) (Choi and Chung, 2003; Hu et al., 2003). A study carried out by Kaithwas et al. (2011) showed that the oral administration of *Aloe vera* gel (100 and 200 mg/kg) for 10 days produced a significant protection against cardiotoxicity induced by doxorubicin (anthracycline anticancer drug) which was evidenced by a significant reduction of serum lactate dehydrogenase, serum creatine phosphokinase, cardiac lipid peroxides, tissue catalase, and tissue superoxide dismutase, along with increased levels of blood and tissue glutathione. More recently, the same research group observed that *Aloe vera* polysaccharides have the ability to reduce the DPPH (2,2-diphenyl-1picrylhydrazyl) radical to the corresponding hydrazine by converting unpaired electrons to paired ones (Kaithwas et al., 2014). Moreover, in vivo assays demonstrated that doxorubicin and its metabolites produced free radical species which attacked lipid components, leading to lipid peroxidation, and coadministration of *Aloe vera* polysaccharides significantly prevented an increase in the levels of thiobarbituric acid reactive substances in doxorubicin-treated animals, which was comparable to standard vitamin E (Kaithwas et al., 2014). Interestingly, the antioxidant activity of *Aloe vera* polysaccharides was reported to be dose-dependent (Kaithwas et al., 2011, 2014). Nevertheless, the potential free radical scavenging mechanism of *Aloe vera* polysaccharides is poorly understood, something which could be attributed to the huge structural diversity of these polysaccharides, resulting in a major hindrance to the establishment of a structure–activity relationship (Kaithwas et al., 2014).

Hypoglycemic and Hypolipidemic Effect

Recent studies have revealed that treatment with *Aloe vera* promotes a significant reduction in blood glucose level and blood pressure, improving the lipid profile in diabetic patients (Alinejad-Mofrad et al., 2015; Choudhary et al., 2011; Huseini et al., 2012; Pothuraju et al., 2016; Sood et al., 2008). In streptozotocin (STZ)-induced diabetic rats, it was observed that *Aloe vera* extract had a similar effect on blood glucose level to that of glibenclamide, an oral hypoglycemic drug commonly prescribed for the treatment of diabetes mellitus (Rajasekaran et al., 2007). In patients who did not respond to glibenclamide alone, it was found that consumption of *Aloe vera* extract for 2 weeks promoted a rapid reduction of blood glucose level (Bunyapraphatsara et al., 1996). Moreover, other studies have also shown the effectiveness of *Aloe vera* extract on the regulation of blood glucose level in diabetic animals (Kim et al., 2009; Rajasekaran and Sathishsekar, 2007). However, a rise in blood sugar levels after consumption of *Aloe vera* extract has also been observed (Koo, 1994), which could be related to the use of different parts of the plant than the gel (Alinejad-Mofrad et al., 2015).

Interestingly, clinical studies have demonstrated that *Aloe vera* gel in powder form improved glycemic control and lowered the blood levels of total cholesterol and low-density lipoprotein (LDL), but did not affect the other blood lipid levels and did not cause any hepatic, renal, or other adverse effects in hyperlipidemic type 2 diabetic patients (Huseini et al., 2012). Moreover, the oral administration of capsules of *Aloe vera* powder (300 mg), twice a day, promoted a significant decrease in the fasting blood glucose level in patients after week 4, whereas the HbA1C level had a significant decrease 8 weeks after

intervention. On the other hand, the administration of capsules of 500 mg of *Aloe vera*, twice a day, reduced the levels of total cholesterol and LDL-D whereas the high-density lipoprotein (HDL)-C level was improved just after 8 weeks of treatment. Interestingly, the triglyceride level showed a significant decrease after 4 weeks with an intake of 500 mg of *Aloe vera* (Alinejad-Mofrad et al., 2015). Thus, *Aloe vera* gel seems to be a safe antihyperglycemic and antihypercholesterolemic agent for hyperlipidemic type 2 diabetic patients whereas in prediabetic patients could revert impaired blood glucose within 4 weeks, but after 8 weeks could alleviate the abnormal lipid profile (Alinejad-Mofrad et al., 2015; Huseini et al., 2012).

Several authors have suggested that the hypoglycemic effect has been attributed to the high molecular weight fractions of acemannan which are degraded by the intestinal microbiota to form oligosaccharides that inhibit intestinal glucose absorption (Boban et al., 2006; Jain et al., 2007; Yagi et al., 2001, 2009). Interestingly, Misawa et al. (2012) proposed that *Aloe vera* suppresses the expressions of gluconeogenic and lipogenic enzymes and increases the levels of enzymes related to glycolysis and lipolysis in the livers of Zucker diabetic fatty rats. However, further studies are required in order to gain more information about the bioactive compounds from *Aloe vera* and, also, about the mechanism involved in the regulation of the blood glucose and lipid levels.

Intestinal Absorption and Purgative Action

It is well known that aloin and other hydroxyanthracene derivatives are the main factors responsible for the laxative effect of *Aloe vera* (Javed and Atta-Ur-Rahman, 2014). Aloin can be metabolized by the colonic flora to the more reactive aloe-emodin; and this last compound is the main component responsible for the purgative activity of *Aloe vera*. Particularly, it has been observed that the administration of aloe-emodin and emodin directly into the caecum acted synergistically with rhein (the main anthraquinone present in *Rheum palmatum* L.) exerting a potential purgative effect on mice (Yagi and Yamauchi, 1999). On the other hand, the gel and whole-leaf extracts from three species of *Aloe* have demonstrated their ability to increase drug permeability across the excised rat intestinal tissue (Beneke et al., 2013). This has been attributed to the opening of tight junctions by *Aloe vera* gel and precipitated polysaccharides since *Aloe vera,* and in particular the acemannan polysaccharide, has showed the capacity to reduce the transepithelial electrical resistance of the Caco-2 monolayer, linked to the ability to open tight junctions between adjacent cells, enhancing the transport of insulin across the cell monolayers (Chen et al., 2009).

CONCLUSIONS

There is no doubt that *Aloe vera* is, probably, one of the most important herbs used in traditional medicine mainly due to the beneficial effects associated to its bioactive compounds. Although initially *Aloe vera* was mainly used in the cosmetic field, nowadays its use as an ingredient of many food products has increased exponentially. In fact, clinical studies have demonstrated that the intake of *Aloe vera* could prevent or reduce the symptoms of different pathologies or disorders, such as diabetes. These beneficial effects have been found to be dose-dependent. Nevertheless, most of these positive effects have been tested by administration of unprocessed *Aloe vera*, either crude extracts or isolated components, while only a few studies have been performed using processed *Aloe vera*. Therefore, there is an urgent need to carry out rigorous scientific studies to assess the potential beneficial effects of the intake of processed *Aloe vera* since most of the current foodstuffs based on *Aloe vera*, which are actually sold in the market in many countries worldwide, contain *Aloe vera* extracts which have been industrially processed.

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Chapter 3.5

Beta vulgaris L.

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INTRODUCTION

Beta vulgaris is an edible plant of the Amaranthaceae family. Its common name is beet and it is distributed throughout Asia Minor, the Mediterranean, and Europe (Kumar et al., 2016). Beets have been used in folk medicines all over the world for different reasons. From the past to the present, the consumption of vegetables and fruits has gained great importance to people in terms of their overall health. A lot of research has indicated that increasing consumption of plant foods like beetroot reduce the risk of obesity, diabetes mellitus, and cardiovascular disease (Kumar et al., 2016). The plant demonstrates benefits for cancer treatment (Chevallier, 1996) and protection against heart disease (Vinson et al., 1998). Ingestion of beetroot, a natural source of nitrate, increases the availability of nitric oxide (NO) as a potential strategy for managing diseases in which NO bioavailability has diminished, like hypertension and endothelial function (Clifford et al., 2015).

Beet contains water-soluble nitrogenous pigments called betalains. They are immonium derivatives of betalamic acid and are divided in two main groups, the red–violet (betacyanins) and yellow (betaxanthins) pigments that have been widely used in food products as natural colorants. More than 80% of all the pigments in red beetroot are composed of betacyanins, mainly betanin and its isomer, whereas vulgaxanthin I represents the predominant betaxanthin (yellow pigment) (Liu et al., 2008). Betalains and anthocyanins have never been found together in the same plant species. The average value of betalains in red beetroot was calculated to be around 1000 mg/100 g of total solids, or 120 mg/100 g fresh weight (Marmion, 1991). Some of the edible and familiar sources of betalains are beetroot (*B. vulgaris* L. ssp. *vulgaris*) and Swiss chard (*B. vulgaris* L. ssp. *cicla*).

Previous in vitro and in vivo studies proved that betalains demonstrate antioxidant and antiinflammatory activities (Vulić et al., 2014). Betalains demonstrate antimicrobial, antiviral, and free radical scavenging activities, as well as inhibiting lipid peroxidation and exhibiting hepatoprotective properties (Delgado-Vargas et al., 2000; Elbe et al., 1974; Georgiev et al., 2010; Jha and Gupta, 2016; Strack et al., 2003; Tesoriere et al., 2003).

PHYTOCHEMICAL CONTENT OF BEETROOT

Betaine is an amino acid generally known as TMG. It is found in a wide range of animals, herbs, and microorganisms. The main role of betaine in herbs is the conservation of cells through osmotic inactivation by adjusting the intracellular osmotic balance. *B. vulgaris* is a rich source of betaine and this compound displays physiological advantages like reducing plasma homocysteine levels, protecting the liver from toxins, and adjusting the osmolyte function of the kidneys. The hepatoprotective effect of the plant occurs through and improvement in antioxidant status and an increase of expression of the phase II enzyme, quinone reductase (Szaefer et al., 2014).

Mroczek and his colleagues determined the triterpene saponin composition in the roots of *B. vulgaris* cultivars. Results of the study indicated that the roots of all the cultivars tested contained 11 saponins consisting of oleanolic acid or hederagenin aglycone. In another study, the bark and pith of the roots were analyzed for their saponin content. The skin and flesh of the roots was found to contain 39 and 37 saponins, respectively. All detected compounds were in their glycoside form.

Apart from betalains, other bioactive compounds were found in the plant such as carotenoids, carbohydrates, phenolics (flavonoids), phytosterols, and amino acids (Georgiev et al., 2010).

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An important and versatile nutrient in beetroot, called choline, has been found to aid sleep, muscle movement, memory, and learning. The compound also prominently assists in the maintenance of cellular membrane structure, transmission of nerve impulses, absorption of fat, and reduction of chronic inflammation (Kumar et al., 2016).

BIOSYNTHESIS PATHWAY OF BETALAINS

Betalains are one of the most prevalent plant pigments alongside chlorophylls, carotenoids, and anthocyanins. The biosynthesis pathway of betalains begins with conversion of L-tyrosine into L-l-3,4-dihydroxyphenylalanine (DOPA) by a hydroxylation enzyme (Fig. 3.5.1). Next, two key enzymes CYP76AD1 and DODA (4,5-dioxygenase) catalyze the conversion of L-DOPA to cyclo-DOPA on the one hand and betalamic acid (compounds with positive nitrogen in a polyene system) on the other hand. Betalamic acid can conjugate with amino acids and amines to produce betaxanthins (yellow pigment) or automatically cyclize with cyclo-DOPA to give betalacyanin (red pigment). The betalaine group comprises around 50 red and 20 yellow pigments (Gandía-Herrero and García-Carmona, 2013).

BETALAINE EXTRACTION

Betalains can be extracted using different methods like ultrafiltration (Roy et al., 2004), solid–liquid extraction (Sturzoiu et al., 2011), diffusion extraction (Wiley and Lee, 1978), and reverse osmosis (Lee et al., 1982). In most cases, pigments can be extracted using water but ethanol solutions also may be useful for efficient extraction (Delgado-Vargas et al., 2000). The application of ethanol–HCl has even been reported as a means to extract the compounds (Barrera et al., 1998). Use of a small volume of acid, like ascorbic acid, in the extraction medium improves betacyanin stability and inhibits oxidation by Polyphenoloxidases (PPOs) (Schliemann et al., 1999; Strack et al., 2003).

BIOAVAILABILITY OF BETALAINS

For a food component the grade and rate at which the active compound (drug) is absorbed into a biological system, or made accessible at the site of physiological activity, must be clear (Toutain and Bousquet-Melou, 2004). In this regard, the bioavailability of the two main bioactive components of *B. vulgaris* including the betalains and inorganic nitrate has been considered in the literature. Human consumption of cooked beetroot demonstrated that the bioavailability of inorganic nitrate is around 100% (106% ± 15%) (van Velzen et al., 2008). In another study, a single oral dose of red beet juice, containing 362.7 mg of betalains, was given to six healthy volunteers. Urine was collected and assessed by spectrophotometer. Betanin and isobetanin were identified at concentrations 0.28% of the ingested dose after 24 h (Frank et al., 2005).

ANTIOXIDANT ACTIVITY OF BEETROOT

Betalain pigments have been recently categorized as a class of antioxidants. Many published research studies show the strong antioxidant properties of beetroot, attributed to its phenolic compounds. Determination of the total content of phenolics and flavonoids, using the Folin–Ciocalteau and aluminum chloride colorimetric methods, showed that the content of polyphenols and total flavonoids in the plant are 660.5 and 29.04 mg/g of sample, respectively (Kapur et al., 2012). In another in vitro study, an ethanolic extract of *B. vulgaris* was tested for its antioxidant capacity using the DPPH radical-scavenging method with the results compared with ascorbic acid as a reference standard. The results revealed the highest antioxidant capacity (90.9%) at concentrations of 500 and 1000 µg/ml (El Gamal et al., 2014).

BEETROOT'S CYTOTOXICITY AND ANTICANCER EFFECT

The beetroot is a highly nutritious food source. There are several reports about the benefits of betalains. In an experiment by Kapadia et al. (2011) beetroot was used by two groups of patients who suffer from cancer. It was found that the extract of beetroot displayed cytotoxic activity in androgen-independent human PC3 and estrogen receptor (ER)-positive human breast cancer cells. In addition, a synergistic antiproliferative effect was observed in breast and prostate cancer cell lines when treated with a mixture of an anticancer drug (doxorubicin) and red beetroot extract (Kapadia et al., 2013). It has been indicated that two major pigment constituents of beetroot, betanin, and betalain, are probably responsible for the cytotoxic activity. Another investigation reported the antitumor activity of red beetroot extract. Oral consumption of water containing 78 mg/ml of extract showed inhibitory activity against *N*-nitrosomethylbenzylamine (NMBA)-induced tumors in the esophagus of rats. Moreover, the plant extract improved apoptosis and decreased angiogenesis and inflammation in cancerous cells in rats (Lechner et al., 2010).

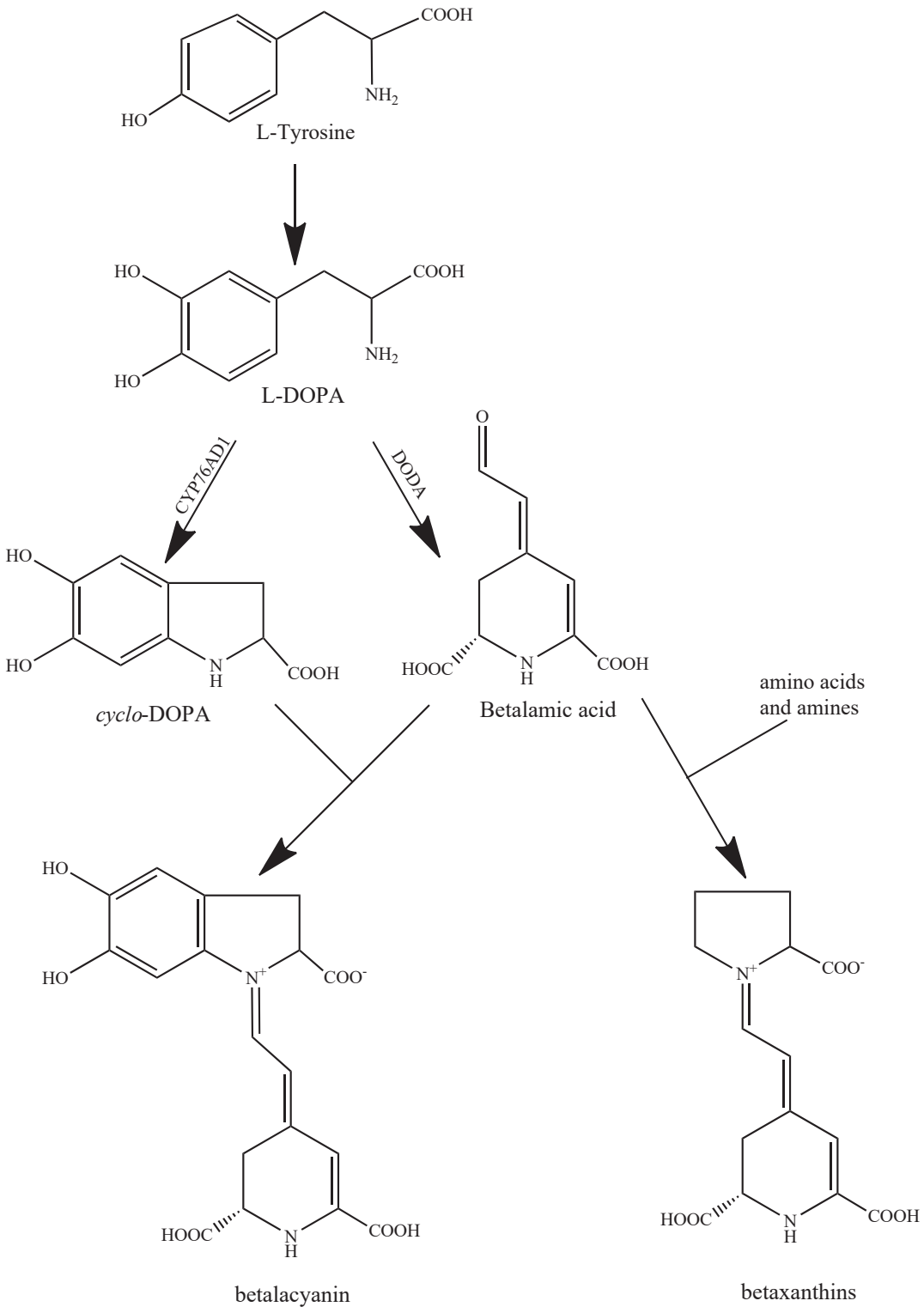

FIG. 3.5.1 Biosynthesis pathway of betalains.

BEETROOT'S EFFECT ON HYPERTENSION

High blood pressure is a common public health problem and is a major cause of heart disease. Recent studies have indicated that consumption of beetroot as a juice supplement or in bread products have a decreasing effect on systolic and diastolic blood pressure (Bailey et al., 2009; Hobbs et al., 2013a,b; Hobbs et al., 2012; Jajja et al., 2014; Webb et al., 2008).

BEETROOT'S EFFECT ON TYPE 2 DIABETES

Type 2 diabetes mellitus is the most common type of diabetes that occurs because of insulin resistance and a relative deficiency of insulin secretion. Several changes arise following diabetes in most parts of the body, like obesity and organ damage (Kumar et al., 2014). Kumar et al. (2014) tested beetroot extract in diabetic rats; the first group was treated with beet ethanolic extract and the second group with glibenclamide, an antidiabetic medicine. The results of the experiment showed that the ethanolic extract of beetroot has a potential antidiabetic activity as seen in diabetic rats and significantly reduced the levels of blood sugar, serum lipid, and urinary sugar with ketone bodies (Kumar et al., 2016). Administration of red beet juice, containing high neobetanin (1.3 g/225 mL) significantly decreased both postprandial insulin response in its early phase (0–60 min) and glucose response in the 0–30 min phase in human volunteers (Wootton-Beard et al., 2014).

BEETROOT'S ANTIINFLAMMATORY EFFECT

In an in vivo study, the antiinflammatory activity of an aqueous extract of *B. vulgaris* was evaluated by the carrageenan-induced rat paw edema method. Animals were treated with 500 and 1000 mg/kg of aqueous extract and the positive control group received indomethacin. Due to the statistical analysis, the aqueous extract of *B. vulgaris* showed significant anti-inflammatory activity in carrageenan-induced paw edema in mice, especially at a concentration of 1000 mg/kg, an effect which was close to that of indomethacin (Jain et al., 2011).

BEETROOT'S RADIOPROTECTIVE ACTIVITY

Based on the available investigation, betalains from red beets provide encouraging radioprotective activity. An in vivo (mice) investigation showed that the betalains of red beets demonstrated a radioprotective activity in mice that were irradiated with gamma-rays. Gamma-ray irradiation reduced the number of white blood cells, spleen cells, and thymus index. Betalains moderated the radiation-induced decline in white blood cells and partly repaired the spleen and thymus index in a dose-dependent way (Lu et al., 2009).

BEETROOT'S EFFECT ON ANXIETY

The consequences of a stressful life can be anxiety and depression disorders. Various illnesses associated with worrying are now being addressed with herbal products. Ethanolic extract of *B. vulgaris* significantly reverses Acute restraint stress (ARS)-induced anxiety in elevated plus maze parameter in the in vivo (adult male Swiss albino mice) experiment (Sulakhiya et al., 2016).

THE COGNITIVE FUNCTION ASSOCIATED WITH BEETROOT

Recent human studies have revealed that a high-nitrate diet (~12 mmol) including beetroot juice increases frontal cortex perfusion, a region responsible for cognitive processes. These experiments showed the influence of beetroot supplements in age-related cognitive diseases, however, long-term clinical trials are yet to be conducted (Clifford et al., 2015). Another study tested a beetroot juice supplement (140 mL/day of nitrate: 9.6 mmol) on cognitive function in healthy, elderly adults (~63 years) for three days. No changes in cognitive performance were detected in comparison with the control group (Kelly et al., 2013). Therefore, an acute serving of beetroot may not measurably improve cognitive performance.

CONCLUSIONS

The effective antioxidant, antiinflammatory, and vascular-protective properties of beetroot and its constituents have been indicated by several in vitro and in vivo studies. Ingestion of beetroot showed notable therapeutic effects in pathologies associated with oxidative stress and inflammation. Beetroot supplements also reduce blood pressure, avert inflammation and oxidative stress, preserve endothelial function, and restore cerebrovascular hemodynamics.

The antioxidative and antiinflammatory effects of beetroot are attributed to betalains and other phenolics, while the cardioprotective, physiological, and metabolic effects of the plant are believed to be mediated by nitrate and its subsequent conversion to NO. However, the precise mechanisms of the beneficial effects of beetroot have not fully been identified. In addition to insufficient evidence regarding the efficacy of beetroot supplements, the long-term safety of beetroot is yet to be fully elucidated. Additionally, little attention has been given to the influence of beetroot in other inflammatory human disorders, something which needs to be considered in future studies.

ABBREVIATIONS

DOPA L-l-3,4-dihydroxyphenylalanine
DPPH 2,2-Diphenyl-1-picrylhydrazyl
ER Estrogen receptor
ESI Electrospray ionization mass spectrometry
HPLC High performance liquid chromatography
MS Mass spectrometry
NMBA *N*-nitroso-*N*-methylbenzylamine
NO Nitric oxide
*PC***3** Prostate cancer cell
PPOs Polyphenoloxidases
TMG Trimethylglycine
ARS Acute restraint stress

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Chapter 3.6

Bilberry (*Vaccinium myrtyllus* L.)

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INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) utilization in human diet and its preparations have a long history (before the Middle Age) and, in fact, since 1700s there are written evidences of decoction preparations from dried fruits to utilize in folk medicine. Bilberry is a perennial shrub (~ 35–60 cm in height) and belongs to the Ericaceae family, which grows in coniferous forests, moors and meadows of northern Europe and America, but it is also present in Asia. It is also known as European blueberry, huckleberry or whortleberry. It blooms from April through June, producing spheroidal blue/black colored fruits (~5-9 mm in diameter) with many seeds that ripe from July through September. Its name is due to the intense color of the fruits and derives from the Danish word "bollebar" (dark berry). Bilberry is commonly known as a "super food", due to its richness in health promoting compounds with several biological properties. Particularly, represents a rich source of anthocyanins, compounds that in the last years have gained research attention due to the numerous functions and applications.

COMPOSITION, UTILIZATION IN HUMAN NUTRITION AND BIOAVAILABILITY

Bilberries are consumed and commercially available almost exclusively as berries (fresh, frozen and dried) as well as in the form of its canned industrial products (jams, juices and concentrates) or to produce syrups, pies, beverages and tarts. Instead, the leaves are used mainly in the preparation of decoctions. Anthocyanins are the principal components isolated and identified in berries (reaching up to 0.1-0.25% of the fresh fruits) and leaves, along with several active constituents including resveratrol, flavonols (quercetin, catechins), ellagitannins, phenolic acids and iridoids (Upton, 2001; Seeram 2008; Smeriglio et al., 2017; Smeriglio et al., 2014). The importance of anthocyanins is so high, that the bilberry extract (BE) has been standardized, according to 8th edition of European Pharmacopea (2014), to a content of anthocyanidins (aglycon of anthocyanins) between 32 and 40%. Sakakibari et al. (2009) reported the identification of fifteen anthocyanins in acidified ethanol extract. The main aglycons identified are: cyanidin, malvidin, delphinidin, petunidin and peonidin. Moreover, the glycosilated forms of cyanidin and delphinidin account for over 60% of the total amount of anthocyanins. Bilberry possesses the highest total anthocyanin content (~300-700 mg/100 g fresh fruit) with respect to other berries (i.e. elderberry, raspberry, strawberry, sour cherry and cranberry) (Smeriglio et al., 2014; Cravotto et al., 2010). This content may substantial changes in function of degree of ripeness, edaphoclimatic conditions and cultivars. Taking into account the daily intake of anthocyanidins (~ 200 mg) (Zafra-Stone et al. 2007), 20-60 g of dried berries or 80-100 mg of fresh fruits are enough to reach it. Fresh bilberry supplies also ascorbic acid (3 mg/100 g), quercetin (3 mg//100 g), and catechin (20 mg/100 g) (Upton 2001; Erlund et al., 2003). Over 20 different kinds of blueberry supplements are available on the market or via internet, but their anthocyanins amount varied considerably among the products (Yamamoto et al. 2012) and, for instance, some of them do not supply the daily minimal dose of 29 mg recommended in Japan (Japan Health Food & Nutrition Food Association, 2009). The bioavailability and distribution of compounds present in the bilberry are available only in mice, while human studies are completely lacking.

Upton (2001) and Sakakibari et al. (2009) described the metabolism of berry anthocyanins. Fifteen minutes after the ingestion of bilberry anthocyanins, the plasma level of these molecules ranged from ~2.5 mg/L (400 mg/kg per os) to ~50 mg/L (100 mg/kg for ethanol extract), followed by a remarkable decrease thereafter. Malvidin-3-glucoside and malvidin-3- galactoside were the main anthocyanins identified in the plasma (Sakakibari et al., 2009). Moreover, anthocyanidins have a specific organotropism, localizing mainly in the lung, kidney, liver and testes, while they seem to be absent in the heart, brain, muscle, fat and eyes.

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BIOLOGICAL PROPERTIES

Beyond its use as food, bilberries are widely used to improve night vision and decrease vascular permeability and capillary fragility. In addition, they have several beneficial effects on human health, although the most interest was focused on the antioxidant effects related to anthocyanins content (Kristo et al., 2016) as can be seen from numerous in vitro and in vivo studies (Veljkovic et al., 2017; Singh et al., 2016; Bujor et al., 2016; Popović et al., 2016). However, although there are many studies that have investigated the biological properties related to anthocyanins-rich extracts or fruit juices, only few investigated the un-processed bilberry fruits. On the whole, of the 17 clinical studies started utilizing this natural matrix, only 11 have been completed and they are available at *www.clinicaltrials.com*. Among them, only one published its results highlighting as a single supplement of a standardized bilberry extract (36 % (w/w) anthocyanins) modified glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle (Hoggard et al., 2013).

VASOACTIVE PROPERTIES

In Vitro Experiments

Bell and Gochenaur (2006) investigated the in vitro direct vasorelaxing activity of a freeze-dried bilberry dry extract, containing 15 different anthocyanins (including cyanidin, peonidin, delphinidin, petunidin, and malvidin), on isolated coronary artery rings from pigs. The total anthocyanins and phenols content were equal to 12.1 g/100 g and 35.7 g/100 g, respectively. The authors concluded that the endothelium-dependent vasorelaxation may be ascribed to endothelial nitric oxide (NO) system because the effect disappeared following administration of L-argine complexed with nitric oxide (L-arginine-μM NO_2). The bilberry anthocyanin-induced vasorelaxation mechanism on thoracic aortic rings obtained from male Wistar rats was investigated by Ziberna et al. (2013). Explants pre-treatment with anti-sequence bilitranslocase antibodies targeting the endothelial plasma membrane carriers, implicated in flavonoids conveyance, resulted in a cyanidin 3-glucoside and bilberry anthocyanins-induced vasodilatation decrease at conventional investigated post-absorption plasma concentrations (nM range).

In Vivo Experiments

The influence of bilberry juice on the capillary fragility was investigated on rats models fed for three weeks with a flavonoids-free diet (EMA, 2015). The capillary resistance, on the depilated skin, was defined following the induction of petechial by vacuum system. The juice, containing 25% of anthocyanidins, was administered intraperitoneally and the capillary fragility evaluated after 2, 4 and 6 hours. After 4 hours, a 200 mg/kg dose resulted in protective effects corresponding to nearly 80% (EMA, 2015).

ANTI-INFLAMMATORY ACTIVITY

In Vitro Experiments

Pomari et al. (2014) evaluated the effects of *Vaccinium myrtillus* L. extract on viability of murine macrophages (RAW264.7). Before testing the cell viability, the optimal experimental conditions, using increasing doses of H_2O_2 and extract at different incubation times, were investigated. A dose of 200 μM of H_2O_2, able to induce mRNA expression of COX2, IL1β, NFE2L2, NFκB1, NFκB2, NOS2 and TNFα and to suppress PPARγ expression, was used. Results showed that extract down-regulated COX2, IL1β, NFκB1, NFκB2, TNFα and NOS2 expression while up-regulated NFE2L2.

In Vivo Experiments

The anti-inflammatory activity of *Vaccinium myrtillus* L. extract, on mice affected by carrageenan-induced edema (450 mg/paw), was evaluated by Nardi et al. (2016). The paw volume was measured immediately after carrageenan or phosphate buffered saline (PBS) administration, at 30, 90, 180, and 300 min post-injection times. The authors showed that doses of 50 and 200 mg/kg of extract lead to a reduction in paw edema of 28.8%. After carrageenan injection, neutrophils migrate to inflamed paw and release enzymes such as myeloperoxidase (MPO). These events, occurring in inflamed tissues, lead to neutrophils accumulation that can exacerbate the inflammatory response. The *Vaccinum myrtillus* L. extract seems modulate the inflammation event by distinct mechanisms, particularly reducing the lipid peroxidation by-products and MPO release. Moreover, the extract was able to reduce acute inflammation by inhibiting the reactive oxygen species (ROS) activity.

ANTIOXIDANT ACTIVITY

In Vitro Experiments

It is well known that anthocyanins are powerful antioxidants able to scavenge free radicals and chelate metal ions. As described by Upton (2001), bilberry and bilberry anthocyanin-rich extract, protect rat liver microsomes against oxidative damage and apolipoprotein B against ultraviolet (UV)-induced oxidative fragmentation. Furthermore, Ogawa et al. (2011) showed that BE significantly decrease the lipid peroxide levels and revealed strong scavenging activity against superoxide and hydroxyl radicals in murine stomach tissue homogenates. The highest capacity to neutralize 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) was found for BE with an IC50 equal to $3.99 \pm 0.14\,\mu g/ml$ (EMA, 2015) while highest Oxygen Radical Absorbance Capacity (ORAC) value was observed for bilberry juice (2359 μmol TE/100g). Furthermore, BE scavenged the superoxide radical, in a dose dependent manner, with an activity of 108 ± 7.2 units of SOD per mg of extract (EMA, 2015).

In Vivo Experiments

The antioxidant animal studies show conflicting results. Lee et al. (2010) have not shown any change in the urinary values of 8-hydroxy-2' -deoxyguanosine (8-OHdG) (a marker of oxidative stress) after rats supplementation for 14 days with an extract enriched in anthocyanins. In other experiments, a significant decrease of malondialdehyde, a biomarker of lipid peroxidation, in brain of OXYS rats fed with BE, was observed. On the contrary, this event was not observed in Wistar rats proving that extract effect occurs only in the presence of high levels of MDA (Kolosova et al. 2006). Choi et al. (2010) observed a lower serum lipid peroxides concentration and an increased glutathione (GSH) level in cardiac muscle cells of doxorubicin-treated rats fed with bilberry extract (1% w/w in the diet). In contrast to the promising results of animal studies, no effect on lipid peroxidation in human volunteers was observed after supplementation with bilberries (Freese et al., 2004).

In fact, the antioxidant effect of the phenolic compounds found in bilberries, not only differs among several polyphenol classes (anthocyanins, ellagitannins, and proanthocyanidins), but is highly dependent on the matrix properties (Heinonen, 2007).

HYPOGLICEMIC EFFECT

In Vitro Experiments

The hypoglycemic effect of bilberry is due, in part, to interference with the α-glucosidase activity (McDougall et al., 2008), as well as on insulin secretion and glucose transport. Jayaprakasam et al. (2005) found that anthocyanins were able to stimulate insulin secretion from cultured rodent pancreatic B cells and particularly that cyanidins and delphinidins (the main bilberry anthocyanins) showed the greatest effect among different anthocyanins investigated.

In Vivo Experiments

Different studies conducted on animals have shown that bilberry extract or juice were able to decrease the glucose blood concentration at least 50% of the maximum values (Grace et al., 2009). Although some human studies about the hypoglycemic effects of berries (e.g., cranberry, chokeberry) are available, strong evidences from human trials are lacking (Helmstädter and Schuster, 2010). In fact, to date, only three studies on patients with type II diabetes are currently available on *www.clinicaltrials.gov*, of which only one with results. This study highlighted the ability of standardized bilberry extract to reduce postprandial glycaemia and insulin values in patients affected by Type 2 Diabetes (Hoggard et al., 2013).

ANTIMICROBIAL EFFECTS

In Vitro Experiments

It is well known that many phenolic substances such as flavonoids, phenolic acids, tannins and lignans have antibacterial activity (Heinonen, 2007). Concerning bilberry, it seems that this activity is attributable to the flavonoid fraction and in particular to the anthocyanins.

The BE, as well as other purified polyphenolic extracts of berries, showed antibacterial effects against human pathogenic bacteria, including *Salmonella* and *Staphylococcus aureus* (Puupponen-Pimiä et al., 2005). Furthermore, BE was able

to inhibit the growth of *H. pylori* and of several *Bacillus*, *Clostridium and Staphylococcus* strains (gram-positive bacteria). On the contrary was showed that pure phenolic compounds inhibit only the gram-negative bacteria, including *Salmonella* species and *Escherichia coli*, and that this activity was correlated to the hydroxylation degree of phenolic compounds (Puupponen-Pimiä et al., 2005). Therefore, can be assumed that whole fruits or phytocomplexes are more effective as antimicrobial with respect to the purified compounds.

INTERACTION WITH SUPPLEMENTS/DRUGS/FOODS

Recently Aichinger et al. (2016) showed how different polyphenols are potentially able to inhibit the cytostatic effects of erlotinib in vitro. Erlotinib is a chemotherapy agent approved for the treatment of pancreatic and non-small cell lung cancer. Its main mechanism of action is the inhibition of epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK). Some anthocyanins and anthocyanin-rich extracts like bilberry extract, showed an inhibition activity on tyrosine kinases, including EGFR.

Paoletti et al. (2011) reported a case of INR reduction to 1.6 (onset time 4 days) in a patient of 80 years, treated with long-term oral anticoagulant, warfarin (27.5 mg/week for 1.5 years) and received bilberry juice (200 ml/day). After this, the co-treatment was suspended, the warfarin dose increased to 32.5 mg/w and the patient fully recovered.

The reduced activity of warfarin is surprising, since the bilberry anthocyanins, with antiplatelet properties, should increase the risk of bleeding using in combination with anticoagulant therapy. The mechanism of this mutual interaction is not clear.

Despite anecdotal cases presented it was not possible to prove a real interaction with anticoagulants and antiplatelet agents at the recommended dose of bilberry formulations.

Recently, the valuation of the in vitro and in vivo potential of several berries highlighted that bilberry, commonly used as herbal supplement, was not able to inhibit uridine diphospho-glucuronosyltransferase. Therefore, it seems does not cause clinically significant herb-drug interactions through this pathway (Choi et al., 2014).

CONCLUSION AN FUTURE TRENDS

Although there are many studies that have investigated the health effects of bilberry extract and its derived products, well-designed human trials using standardized extracts of bilberry are still lacking. In addition, among the few available, only one, to date, has published its results, demonstrating that a standardized extract of bilberry can be useful to reduce postprandial glycaemia and insulin values in patients affected by Type 2 Diabetes; but this is just one of many diseases for which, a bilberry role was hypothesized. In light of this, it is essential to increase the clinical studies in order to translate the several observed in vitro and in vivo evidences to humans elucidating the often, neglected pharmacological and toxicological aspects.

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Chapter 3.7

Borage (*Borago officinalis* L.)

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OVERVIEW

Borage (or burrage, bourrache, bugloss) also known as starflower is botanically equated with *Borago officinalis* L. belonging to the family Boraginaceae (Asadi-Samani et al., 2014; Bianco et al., 1996; Larson et al., 1984). The plant is native to Europe, North Africa, India, Iran, and widely distributed in the Mediterranean countries (Kalhor and Ashrafian, 2017; Mhamdi et al., 2009; Zemmouri et al., 2014). In the middle east, tea obtained from borage leaves infusion is being consumed since long back (Kalhor and Ashrafian, 2017). Various species are used as borage in different parts of the world. In India, two plants namely *Plectranthus amboinicus* Benth. (Indian borage) (Gupta and Negi, 2016) and *Echium amoenum* Fisch. & C.A. Mey. (Adel Pilerood and Prakash, 2014) are used as borage. In Iran, *Echium amoenum* (Abolhassani, 2004) and *Borago officinalis* (Kalhor and Ashrafian, 2017; Mirsadraee et al., 2016) are used. *Trachystemon orientalis* (L.) G. Don is used as borage in east Bulgaria (Alici and Arabaci, 2016) and *Borago officinalis* L. is used in Algeria (Zemmouri et al., 2014). Borage possesses a wide range of therapeutic potential covering a wide array of diseases including renal diseases, respiratory and gastrointestinal tract disorders. It is also considered to act as an antihypertensive, diuretic, antipyretic, antispasmodic, demulcent and aphrodisiac (Singh et al., 2016). An extensive description pertaining to curative properties of borage have been described in therapeutic subsection of this chapter.

Borage is a herbaceous, hairy annual plant with 70 to 100 cm height (Farhadi et al., 2012). The leaves are simple, alternate and fibrous (Yazdani et al., 2004). Flowers of borage are blue in color and possess five flags with anthers in close proximity to each other. Borage bears brownish, oval shaped and wrinkled nutlet fruit (Asadi-Samani et al., 2014).

Borage oil has gained substantial interest among various research groups over the world, due to its therapeutic and nutritive potential, which is, in general, due to the presence of high γ-linolenic acid (GLA) content. The oil also possesses antioxidant and free radical scavenging properties (Bandoniene and Murkovic, 2002; Huang et al., 1995; Singh et al., 2016). Several fatty acids such as GLA, linoleic, stearic, palmitic acid are present in borage (Fig. 3.7.1) (Asadi-Samani et al., 2014).

Due to the presence of linolenic acid in its seeds, the agricultural interest of this plant is also increasing (Asadi-Samani et al., 2014; Mhamdi et al., 2009) since GLA, a volatile fatty acid present in seeds is unique to borage with exception of very few other plants (Yang et al., 2002).

Major sources of GLA are evening primrose and borage. GLA is an omega-6 polyunsaturated fatty acid (omega-6 PUFA) which cannot be synthesized in the body and hence falls into the category of essential fatty acids (Horrobin, 1992). GLA is a product of conversion of linoleic acid, a parent omega-6 essential fatty acid. This conversion is catalyzed by a Δ6-desaturase enzyme in the body which is a rate limiting step of this metabolism (Guil-Guerrero et al., 2017; Kapoor et al., 2015). In the human body, GLA serves as a precursor to dihomo-γ-linolenic acid (DGLA) (Guil-Guerrero et al., 2017; Horrobin, 1992; Maldonado-Menetti et al., 2016) which is further converted to arachidonic acid by Δ5-desaturase (Chilton et al., 2008). DGLA (8,11,14-eicosatrienoic acid) is an omega-6 polyunsaturated fatty acid that leads to the production of several eicosanoids (Goodman, 1996) viz. series-1 prostaglandins like prostaglandin E_1 (PGE_1); thromboxane A_1 (TxA_1), series-3 leukotrienes; as well as 15-hydroxyeicosatrienoic acid (15-HETrE) through respective cyclooxygenase and lipoxygenase pathways (Fig. 3.7.2).

These eicosanoids serve multiple physiological functions and therefore possess beneficial effects in numerous disease processes. The therapeutic effects are specifically due to the anti-inflammatory and immunomodulatory properties of these eicosanoids (Guil-Guerrero et al., 2017; Kapoor and Huang, 2006). On the other hand, arachidonic acid (5,8,11,14-eicosatetraenoic acid) leads to the production of inflammatory mediators like series-2 prostaglandins and

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00023-0

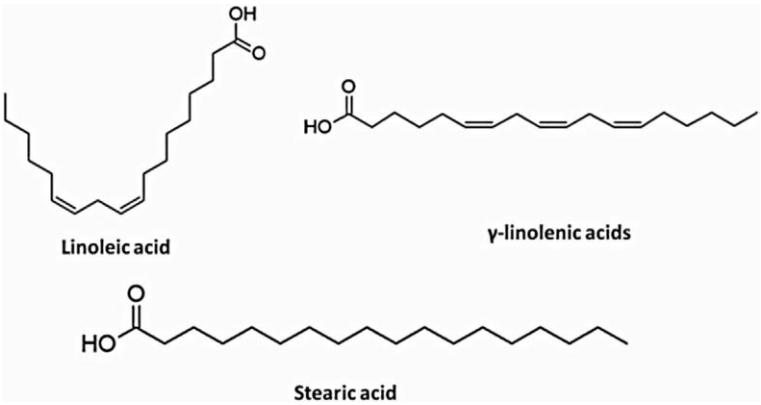

FIG. 3.7.1 Major chemical constituents present in Borage.

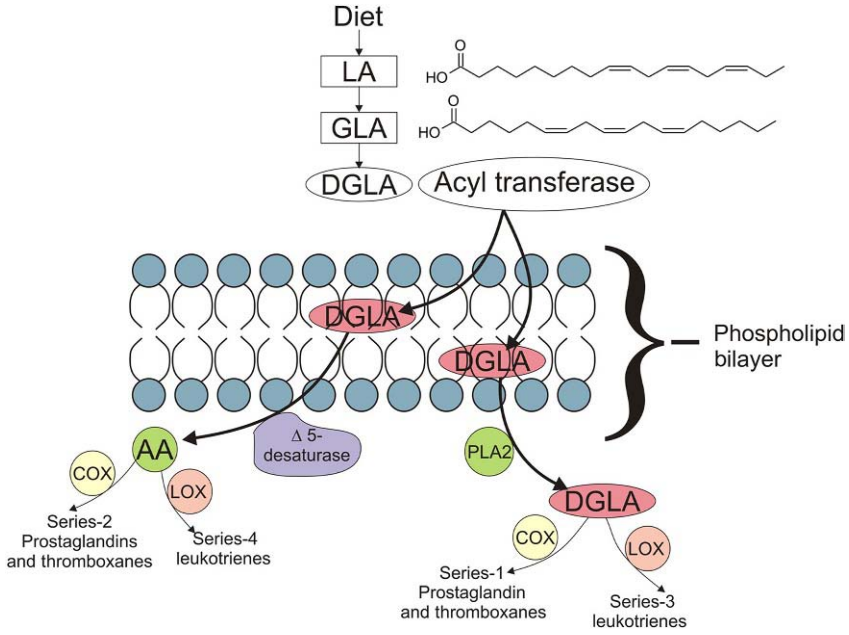

FIG. 3.7.2 Schematic representation of DGLA mediated biosynthesis of prostaglandins and leukotrienes.

series-4 leukotrienes (Belch and Hill, 2000). This explains the importance of GLA as a dietary nutrient which is speedily gaining importance in therapeutics for prevention and treatment of various diseases.

Borage is being used in diverse areas including pharmaceutical and beverage industry, for forage, as dietary supplement and salads (Sayanova et al., 1999, 1997). Borage contains about 30-40% of oil, of which GLA constitutes approximately 20-30%. Although, borage is better in terms of GLA yield, as the amount of GLA present in borage is around twice than that of evening primrose oil. However, 90% of GLA oil selling rate is associated with evening primrose (Asadi-Samani et al., 2014; Belch and Hill, 2000; Sayanova et al., 1999).

BORAGE AND THERAPEUTICS

Borage oil is one of the richest sources of GLA (Tasset-Cuevas et al., 2013) and is therefore, being extensively explored for its possible beneficial effects on a wide range of diseases. Some important aspects of borage in therapeutics have been discussed here.

Borage oil is known to offer protection in atherosclerosis or arterial plaquing which is an inflammatory disorder characterized by loss of integrity and function of the arterial endothelium (Shewale et al., 2015). This loss of integrity and function of the endothelium triggers a cascade of events which basically induces chemotactic migration of monocytes to the necrotic site that differentiate into macrophages. These macrophages are mainly responsible for recruiting and depositing cholesterol, mainly low-density lipoproteins in the arterial lumen leading to plaque formation (Falk, 2006; Jefferson and Topol, 2005; Lorkowski and Cullen, 2007). Borage oil reduces plasma VLDL (very low-density lipoproteins) levels and expression of inflammatory genes, especially those of macrophages like macrophage ABCA1 and ABCG1 that are involved in atherosclerotic disease progression (Shewale et al., 2015). Additionally, borage oil is known to decelerate cardiac remodeling after myocardial infarction (MI) and thus prevent cardiac failure. The involved mechanism seem to be the reduction of the inflammatory response of lymphocytes and macrophages, curtailment of fibrosis to infarcted tissue, and prevention of ventricular distension (Maldonado-Menetti et al., 2016).

Borage oil also possesses therapeutic efficacy against rheumatoid arthritis (RA). RA is a chronic inflammatory disorder that afflicts joints. Tumor necrosis factor-α (TNF-α) is known to have a key role in the pathophysiology of RA, as this very cytokine induces the production of the pro-inflammatory interleukins viz. IL-1 and IL-6; generation of chemokines that promote migration of leukocytes to the inflamed tissue; and stimulate bone destruction and resorption (Brennan and McInnes, 2008; Kalden, 2002; Moelants et al., 2013). Borage oil is known to reduce TNF-α levels, by promoting the generation of PGE_1 in the body. PGE_1 generation enhances intracellular cyclic adenosine monophosphate (cAMP) levels that share an inverse relationship with TNF-α (Kast, 2001). PGE_1 with its anti-inflammatory tendency also aids in the gradual alleviation of this chronic disease (Belch and Hill, 2000).

Borage oil is also known to reverse the neurotoxic and hippocampal long-term potentiation reduction effects of β-amyloid-mediated neurodegeneration and loss of cognitive functions in Alzheimer's disease. This effect is due to the antioxidant and immunomodulatory property of its GLA content (Freir et al., 2001; Zargooshnia et al., 2015).

In asthma, borage oil seems to subdue leukotriene B_4 (LTB_4) level which is strongly implicated in the pathophysiology of asthma. DGLA content that accumulates in polymorphonuclear granulocytes on administration of borage oil gets converted to 15-hydroxyeicosatrienoic acid (15-HETrE) through 5-lipoxygenase pathway which strongly inhibits LTB_4 production. Moreover, DGLA itself halts the production of pro-inflammatory mediators. Despite zeroing on such a crucial target of asthma, still, borage oil doesn't seem to have a very promising effect on clinical symptoms of asthma and requires further exploration.

Borage oil is also known to have hepatoprotective effect in hepatic steatosis or fatty liver disease (Lukivskaya et al., 2012). This effect is due to its GLA content that has potential to inhibit the activation of liver X receptor (LXR) which in turn is responsible for the transcription of sterol regulatory element-binding protein-1c (SREBP-1c) gene which is involved in hepatic fatty acid synthesis (Ou et al., 2001; Shewale et al., 2015).

Apart from these activities, due to the presence of carvacrol, borage possesses larvicidal activity against *Anopheles gambiae*, a highly prevalent vector for malaria parasites in Africa (Kweka et al., 2012).

DRUG INTERACTION AND TOXICITY WITH BORAGE

Borage possesses potential to lower seizure threshold maintained by various anticonvulsants due to its gamolenic acid content. Therefore its use is contraindicated in combination with anticonvulsant drugs (Dasgupta, 2003; Ikegami et al., 2004; Williamson, 2005). This seizure threshold lowering effect of borage oil is quite prominent when administered with Phenobarbital, and leads to dose enhancement of Phenobarbital to obtain an anticonvulsant effect (Dasgupta, 2003). Additionally, simultaneous use of borage with other drugs like tricyclic antidepressants and phenothiazines with a potential to lower seizure threshold should be avoided (Miller, 1998). Contrarily, some studies have reported the absence of seizures while using borage, and studies on rats have showed that GLA even exhibits antiseizure activity (Samuels et al., 2008; Voskuyl et al., 1998; Yehuda et al., 1994).

Borage seed oil interacts with warfarin and can potentiate the bleeding effect of warfarin. Few beneficial interactions of borage seed oil have also been reported, like the enhanced cytotoxicity of human breast cancer cell line *in vitro* was evident due to the synergistic effect of gamolenic acid on paclitaxel and vinorelbine (Menendez et al., 2002; Williamson, 2003). In a study conducted to scrutinize the interaction of non-vitamin and non-mineral supplements with prescription and non-prescription drugs, borage exhibited interaction with anticonvulsants, estrogen, and progesterone (Wold et al., 2005). Here it is important to state that, the use of borage oil is contraindicated in pregnancy as it can lead to labor inducing and teratogenic effects of prostaglandin E agonists (Kast, 2001). The use of borage should also be avoided with hepatotoxic drugs like phenothiazine, anabolic steroids and ketoconazole (Miller, 1998).

PHARMACEUTICAL FORMULATIONS WITH BORAGE AS AN INGREDIENT

Various formulations using borage oil have been prepared including emulsions and syrups (Kapoor et al., 2015; Mirsadraee et al., 2016). Green synthesis of nanoparticles from borage leaf extract have also been reported (Singh et al., 2016). Borage oil is also used in the preparation of various drug delivery systems as an absorption enhancer (Modi, 2001, 2000). Moreover several patents are available for drug delivery systems and dietary supplements with using borage oil as an ingredient. Borage seed oil is used as an anti-irritant and found potentially effective in plummeting hydroxy acids/retinoids induced irritation (Habif et al., 1997). A dietary supplement containing borage oil along with some other ingredients from plant sources has been invented that acts as a performance enhancer under stressful conditions for humans (Bell et al., 2000). In the form of capsules it is available for cosmetic applications for the treatment of dry skin and hair (Alvarez and Rodríguez, 2000). Borage oil has also been utilized in the formulation of nanospheres for different topical formulations (Keefe et al., 2003). In addition to these formulations some of the fermented dairy products were also developed containing borage and evaluated for bioavailability studies (Puch et al., 2008). These inventions and studies undoubtedly reveal the diverse potential and applicability of borage oil in a vast array of pharmaceutical formulations. In comparison to the extensive ongoing research on exploring the tremendous potential of borage oil for preventing and curing numerous diseases, its exploitation for the development of formulations targeting major diseases has been very limited. Therefore, substantial research is required in the field of formulation development involving borage oil and its suitability for self emulsifying drug delivery systems can also be explored.

CONCLUSION AND FUTURE PROSPECTIVE

Recently, dietary supplements have garnered lot of research interests as alternatives to conventional medicine for various disease conditions. Similar interests have lead to the burgeoning of the use of borage in pharmaceutical and nutraceutical industries. This is mainly due to the presence of its main compound GLA, which is having substantial role in therapeutics. Despite extensive ongoing research on borage, there is a lot to be explored in this non-vitamin and non-mineral nutrient. Some of the key areas could be clinical and adverse events, molecular mechanism and validation of the dose of borage for human consumption.

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Chapter 3.8

Brown Seaweeds

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INTRODUCTION

Throughout time, marine algae have developed complex mechanisms to promote adaptation to external factors (e.g., ultraviolet (UV) radiation, salinity and temperature stress) as well as to defend themselves from biological pressures such as competitors, grazers, or parasites, since they live in an extremely stressful and fluctuating environment. To do so, these organisms divert resources into producing unique bioactive compounds not present in terrestrial food sources that, when adequately processed, have various applications for humankind. Adding the fact that approximately half of the global biodiversity exists in marine environments, the sea and its inhabitants provide a large source of novel, and potentially revolutionary, bioactive compounds (Gupta and Abu-Ghannam, 2011a).

Edible macroalgae are ubiquitous in east-Asian (Japanese, Korean, Chinese) and Pacific (Indonesian, Maori, Hawaiian) diets, with *Porphyra* spp., *Caulerpa* spp., *Laminaria* spp., and *Sargassum* spp. serving as common ingredients in many salads, soups, garnishes, among other dishes. Having previously been chosen due to their availability and nutritional value, fresh and dried seaweeds are enjoying a renewed interest from today's consumers. Their unique sensory properties have earned macroalgae of all types a frequent presence in gourmet cuisine, and the improved understanding of their chemical composition has made them a popular product within a consumer base that is increasingly more aware of healthy eating habits (Fleurence, 2016; Yuan, 2008).

While seaweeds have had a minimal role in human nutrition throughout most of its history, recent studies are shedding light over the nutritional and nutraceutical potential of these products. Readily available algae, such as *Palmaria palmata*, were identified as excellent sources of protein, polyunsaturated fatty acids (PUFA), and both soluble and insoluble dietary fibers. Selected micronutrients, such as iodine, are abundantly found in brown seaweeds, with exceptional in-vitro bioavailability (Romarís-Hortas et al., 2011; Yuan, 2008) Rich sources of antioxidant and anti-cardiovascular disease (CVD) bioactive compounds have also been identified among commonly consumed seaweed species. These findings were further motivated by epidemiological evidence between the regular consumption of seaweeds and reduced risk of diet-related cancers and CVD, in particular, between Asian and Western populations. Edible seaweeds have also found use as sources of thickening, gelling, and emulsifying agents for the food industry. Functional polysaccharides, such as alginates, are extracted from several species of brown kelp, such as *Macrocystis* and *Laminaria* spp. (Shahidi, 2008; Yuan, 2008).

Brown macroalgae (*Phaeophyceae*) have had a long history of use as a biomass source for animal feed throughout the coastal regions of the Northern Hemisphere. Research concerning the cultivation of brown seaweeds has gained momentum in recent years, attempting to take advantage of its robust tolerance to different temperature and light conditions, a feature of many brown kelp species. Most of these cultures are then used as a source of low-value products, such as food, functional phycocolloids, fertilizers, and biofuels. The use of extracts of brown algae or their bioactive compounds as a food supplement is still a growing area with the majority of studies assessing the possible effects of their implementation on animal nutrition (Hayes, 2015).

In this chapter, we will discuss in more detail the nutraceutical applications of some of the most promising bioactive compounds from brown algae, emphasizing the importance of continuous research and future development in this field.

BROWN ALGAE AS A FOOD PRODUCT

The presence of seawater, photosynthesis-enabling light, and a firm attachment point are the dominating environmental requirements for the ecology of macroalgae. As a consequence, the vast majority of seaweed diversity occurs in rocky littoral

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areas. While there are some exceptions, these ecological limitations mean that, among the many marine resources available on our planet, seaweeds are some of the most readily available and easy to exploit. As such, many species of algae have a long history of human consumption, whether for food purposes or otherwise. Brown macroalgae are often very conspicuous among the seashores they inhabit, with rockweeds (*Ascophyllum* spp.) and leathery kelps cited as common examples. A great amount of the species that constitute this class of marine algae has found use by human settlers of coastal regions, being used as food, for pharmaceutical purposes, or as a source of functional compounds (Fleurence, 2016; Kilinç et al., 2013).

Laminaria spp. ("*kombu*") and *Undaria* spp. ("*wakame*") are among the most important of algal resources in China and Japan, with a long history of human consumption, and a recent worldwide boom in popularity as a common ingredient in Eastern cuisine. Several species of the *Laminaria* genus are very common in the island of Hokkaido, Japan, and they have frequent use in local dishes. *Sargassum* spp., *Rosenvingea* spp., *Colpomenia* spp., *Hydroclatharus* spp., and *Dictyota* spp. have been a part of human diets from China to Hawaii, as ingredients in soups and salads (Fleurence, 2016; Novaczek, 2001).

BROWN MACROALGAE AS A SOURCE OF BIOACTIVE COMPOUNDS

Brown algae are constituted by a number of biologically active substances such as polysaccharides, carotenoids, proteins, lipids, and ω-3 fatty acids and also by secondary metabolites, such as terpenes and polyphenols. The presence of these constituents has granted these organisms wide usage in many different industries such as food, cosmetics, biotechnology, and pharmaceutical. Already a known source of powerful bioactives among the medical and pharmaceutical industries, brown algae are commonly used in the treatment of rheumatic processes, arteriosclerosis, menstrual disorders, hypertension, gastric ulcers, goiter, skin diseases, and syphilis. With widely confirmed antitumor, antioxidant, antiviral, antimicrobial, anticoagulant, and antiinflammatory activities, *Phaeophyceae*-sourced compounds still harbor great potential benefits to human health (Kristinsson, 2008; Yuan, 2008).

While many red algae have gained attention from nutritionists and food researchers for their very significant protein content, their brown counterparts have gained a more specialized attention, with particular focus on their protein hydrolysates, which have shown promising antibacterial activity. Not to undermine the potential dietary value of brown algae protein, as when evaluating the quality of dietary protein, amino acid profiles, and protein digestibility and bioavailability are of the utmost importance, and should be considered when determining how suitable any given food product can be as a source of protein. Many of the edible seaweeds contain amino acid profiles that adequately cover nearly 50% of the total amino acids. In this regard, several species of brown algae stand out in particular, with *Sargassum hemiphyllum* and *Durvillaea antarctica* covering 56.4% and 57.7% of the total essential amino acids in their protein content (Kristinsson, 2008).

Protein concentrates obtained from seaweeds have demonstrated outstanding functional properties, with foaming, emulsifying, water-holding, and fat absorption capabilities on par with, if not better than, conventional plant-sourced concentrates. These are particularly desirable features when attempting to develop new value-added food products. Seaweed proteins can also be used in the synthesis of bioactive peptides, as a result of proteolytic cleavage of the original chains. The beneficial health effects of these molecules can far surpass those of regular protein that would otherwise be consumed upon ingestion of the unprocessed seaweed. Several authors have reported a myriad of beneficial bioactivities gained from hydrolyzed protein fractions of marine plants, including antioxidant, antiinflammatory, antiatherosclerotic, opioid, and potential antiobesity, antidiabetic, and heart health benefits. The role of these peptides in the prevention of chronic diseases, such as Alzheimer's and Parkinson's are currently being investigated (Kumar et al., 2014; Hayes, 2015).

Brown seaweed protein hydrolysates are frequently studied in parallel with other algae and often achieve very comparable bioactivities, a positive outcome for the exploitation of readily available species of brown seaweed. The generally lower amount of phenolic compounds in these algae can also be advantageous when attempting to extract and make use of their hydrolyzed proteins. Lower extraction yields are often related to higher phenolic content in a multitude of seaweed samples. It should also be said that high quantities of structural polysaccharides, such as xylans, agar, carrageenan, and alginates, have been related to reduced protein bioavailability, and should thus be considered when evaluating the dietary potential of any given seaweed.

Phlorotannins are a class of polyphenols that consist on polymers of phloroglucinol. These are synthesized exclusively by brown algae, as part of their secondary metabolism. Phlorotannins extracted from *Ecklonia cava*, including phloroglucinol, eckol, and dieckol, were reported to have strong antioxidant activity by preventing oxidative stress–induced cell damage in lung fibroblast cells (Wang et al., 2012; Kang et al., 2015). Tierney et al. (2013, 2014) have also conducted studies on the degree of polymerization and molecular weight of phlorotannins obtained under differing food-compatible extraction conditions. This research was further supplemented by attempts to improve the antioxidant potential of the extracts through the removal of interfering compounds with molecular weight cutoff membranes (Tierney et al., 2014). ACE-I inhibitory activity has also been associated to this molecule. While the interaction mechanisms between phlorotannins and the ACE-I

enzyme are not yet fully understood, the inhibitory effects found were similar to those seen in peptides from enzymatic fractions of flaxseed, which have known antihypertensive capabilities (Tierney et al., 2010; Udenigwe and Howard, 2013).

The lipid-soluble content extracted from brown seaweeds has been of particular interest in recent studies. While very few species actually contain high crude lipid content, the presence of omega-3 PUFAs, omega-6 arachidonic acid, fucoxanthin, and fucosterol make this small fraction of algae weight very relevant nonetheless (Shahidi, 2008; Miyashita et al., 2013). Unique to certain species of brown macroalgae, fucoxanthin is a carotenoid that has displayed multiple major physiological effects in molecular mechanisms involved in the development of type 2 diabetes and general obesity (Miyashita et al., 2011).

Sulphated polysaccharides, particularly those of marine origin, are getting a steadily increasing amount of attention from both the biochemical and medical fields, due to reported immunomodulatory and anticancer activities (Wijesekara et al., 2011). While their quantities are highly dependent on season, species, age, and geographic location, polysaccharides are still the major component in most members of the *Phaeophycea* class, with percentages (dry weight) generally ranging between 40% and 60% (Fleurence, 2016; Ponce et al., 2003). Polysaccharide-based cell walls provide the plant with the needed strength and flexibility to withstand environmental physical stress, as well as regulating ionic equilibrium. Brown seaweeds display a variety of different polysaccharides, including alginates, fucoidans, and laminarans, which can be selectively extracted using either water or alkali solutions (Gupta and Abu-Ghannam, 2011b).

While the *Fucales* and *Laminariales* classes are particularly rich in fucoidan, the molecule is found in every brown algae to some degree. Alekseyenko et al. (2007) isolated fucoidan from *Fucus evanescens* and verified its antitumor and antimetastic activities in mice upon administrating doses ranging from 10 to 25 mg/kg (Alekseyenko et al., 2007). Antiviral properties have also been attributed to specific types of fucoidans, including galactofuran and uronofucoidan (Ponce et al., 2003).

Serving as an energy reserve for most brown algae species, *Laminarian* have been regarded as an important bioactive and promising ingredient in food supplements. The ability to form complex, hydrolysis-resistant structures means that they remain mostly intact throughout the upper gastrointestinal tract and therefore function as dietary fibers. Antibacterial and antitumor activities have also been reported (Neyrinck et al., 2007).

Alginate is another marine polysaccharide exclusive to brown seaweeds, found as calcium or sodium alginate. It is one of the most well-known, and technologically employed, molecules of marine origin, with common use in the food and pharmaceutical industries, both for its gelling and metal-chelating capabilities. Reported antitumor and immunomodulatory activities open a slew of possibilities for further pharmaceutical uses of alginates, though their mechanisms of action make these properties difficult to exploit in functional foods (Sousa et al., 2007; Andrade et al., 2004).

Seaweeds are also particularly rich in trace elements that are essential for human health. Brown algae in particular have exceptionally high levels of iodine and bromine and, while studies about this topic are few in number, they display high bioavailability both in vitro and in vivo. The results are difficult to extrapolate upon, but they indicate a likely possibility that regular consumption of seaweed, in which species of brown algae are represented as *Laminaria sacharina*, *Laminaria ocholeuca*, and *Undaria pinnatifida*, should fulfill the suggested daily intakes of iodine, or act as a supplement in cases of deficiency (Romarís-Hortas et al., 2011; Aquaron et al., 2003).

BROWN SEAWEED AS A FOOD AND FEED SUPPLEMENT

The use of extracts of brown algae or their bioactive compounds as a food supplement is still a growing area, with a large number of studies focusing on in vitro potentials and the possible effects of their implementation on animal nutrition.

Walsh et al. (2013) investigated the influence of dietary supplementation of purified laminarin and fucoidan from *Laminaria* spp. independently or in combination on growth performance, coefficient of total tract apparent digestibility, selected faecal microbial populations, and volatile fatty acid concentrations in weaned pigs. The authors discovered that the inclusion of 300 mg/kg of laminarin showed the greatest benefit in growth performance with improvements in average daily gain and gain-to-feed ratio partially due to an increased coefficient of total tract apparent digestibility and lower faecal score. Curiously, combining fucoidan with laminarin showed no beneficial effect on the overall growth performance of the weaned pigs. In another case, Hong et al. (2015) examined the effects of supplementing by-products from *Undaria pinnatifida* on ruminal fermentation characteristics in vitro as well as on growth performance, endocrine response, and milk production in Holstein cows (in vivo), and concluded that dietary supplementation with by-products from brown seaweed did not compromise ruminal fermentation nor daily milk yield and composition, which may have potential to be used as a safe food supplement in dairy cows. More recently, Belanche et al. (2016) studied the effect of two brown seaweeds (*Ascophyllum nodosum* and *Laminaria digitata*) on rumen function in rusitec fermenters and observed that these seaweeds had no substantial effect on rumen fermentation, feed degradability, or methane emissions. However, specific

effects depending on species were noticed such as a substantial decrease in nitrogen degradability promoted by *A. nodosum* due to its high phlorotannin content, which led to a change of bacterial community, having a negative impact. In contrast, *L. digitata* did not have these effects because it has much lower phlorotannin content and therefore, promoted a greater efficiency of microbial protein synthesis. These results suggested that special attention must be taken with seaweeds that present higher phlorotannin concentrations.

It should still be mentioned that research surrounding the potential of brown algae as a source of bioactives for pharmaceuticals and food supplements for human consumption is being carried out, and seems to be gaining significant traction. Lin et al. (2017) evaluated the combined effects of low molecular weight fucoidan and fucoxanthin from *S. hemiphyllum* in terms of antihyperglycemic, antihyperlipidemic, and hepatoprotective effects in mouse models of type 2 diabetes. Positive effects in several of these activities, including lowered blood sugar and increased serum adiponectin levels were identified upon administration of 300 mg/kg (of body weight) of low molecular weight fucoidan and fucoxanthin, as well as upon ingestion of a 50:50 mixture of these compounds. The mixture had a greater effect over increased hepatic glycogen levels and antioxidant enzyme activities, hinting at a synergic effect. Fan et al. (2017) evaluated the anticancer effects of a polysaccharide exclusively found in *Sargassum fusiforme*, and previously extracted by Cen et al. (2005) and Chen et al. (2012). In this study, human hepatocellular carcinoma cells HepG2 were inoculated in mice supplied with a daily oral dose of 100, 200, and 400 mg/kg (of body weight) of the aforementioned polysaccharide. Significant in vitro HepG2 cytotoxicity was found, as well as tumor growth inhibition in mice. The authors also attributed the antitumor activity of this polysaccharide to possible immunomodulatory effects. In a pilot study, Lee et al. (2012) studied the safety and effects of Seapolynol™, a commercial polyphenol extract obtained from *E. cava*. In this study, 46 individuals with hypercholesterolemia were subjected to a 12-week treatment period with a daily oral dose of 400 mg. The researchers verified a significant decrease in hip circumference, total cholesterol, low-density lipoprotein cholesterol, and c-reactive protein. No significant adverse effects were identified. Rhanasto-Rilla et al. (2017) performed an in vitro evaluation of the SIRT6 activation potential of extracts obtained from brown seaweeds. These were obtained from *Fucus dichitus*, *Fucus vesiculosus*, *Cytoseira tamariscofolia*, *Cytoseira nodacaulis*, and *Alaria esculenta*. The activation of SIRT6 was evaluated through measurement of H3K9 deacetylation. Significant H3K9 deacetylation was verified in the presence of *F. dichitus*, *F. vesiculosus*, and *C. tamariscofolia* extracts. From the *F. dichitus* extracts, the researchers identified fucoidan as the compound responsible for the verified activities, using mass spectrometry. Yu et al. (2017) studied the neuroprotective effects of fucoxanthin from *Sargassum horneri* on H_2O_2-induced toxicity, in SH-SY5Y cells and in cerebellar granule neurons. The authors verified significant protection against neural apoptosis and intracellular reactive oxygen species. Cells treated with fucoxanthin displayed higher survival rates and restored enzymatic activity, which had been disrupted by exposure to H_2O_2.

Based on the evaluated research, the use of brown seaweeds as dietary supplements appears to be promising, even if there is a lack of human studies. Further studies are needed regarding the positive and negative impacts of dietary supplementation with extracts or pure compounds from brown seaweeds under in vitro and in vivo conditions in both humans and animals to understand their mechanisms of action and their true potential.

CONCLUSIONS

There is little doubt regarding the potential of marine bioactives in the development of new food products, nutraceuticals, and diets. Studies regarding the biochemistry of these organisms show that both the extraction of select compounds and the incorporation of the seaweeds themselves in traditional diets can greatly benefit human health. However, while our understanding of these compounds keeps expanding, it is important to monitor how they can be introduced in the market and our diets, while being sure that no nefarious effects can manifest. When talking about products whose origin lies in brown seaweeds, research is incredibly slim. The widespread availability of these organisms and the ever-increasing demands for feeding livestock have conducted most of the harvested biomass to be directed to feed and feed supplements, which in turn has made research focus heavily on this field.

The information gathered here suggests that both the availability and gastronomical familiarity of brown seaweeds should, together with the vast biochemical knowledge, favor the development of innovative functional foods and supplements. Proper research on these products should then be conducted to provide safe choices to consumers, as well as for the continuous development of better nutrition for humankind.

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Chapter 3.9

Coffee Supplements

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GREEN COFFEE BEAN PRODUCTION AND PROCESSING

The majority of coffee supplements are made with green coffee, which is obtained from the seeds of the coffee plant, i.e., green coffee beans (GCB).

The coffee plant (*Coffea* sp., family Rubiaceae) is particularly abundant in tropical areas, and among the more than 90 different species, only *Coffea arabica* and *Coffea canephora* (also known as *Coffea robusta*) have major commercial importance and, respectively, account for 60% and 40% of the world's coffee production (Farah and dos Santos, 2015). However, wild varieties with interesting properties, such as the caffeine-free *C. arabica* variety, have been discovered and are still under study in order to find ways to transfer these traits to commercial varieties (Favoretto et al., 2017; Silvarolla et al., 2004).

Usually, berries are harvested when ripe. After harvesting, the outer layers of the coffee drupe (i.e., the hull and pulp) are easily removed, while the mucilage, parchment, and silverskin remain attached to the beans. The removal of these layers can be carried out using three different methods, referred to as dry, wet, and semidry processing (de Melo Pereira et al., 2016). Wet processing is mainly used for Arabica coffee: the ripe fruits are depulped and then submitted to 24–48 h of fermentation in a water tank and dried until a final water content of 10%–12% is achieved (Murthy and Naidu, 2012; Silva, 2014). In contrast, in dry processing, whole coffee fruits are dried (in the sun) on platforms and/or on the floor, without prior removal of the pulp (Silva et al., 2008). Semidry processing is a combination of both methods in which the coffee fruits are depulped, but the fermentation process occurs directly under the sun on a platform (Vilela et al., 2010).

The GCB obtained by these methods are further sorted to separate and count defective beans, and coffee lots are graded based on various international systems (Farah and dos Santos, 2015).

Unroasted coffee beans are used to prepare dietary supplements because it is well known that the conventional roasting process can cause a loss of some important bioactive components, such as phenolic compounds, which in GCB are mainly represented by chlorogenic acids (CGA). However, Vella and Amen (2015) noted that processing, preserving, and packaging coffee beans in their nutritious, unroasted green state can be difficult, expensive, and sometimes not feasible, and this could be the reason why only extracts and not whole GCB are generally used in supplements. Therefore, these researchers proposed a method to produce whole or partial GCB for tableting, encapsulation, or use in mixes, additives, and supplements that consists of the following: (1) selecting coffee beans with high levels of CGA; (2) sterilizing and heating or drying the coffee beans in order to kill molds, yeasts, and bacteria and to extend the shelf life of the product containing the GCB; (3) using a grinding process that avoids heating the material at temperatures over 54°C for excessively long times and risking degradation of its nutritional components; (4) stabilizing, which includes the addition of a drying agent, such as magnesium silicate, or silicon dioxide, which prevents the material from clumping during storage and helps the process of tableting or encapsulation; (5) testing, which includes the analyses of parameters such as color, odor, taste, appearance, moisture, microbial levels, CGA, and caffeine levels; and (6) packaging as a bulk powder, compressed into tablets, inserted into capsules, added to food, or delivered in a medium for topical and cosmetic use.

BIOACTIVE COMPOUNDS FROM GREEN COFFEE

The chemical composition of green coffee depends primarily on intrinsic factors such as genetic aspects, especially the species and, to a lesser extent, on extrinsic factors such as soil composition, climate, agricultural practices, and storage conditions (Farah and Donangelo, 2006). The bioactive compound fraction in green coffee is mostly represented by CGA (Fig. 3.9.1; Ludwig et al., 2014), caffeine, diterpenes (cafestol and kahweol), trigonelline, soluble fibre, magnesium, and potassium (Jeszka-Skowron et al., 2015; Ludwig et al., 2014).

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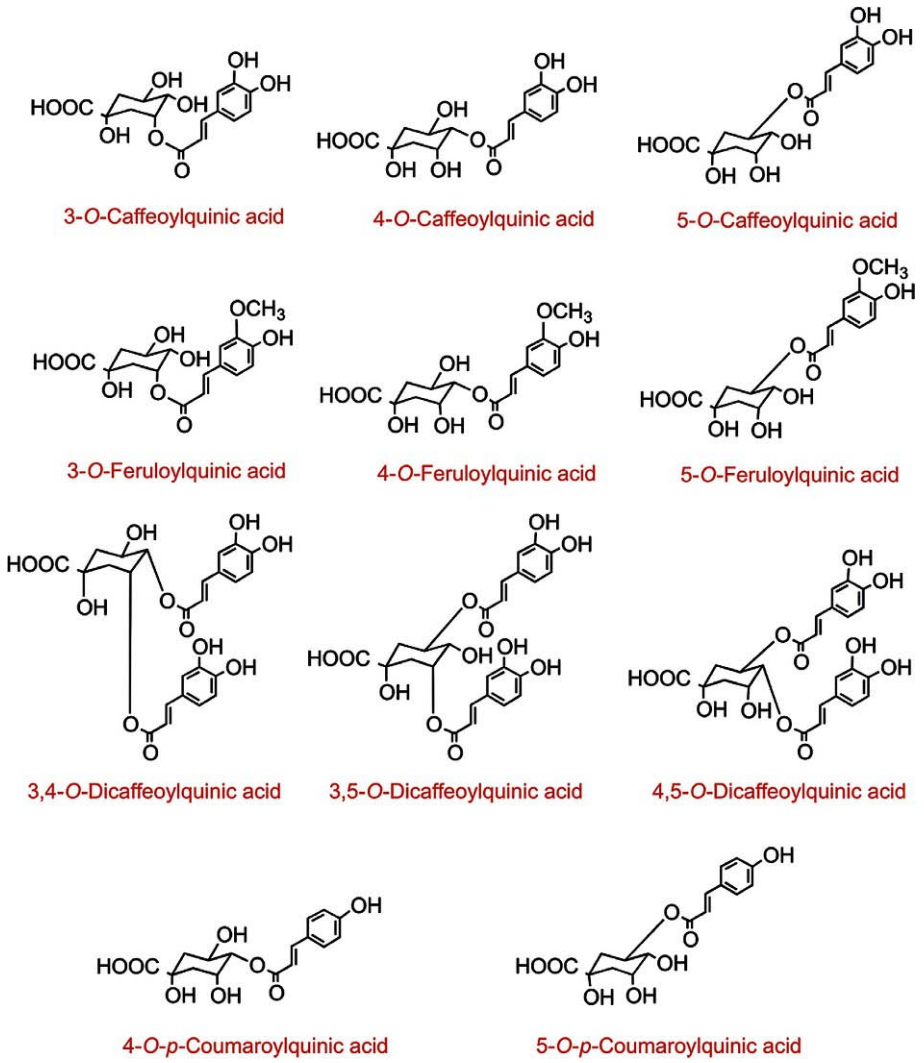

FIG. 3.9.1 Structures of the main chlorogenic acids in green coffee beans. *(Data taken from Ludwig, I.A., Clifford, M.N., Lean, M.E.J., Ashihara, H., Crozier, A., 2014. Coffee biochemistry and potential impact on health. Food Funct. 5, 1695–1717.)*

CGA are a family of esters formed between quinic or shikimic acids (the first being the most common) and specific *trans*-cinnamic acids (Clifford, 1999), which in turn, according to the substitution on their ring, give rise to different compounds such as *p*-coumaric, caffeic, ferulic, sinapic, and other acids (Baeza et al., 2016). Moreover, cinnamic acids can also be conjugated with amino acids, polysaccharides, and glycosides (Baeza et al., 2016). Therefore, a great number of compounds have been identified and quantified in GCB, mainly by the means of liquid chromatography coupled with ultraviolet and mass spectrometry detection systems (Jeszka-Skowron et al., 2015). Recently, new cinnamate esters belonging to six different chemical groups were further identified in Arabica GCB, showing that research on the chemical characterization of coffee antioxidants is ongoing (Baeza et al., 2016). Among CGA, caffeoylquinic acids (CQA), and in particular 5-caffeoylquinic acid, is the most abundant in GCB, accounting for 50%–60% of the total polyphenols, while feruloylquinic acids and dicaffeoylquinic acids are present in lower amounts, 20% and 10% of the total, respectively (Mehari et al., 2016; Monteiro and Farah, 2012; Perrone et al., 2008). Amounts between 36.3 and 61.0 mg/g of dry matter (DM) of CQAs have been reported for Arabica GCB (Alonso-Salces et al., 2009; Baeza et al., 2016), while CQA levels of 38.5–42.8 mg/g of DM were found in Robusta GCB (Alonso-Salces et al., 2009).

Caffeine (1,3,7-trimethylxanthine) is the major alkaloid in GCB, and caffeine content ranges from 0.7%–1.6% and 1.5%–4.0% for Arabica and Robusta, respectively (Hečimović et al., 2011; Ky et al., 2001). Among the other methylxanthines, theobromine has been found in some Arabica coffee at a content of 0.0018%–0.032% (Baeza et al., 2016), while theophylline has been found in some Robusta coffee at 0.077%–0.120% (Alonso-Salces et al., 2009).

Diterpenes such as kahweol and cafestol occur as fatty acyl esters in coffee (Ludwig et al., 2014). Kahweol has been proposed as a marker for Arabica coffee (Monakhova et al., 2015), but recently, it has also been found in Robusta coffee, although at considerably lower amounts (Finotello et al., 2017). The concentration values for the two diterpenes in GCB reported in the literature are very different depending on the extraction and the analytical methods used and have been reported in ranges of 8.7–9.7 g/kg (Kitzberger et al., 2013), 4.5–8.9 g/kg (Tsukui et al., 2014), and 1.5–2.7 g/kg (Finotello et al., 2017).

Trigonelline is the second major alkaloid in GCB, and its content is slightly higher in Arabica than in Robusta (Farah, 2012). Trigonelline contents in the range 0.74%–1.54% have been reported for Arabica GCB (Duarte et al., 2010; Gichimu et al., 2014; Mehari et al., 2016).

Soluble dietary fibre in coffee consists of high molecular–weight polysaccharides, mainly galactomannans and arabinogalactans, and the content of these polysaccharides does not vary between Arabica and Robusta GCB (Fischer et al., 2001). However, Farah (2012) reported polysaccharide ranges of 34–44 g/100 g for Arabica and 48–55 g/100 g for Robusta GCB.

COFFEE BY-PRODUCTS AS SOURCES OF BIOACTIVE COMPOUNDS

Recently, great attention has been devoted to coffee by-products as more economical sources of the same bioactive compounds found in GCB extracts (Fig. 3.9.2; Campos-Vega et al., 2015). Among coffee by-products, coffee silverskin (CS) has been intensively studied. CS is mainly composed of carbohydrates, in particular 30% lignin, 17.8% glucan, 4.7% xylan, 2% arabinan, 3.8% galactan, and 2.6% mannan (Mussatto et al., 2012), but it also contains some bioactive components such as CGA, caffeine, and dietary fibre. Among those, CGA, 5-caffeoylquinic, 3-caffeoylquinic, and 4-caffeoylquinic acids are the most abundant, with amounts of 198.9, 147.8, and 84.9 mg/100 g, respectively (Bresciani et al., 2014). Regazzoni et al. (2016) showed that CS is a valuable source of polyphenols, as the total amount of CGA in the corresponding extracts were similar to that of GCB (CS: 199.97 mg/g of dry extract; decaffeinated CS: 227.47 mg/g of dry extract; GCB: 212.01 mg/g of dry extract) but were lower when compared with the raw material (CS: 2.60% w/w; decaffeinated CS: 3.42% w/w; GCB: 4.39% w/w). As reported for the GCB, the amount of CGA found in Robusta CS is higher than that found in the Arabica variety (Martinez–Saez et al., 2014).

Caffeine is the main alkaloid present in CS (Toschi et al., 2014) with amounts that range from 4.1–4.4 mg/g of CS (Narita and Inouye, 2012) to 13.7 mg/g of CS (Napolitano et al., 2007), depending on the coffee variety and on the extraction method used. As reported for CGA and also for caffeine, the content in Robusta samples is higher than in the Arabica samples (Martinez–Saez et al., 2014).

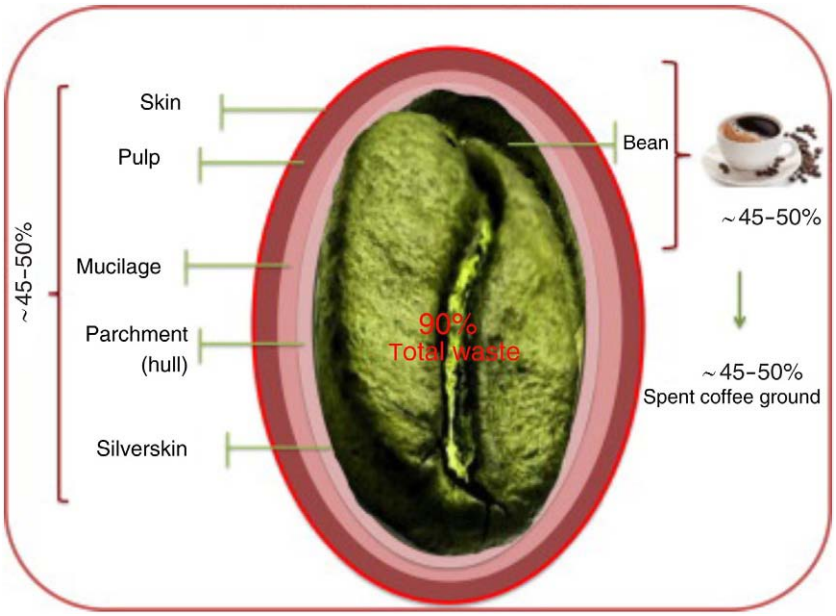

FIG. 3.9.2 Coffee cherry fruit wastes. *(Data taken from Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H.A., Oomah, B.D., 2015. Spent coffee grounds: a review on current research and future prospects. Trends Food Sci. Technol. 45, 24–36.)*

CS contains the highest quantities of total dietary fiber (80%; Murthy and Naidu, 2012) of all coffee by-products. Of the total fibre, 15% was found to be soluble dietary fibre and 85% insoluble dietary fibre in CS (Borrelli et al., 2004; Pourfarzad et al., 2013).

Recently, GCB extract, green coffee powder, or CS have been used in order to functionalize food and for the fortification of food and beverages, such as bread, soy milk (Dziki et al., 2015; Pourfarzad et al., 2013; Seczyk et al., 2017; Świeca et al., 2017), beverages for reducing body fat accumulation (Martinez–Saez et al., 2014), and biscuits (Garcia-Serna et al., 2014). In addition to these applications, due to the ability of CGA to increase the growth of total bacteria, some authors suggest its use as a prebiotic in food products such as yoghurt and yoghurt derivatives (Mills et al., 2015; Regazzoni et al., 2016). The direct application of the natural antioxidants found in coffee to food has some limitations including the degradation of the antioxidants, unpleasant flavor, and bioavailability (Aguiar et al., 2016; Chao et al., 2011; Lozano-Vazquez et al., 2015; Nedovic et al., 2011; Poshadri and Kuna, 2010; Wilson and Shah, 2007). A promising approach to overcome these problems is microencapsulation, since it protects antioxidants from heat, light, and oxygen, masks flavors, and controls the release of components (Aguiar et al., 2016; Fang and Bhandari, 2010).

AVAILABLE FORM OF SUPPLEMENTS

A number of supplements containing GCB extracts are available on the market in a variety of forms, including capsules, tablets, and powders. Among them, one of the most known is Svetol® (Naturex), a decaffeinated green coffee extract with a standardized CGA content and a specific ratio of 5-caffeoylquinic acid and other CGAs, which claims to have a slimming effect (Dellalibera et al., 2006; Henry-Vitrac et al., 2010). Fig. 3.9.3 shows some of the available products containing Svetol® as the active ingredient. Together with freeze dried coffee, Svetol® is one of the ingredients in CoffeeSlender® (Body Slender, 2018), a supplement that reduces body weight and fat (Thom, 2007). A novel, patent-pending (PCT/IN2015/000236 dated June 10, 2015), water-soluble GCB extract, named GCB-70 (total CGA 70%; CQA 45%, and caffeine <1%), has been developed as an antioxidant supplement and is effective in healthy weight management (Bagchi et al., 2017; Swaroop et al., 2014). Other products contain green coffee as one of the ingredients, such as Lisopresol®,

FIG. 3.9.3 Different types of products using Svetol® as an ingredient. *(Data taken from www.svetol.com).*

which is a nutraceutical mint-flavored gum containing *Garcinia cambogia*, green coffee extract (GCE), and L-carnitine that claims to aid in the control of snack intake (Bobillo et al., 2016). A tablet formulation combining GCB extract (from Svetol®) with olive leaf extract and beet powder (from two other commercial supplements) has also been tested for its antihypertensive potential (Wong et al., 2014)

RELATION WITH HEALTH

The main reason that supplements containing GCB or GCE are considered beneficial for human health is the presence of CGA. By acting as potent antioxidants, CGA scavenge free radicals, chelate metals, reduce lipid peroxidation, and inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity (Clifford, 1999). It is well known that reactive oxygen species, increasingly observed as primary upstream agents in the signaling cascades involved in inflammatory responses, are implicated in the pathogenesis of most chronic diseases (including diabetes, cancer, and cardiovascular disease). Thus CGA can play an important role in their treatment. Very recently, Tajik et al. (2017) reviewed the potential health effects of CGA by a systematic examination of in vivo animal and human studies. A summary of the principal results from the human studies, although limited in number, is reported here. The consumption of GCB extracts or CGA causes a significant reduction in systolic and diastolic blood pressure both in mildly hypertensive patients and in healthy adults (Kozuma et al., 2005; Mubarak et al., 2012; Ochiai et al., 2004; Revuelta-Iniesta and Al-Dujaili, 2014; Watanabe et al., 2006; Yamaguchi et al., 2008), while controversial results were obtained regarding their effects on endothelial function (Mubarak et al., 2012; Olthof et al., 2001), the impairment of which develops in diseases such as hypertension, diabetes, and metabolic syndrome and can contribute to the development of arteriosclerosis. Lecoultre et al. (2014) showed that the consumption of both caffeinated and decaffeinated coffees has positive effects on glucose and lipid metabolism in healthy subjects, while CGA and trigonelline reduced glucose and insulin concentrations in overweight men (following an oral glucose tolerance test) compared to a placebo (Van Dijk et al., 2009). It has also been reported that drinking three to four cups of decaffeinated coffee with a high content of CGA reduced the risk of type 2 diabetes mellitus by 30% (Huxley et al., 2009). Insulin resistance is a main obstacle in diabetes treatment and plays a pivotal role in the pathogenesis and clinical course of several important diseases. The consumption of coffee rich in CGA is able to stimulate the secretion of glucagon-like peptide-1, which is known to augment insulin secretion, after oral glucose consumption in healthy volunteers (Johnston et al., 2003); however, Olthof et al. (2011) did not obtain the same results in overweight men. Obesity is one of the main risk factors for cardiovascular disease (Ogden et al., 2007), and medicinal, and an increasing number of nutraceutical, interventions address this issue. The results for the effectiveness of supplements based on GCB extracts/CGA are controversial. Favorable effects of the intake of coffee rich in CGA or GCB extracts rich in CGA on body weight control in overweight adults have been observed in studies carried out by Dellalibera et al. (2006) and Thom (2007). However, according to the European Food Safety Authority (EFSA) scientific panel (EFSA, 2011), important methodological limitations of these studies prevent the scientific substantiation of the claimed effect. Other human studies performed in 2010 and 2011 also showed that the intake of CGA significantly reduces body weight (Bakuradze et al., 2011; Onakpoya et al., 2011; Revuelta-Iniesta and Al-Dujaili, 2014), while the same effect was not observed by Kotyczka et al. (2011) and Watanabe et al. (2006).

It has been observed that the activation of the Nrf2/ARE pathway is an important mechanism for protecting cells and tissues from carcinogenesis and carcinogenic metabolites (Boettler et al., 2011). Studies conducted on healthy volunteers showed that the consumption of coffee rich in CGA modulated ARE-dependent transcription and significantly increased Nrf2 transcription (Bakuradze et al., 2014; Volz et al., 2012).

Human studies on the effect of CGA on brain functions have also been performed (Camfield et al., 2013; Cropley et al., 2012; Reyes-Izquierdo et al., 2013). The major concern seems to be that CGA do not improve cognitive functions (attention, decision time, and alertness) but were found to improve headache symptoms (Camfield et al., 2013). Instead, it is caffeine that has the most important effects on the central nervous system (CNS), as it promotes wakefulness, enhances mood and cognition, and produces stimulatory effects (Haskell et al., 2005; Nehlig et al., 1992). Moreover, a number of studies have demonstrated a protective role of caffeine from neurodegenerative diseases, such as Alzheimer's, Parkinson's, and multiple sclerosis (Madeira et al., 2017; Rivera-Oliver and Díaz-Ríos, 2014). The neuroprotection effect of caffeine seems to be linked to the blockage of one of the adenosine receptors (A_{2A}) in microglial cells, which are regulators of homeostasis in the CNS but are also responsible for the inflammatory response (Madeira et al., 2017).

The diterpenes kahweol and cafestol are instead reported to be responsible for increasing serum cholesterol (Jee et al., 2001; Rebello and van Dam, 2013; Urgert and Katan, 1997). For this reason, the consumption of filtered coffees, which contain smaller amounts of these compounds, has been suggested (Ludwig et al., 2014).

DOSE AND ADVERSE EFFECTS

An appropriate range for the dosage of coffee supplements has not yet been established. However, if caffeinated products are assumed, it should be taken into account that the safe daily intake of caffeine for adults has been estimated to be 400 mg, equivalent to approximately seven cups of coffee, while specific populations such as pregnant women, elderly people, or hypertensive subjects should limit their intake to 200 mg/day (da Justa Neves and Caldas, 2017; EFSA, 2015). Even if the response to the caffeine dose strongly varies between individuals (Rogers et al., 2010), it can be assumed that caffeine consumption greater than the safe dose can cause adverse effects, such as tachycardia, insomnia, nervousness, headaches, abdominal pain, nausea, vomiting, diarrhea and dieresis (Clark and Landolt, 2017; Higdon and Frei, 2006; Nawrot et al., 2003; Riksen et al., 2011). Concerning CGA, the other main constituent of coffee supplements, appropriate doses have not yet been recommended; different amounts of CGA, between 100 mg and 2 g, have been administered for variable periods (2 h–16 weeks) to humans in the studies reported above (Camfield et al., 2013; Cropley et al., 2012; Kozuma et al., 2005; Mubarak et al., 2012; Ochiai et al., 2004; Olthof et al., 2001; Revuelta-Iniesta and Al-Dujaili, 2014; Van Dijk et al., 2009; Watanabe et al., 2006; Yamaguchi et al., 2008). However, the long-term effects of CGA at these doses have not yet been examined (Stohs and Badmaev, 2016). With regard to the safety of the coffee dietary supplements, another fact that should be considered is that toxic compounds, such as mycotoxins and derived carboxyatractyligenin compounds, have been found in commercially available products (Lang et al., 2014; Vaclavik et al., 2013). Therefore, there is an urgent need to monitor the quality of these products and assure their safety because such products are already available on the market, and they are being consumed daily and in large amounts.

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Chapter 3.10

Chlorella

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INTRODUCTION

Microalgae are a diverse group of photosynthetic, prokaryotic or eukaryotic microorganisms that exhibit a high growth rate due to their extremely simple structure. These include species of different phyla, such as Cyanophyta, Chlorophyta, Cryptophyta, Haptophyta, Pyrrophyta, Streptophyta, and Heterokontophyta (Guedes et al., 2014; Santhosh et al., 2016). Microalgae are unicellular or multicellular organisms that have the capacity to grow with little water, nutrients, or carbon dioxide, can absorb solar energy, and have the capacity to use photosynthesis as a mechanism to acquire energy (Ahmad et al., 2011). Microalgae can be found mostly in fresh or salty water, but also on surfaces of different types of soil (Katarzyna et al., 2015; Tomaselli, 2004). They are of great ecological importance for their ability to adapt to conditions with adverse temperature variations, light, pH, salinity, and humidity, as well as their reduced need for nutrients and their ability to grow in inhospitable environments such as deserts (Guedes et al., 2014; Katiyar et al., 2017). In the early 1950s in the USA, Japan, and Israel large-scale experiments with biotechnological purposes were started (Guedes et al., 2011), including industrial-scale cultivation of *Chlorella* sp. Later, in the 1980s, there were about 60 production units in Asia.

Thus, in recent years a diverse range of microalgae applications have been achieved, from their use in the production of biofuel to their use in the traditional production of biomass for human and animal nutrition, soil conditioning, bioremediation, formulation of cosmetic products and pharmaceuticals, high-technology applications, based on the discovery of new compounds within the microalgae, and for medical research and diagnosis (Katiyar et al., 2017; Santhosh et al., 2016; Velmurugan and Incharoensakdi, 2017). Thus, microalgae have attracted high levels of commercial interest due to their potential to produce valuable products and high levels of biomass (Guedes et al., 2014). In addition, although each resource has its own benefits and obstacles, on an industrial scale, considering all the energy resources currently available, microalgae appear to be a fantastic alternative, in all respects due to their high rate of production in terms of biofuels, to other energy crops and microorganisms (Katiyar et al., 2017).

Some microalgae representing the Chlorophycea class, such as the genera *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Chlamydomonas*, *Botryoccocus*, and *Dunaliella*, have been reported in the literature due to their high production of biomass. Among the various organisms in this class, it is possible to highlight the genus *Chlorella*, which is unicellular green algae, spherical, showing asexual reproduction. Moreover it is autotrophic and it contains the photosynthetic pigments chlorophyll a and chlorophyll b, which are located in its chloroplasts. These microalgae are representative of the phytoplankton of lakes and ponds and can also be found in soils (Qiao et al., 2009). The demand for this type of microalgae has been continuously increasing due to its high nutritional value, leading to its addition to diathetic substitutes, cosmetic products, antiinflammatory products, among others (Katiyar et al., 2017).

SOURCES OF *CHLORELLA*

Currently, it is estimated that there are 50,000 different microalgae species, these microorganisms have been classified according to the chemical nature of the pigments resulting from their metabolism and their respective cell wall constituents (Hu et al., 2008). The most common classification among the scientific community divides microalgae into two distinct groups depending on their cellular structure. Thus, prokaryotic microalgae belong to the Cyanophyta and Prochlorophyta divisions, whereas the eukaryotic microalgae are grouped into the following divisions: Chlorophyta, Euglenophyta, Rhodophyta, Haptophyta (Prymnesiophyta), Heterokontophyta (Bacillariophyceae, Chrysophyceae, Xantophyceae, among others), Cryptophyta, and Dinophyta (Derner et al., 2006). *Chlorella* is a group of eukaryotic green microalgae with a high

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capacity for photosynthesis, which able the reproduce in several hours and requiring only sunlight, carbon dioxide, water, and a small amount of nutrients (Mata et al., 2010; Nigam and Singh, 2011). These microalgae are easy to grow, have simple life cycles and metabolic pathways similar to higher plants and therefore have been employed as model organisms for research on the mechanisms of photosynthesis and assimilation of carbon dioxide. In addition, due to its high protein content and richness in carotenoids, vitamins, and minerals, *Chlorella* have been widely produced as a health food source in Germany, China, Japan, and several other Asian countries, having been proposed as a food substitute for humans. On the other hand, due to the energy crisis and the public interest in green, renewable fuels, *Chlorella* have also been described as promising candidates for the raw materials required for the production of biofuels, with a growing interest from scientific and industrial communities (Liu and Chen, 2014). Outdoor cultivation of these microalgae began in the late 1940s with an almost simultaneous launch in the USA, Germany, and Japan (Burlew, 1964). The cultivation of *Chlorella* has become one of the most interesting topics for many researchers in biotechnology leading to the development of several crop systems, which may be phototrophic, heterotrophic, or mixotrophic in open-culture or closed-culture systems (Liu et al., 2014). Over the past few years several researchers have demonstrated different applications for this microalgae in several industrial areas, namely, food, pharmaceutical, cosmetic, among others (Priyadarshani and Rath, 2012), having already extracted high-value chemical compounds, such as carotenoids (astaxanthin, lutein, β-carotene, violaxanthin, and zeaxanthin), antioxidants, vitamins, polysaccharides, proteins, peptides, fatty acids, among others (Mata et al., 2010).

FATTY ACIDS

Due to changes in the human diet over the past few centuries, and to the rise of a number of diseases related to the low consumption of fatty acids, there has been a great focus on the search for potential sources of compounds with nutritive properties. *Chlorella* is a rich source in mono/polyunsaturated fatty acids and may be comprised of 30%–60% of these compounds (Yusof et al., 2011). In this way, *Chlorella* extract has been applied as a supplement, with health benefits, or even in foods (Yusof et al., 2011). However, the fatty acid content of this microalgae can be influenced by the environmental conditions in which it grows (Petkov and Garcia, 2007).

CAROTENOIDS

Carotenoids are natural pigments biosynthesized by all photosynthetic plants, protists, bacteria, among others. Humans are unable to synthesize carotenoids and require a dietary intake to meet their daily health requirements. Microalgae are a rich source of carotenoids as in the case of *Chlorella* which contains up to 0.2% (Guedes et al., 2011; Varela et al., 2015). The carotenoids identified in these microalgae are astaxanthin, zeaxanthin, violaxanthin, and lutein which are already industrially produced synthetically for use in a variety of food products and cosmetics. Therefore, these caratenoids are not only a valuable source of natural compounds, they are also potential functional foods that are currently being studied as chemopreventive agents against inflammation and cancer (Guedes et al., 2011; Jin et al., 2003). Soontornchaiboon et al. (2012) evaluated the antiinflammatory effects of the carotenoid violaxanthin, isolated from *Chlorella ellipsoidea*, on RAW 264.7 cells stimulated with lipopolysaccharide (LPS). Violaxanthin inhibited significantly nitric oxide (NO) and prostaglandin E2 (PGE2) expression. Moreover, this carotenoid effectively inhibited the LPS-mediated nuclear factor-κB (NF-κB) p65 subunit translocation into the nucleus, suggesting that violaxanthin's antiinflammatory activity may be based on inhibition of the NF-κB pathways.

SULFATED EXTRAPOLYSACCHARIDES

Sulfated extrapolysaccharides (EPSs) are part of a class of high value-added biopolymers with a wide variety of applications. Microbial cells can be a rich source of carbohydrate molecules. Some of these are components of cell walls, while others can be found completely dissociated from the cell and are known as exopolysaccharides (Liu et al., 2016). *Chlorella* is a rich source of exopolysaccharides containing about 8% of these compounds (Guzmán et al., 2003; Hasui et al., 1995; Staats et al., 1999). These compounds have been shown to have beneficial effects such antiinflammatory, antioxidant, anticoagulant, antimicrobial, etc. Kaplan et al. (1987) compared the ability of exopolysaccharides, isolated from *Chlorella stigmatophora*, to bind to toxic heavy metals compared to other species of the genus *Chlorella*, noting the composition of polysaccharides, not the content of uronic acids. However, the toxicity and bioavailability of these compounds have not yet been studied in humans (Raposo et al., 2013).

SUPPLEMENTS AND FUNCTIONAL FOODS OBTAINED FROM *CHLORELLA* GENUS

The evolution of the human diet over the last few years has adversely affected health, leading to an increase in the incidence of chronic diseases, including obesity, diabetes mellitus, cardiovascular diseases, hypertension, strokes, and cancers

(Booth et al., 2012). The increase in demand by consumers for healthy, "beneficial" products led to an increased consumption of functional foods and ingredients, due to their biological activities. Moreover, an increase in health care costs and average life expectancy, as well as the general population's demand for a better quality of life, boosted studies on bioactive compounds. The health benefits attributed to foods rich in phenolic compounds and other antioxidants (such as ascorbic acid and carotenoids), in terms of their chemical composition, has increased the demand for new botanical species that have, in addition to this property, a relevant complementary biological activity (Céspedes et al., 2008). In this perspective, microalgae can play an important role as source of natural products and bioactive compounds, and can be used instead of synthetics (Mariutti and Bragagnolo, 2007). The genus *Chlorella* is often used as a food supplement as well as in the cosmetics and pharmaceutical industry, since it contains in its composition proteins, carotenoids, some immunostimulators, polysaccharides, vitamins, and minerals (Nicoletti, 2016). In the particular case of *Chlorella vulgaris*, this species has been shown to be rich in ascorbic acid, tocopherol, vitamins, minerals, protein, polysaccharides (da Silva Vaz et al., 2016; Vijayavel et al., 2007), carotenoids and chlorophylls—particularly lutein, chlorophyll a and b, and pheophytin a—being one of the most important compounds in terms of the antioxidant capacity of this species (Cha et al., 2010). The fact that this microalgae has high productivity has resulted in it being one of the microorganisms most commonly used in the production of algae biomass used for different biotechnological applications, showing promising results mainly in the nutraceutical and pharmaceutical industries. Although the introduction of microalgae in human food, as a source of protein, dates back to distant times, it was only in the 20th century that the commercialization of microalgae as a nutritional food source started, with it only being considered a functional food a few years ago (da Silva Vaz et al., 2016; Kent et al., 2015; Kovač et al., 2013). *Chlorella* sp. have been incorporated into various types of human food, such as natural food or food supplements, and can be found in various formulations: powders, tablets, capsules, or extracts (Sousa et al., 2008). In addition, extracts from these microalgae have also been incorporated in the form of pasta, snacks, sweets, and beans and marketed as a food supplement in the form of food additives, dyes, and emulsifiers, improving the nutritive properties of these products (da Silva Vaz et al., 2016). Fradique et al. (2010) added extract of *C. vulgaris* in fresh spaghetti and compared the quality of the pasta and the cooking, color, texture, and sensorial parameters. These authors concluded that the addition of this microalga to the spaghetti originated a product with better chemical composition without affecting the cooking quality. Gouveia et al. (2007) used *C. vulgaris* to give a greenish color to buttery biscuits. Merchant and Andre (2001) evaluated the potential of dietary supplements of *Chlorella pyrenoidosa* in improving quality of life and normalization of body functions in people with chronic diseases, specifically fibromyalgia, hypertension, and ulcerative colitis. These authors verified that the use of this microalga in the form of dietary supplements reduced the high blood pressure and cholesterol levels in patients with chronic diseases. In addition, *Chlorella* (*Chlorella* sp.) as a dietary supplement containing biologically active vitamin B12 (Tang and Suter, 2011; Watanabe et al., 2014) is already being marketed, allowing patients who have a vitamin deficiency to restore their levels, reducing the risks of developing anemia and/or neurological disorders (O'Leary and Samman, 2010).

CLINICAL ASPECTS AND SCIENTIFIC EVIDENCE OF ISOLATED COMPOUNDS FROM *CHLORELLA* GENUS

The demand for bioactive compounds from microalgae is increasingly being met, because commercial cultivation has started, allowing specific molecules of interest to be obtained. Thus, due to the enormous biodiversity of microalgae, as well as their biochemical and molecular strategies to deal with stressful environments, they can synthesize several bioactive compounds (Mimouni et al., 2012). Among the various groups of microalgae, green algae provide an exceptional source of bioactive compounds, such as the genus *Chlorella* (da Silva Vaz et al., 2016; Safafar et al., 2015). Several studies on the bioactive compounds of microalgae have shown that these compounds may have beneficial biological activities, such as antitumour, chemopreventive, antiinflammatory, antimicrobial, and antioxidant properties (da Silva Vaz et al., 2016; Olasehinde et al., 2017; Safafar et al., 2015; Talero et al., 2015; Thomas and Kim, 2013). Several researchers have demonstrated the production of secondary metabolites from *C. vulgaris* that behave as an antibiotic against Gram-positive and Gram-negative bacteria, as well as acting as an antifungal (Cha et al., 2010; Guedes et al., 2014). Other researchers have reported that *Chlorella* have high levels of chlorophyll when compared to several other species of microalgae (Morais et al., 2015). The high activity of chlorophyll has shown pharmaceutical benefits especially in the treatment of ulcers in the liver. Chlorophyll isolated from this microalga was also investigated as a source of pigments for cosmetics (Cuellar-Bermudez et al., 2015; Yaakob et al., 2014). An example of a product commercially available from PROTEC Ingredia is an extract of *C. vulgaris* which stimulates the synthesis of collagen in the skin, regenerating tissues, contributing to a reduction in aging (Ariede et al., 2017). Maadane et al. (2017) evaluated antimicrobial activity against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* yeast, and *Aspergillus niger* fungi. These authors verified that ethanolic extract from *Chlorella* sp. did not exhibit antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *A. niger*

fungi, but showed antimicrobial potential against *C. albicans* yeast (ICM: > 5mg/mL). Liu and Chen (2014) have described that a carotenoid, astaxanthin, isolated from *Chlorella zofingiensis* has the potential to protect organisms against a wide range of diseases with promising applications in human health. Astaxanthin alone has been shown to have antitumour, antioxidant, and antiinflammatory effects (Liu and Chen, 2014; Yuan et al., 2011). The carotenoid lutein was also isolated from *Chlorella sorokiniana* and presented antitumor, antioxidant, and antiinflammatory potential (Cordero et al., 2011; Shi and Chen, 2002). On the other hand, Nomoto et al. (1983) isolated sulfated polysaccharide B-(1,3)-glucan from *Chlorella regulis* and tested its antitumour effect against murine transplanted tumors. They observed that this sulfated polysaccharide demonstrated antitumor activity. This polysaccharide was also isolated by Guzmán et al. (2003) from *C. stigmatophora*, which demonstrated having an antiinflammatory effect in the paw edema test as well as having an immunosuppressive effect (Table 3.10.1).

Regarding clinical evidence, there are several clinical studies that have already showed the great potential of *Chlorella*. The dietary supplementation of humans diagnosed with mild hypertension or moderate hypertension with *Chlorella* led to a significant reduction in total serum cholesterol and both lipoprotein cholesterol of low-density and high-density. The results obtained by these authors suggested that daily dietary supplementation of *Chlorella* alone may contribute to reducing or stabilizing the clinical condition of mild or moderate hypertension (Merchant et al., 2002). Ryu et al. (2014) also observed that daily consumption of *Chlorella* supplements by mildly hypercholesterolemic subjects promoted a reduction of serum lipid risk factors, mainly triglycerides and total cholesterol. On other hand the administration of *C. vulgaris* in chronic smokers over a 6-week period enhanced antioxidant status and reduced lipid peroxidation suggesting that its use may contribute to prevent the problems inherent with smoking (Panahi et al., 2013). Moreover the use of *C. vulgaris* as an adjuvant in the therapeutic regimen of nonalcoholic fatty liver disease (NAFLD) promoted several beneficial effects, namely, sensitivity to insulin and the reduction of triglyceride and transaminase levels in serum (Panahi et al., 2012). Recently, Ebrahimi-Mameghani et al. (2016) also observed that the incorporation of *C. vulgaris* in the diet of NAFLD patients allowed them to reduce weight and reduce their high sensitivity to C-reactive protein, improving their glycemic condition and contributing to their liver function improvement. The addition of *C. pyrenoidosa* in the diet of patients with malignant glioma provided several benefits including maintaining cellular components and functions of the immune system near to

TABLE 3.10.1 Compounds Obtained From the *Chlorella* Genus and Their Biological Activities

	Compound	Source	Biological activity	References
Carotenoid	β-carotene	*Chlorella sorokiniana*	Antiinflammatory anticancer	Cordero et al. (2011)
	Astaxanthin	*Chlorella zofigiensis*	Antioxidant Antiinflammatory anticancer	Liu et al. (2014)
	Lutein	*C. sorokiniana* *Chlorella prothecoides*	Antioxidant Antiinflammatory anticancer	Cordero et al. (2011); Shi and Chen (2002)
	Violaxanthin	*Chlorella tertiolecta*	Antiinflammatory	Soontornchaiboon et al. (2012)
	Zeaxanthin	*Chlorella saccharophila*	Antioxidant Antiinflammatory	Singh et al. (2013)
Polysaccharide	Sulfated polysaccharide β-(1,3)-glucan	*Chlorella stigmatophora* *Chlorella vulgaris*	Inmunomodulating anticancer	Guzmán et al. (2003); Nomoto et al. (1983)
	Exopolysaccharide	*C. stigmatophora*	Metal-binding	Kaplan et al. (1987)
Protein	Peptides	*C. pyrenoidosa*	Antioxidant Antiinflammatory anticancer	Wang and Zhang (2013)
Others	Phenolic compounds	*Chlorella ellipsoidea*	Antioxidant	Abd El-Baky et al. (2010); Cha et al. (2011)

Source: Adapted from Talero, E., García-Mauriño, S., Ávila-Román, J., Rodríguez-Luna, A., Alcaide, A., Motilva, V., 2015. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Mar. Drugs 13, 6152–6209.

normal levels and reducing side-effects in patients undergoing chemotherapy and/or taking immunosuppressive medication. In addition, these patients also presented reduced occurrence of respiratory infections and flu-like illnesses (Merchant et al., 1990). A nutritional supplement derived from *C. pyrenoidosa* was added in the diet of some patients with fibromyalgia syndrome, reducing the symptoms of the disease (Merchant et al., 2000). Nakano et al. (2010) studied the preventive effects of *Chlorella* supplements on the development of anemia during pregnancy. The study, which included 70 pregnant women, demonstrated that the inclusion of *C. pyrenoidosa* in the diet reduced the risk of anemia, proteinuria, and edema.

INTERACTIONS OF SELECTED SUBSTANCES WITH OTHER FOOD SUPPLEMENTS/DRUGS/FOODS (SAFETY AND TOXICITY)

As described earlier in this chapter, *Chlorella* supplements demonstrate several health benefits. However, they should be used with care, since they can interact with drugs, other food supplements, or foods. Regarding interactions with drugs, *Chlorella* due to its high vitamin K content, may interact with warfarin, inhibiting its anticoagulant effect (Ohkawa et al., 1995). Moreover, the inclusion of *Chlorella* in the diet has also demonstrated a reduction in blood pressure (Merchant and Andre, 2001). Consequently, caution is required when used on patients that are hypotensive or taking antihypertensive drugs. Due its immune-stimulating properties, patients that are administered immune-suppressing drugs should avoid taking *Chlorella* supplements. Since several food supplements, or foods, mediate similar effects to those exhibited by *Chlorella* supplements, their combinations may induce synergistic effects which are dangerous to human health. For instance plants such as garlic commonly used in the human diet are described as to possess hypotensive properties (DalBó and de Aguiar Amaral, 2017). Consequently, the interaction of bioactives should be evaluated in order to ensure a safety use of these drugs/food supplements/foods in combination.

CONCLUSIONS

The cultivation of *Chlorella* for the production of biomass and derivative products is an industrial activity that has already been established on a commercial scale in several countries. The interest in these microalgae is due to their rapid growth and simple life cycles, allowing in-depth studies of their mechanisms and use as a food substitute due to their high protein, carotenoids, vitamin, and mineral content. In this way, *Chlorella* have been extensively studied to obtain biomass or to extract bioactive compounds with potential applications in functional food supplements, as well as nutraceuticals, cosmetics, and pharmaceuticals. This advantage for human and clinical nutrition is due to their biological functions with health benefits. Several studies of the bioactive compounds of microalgae have shown that these compounds may have beneficial biological activities, not limited to antitumour, antiinflammatory, antimicrobial, antioxidant, and anticoagulant properties. In this way, *Chlorella* can become a source of bioactive compounds or even be able to be applied as a functional food promoting the prospect of sustainable health benefits.

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Chapter 3.11

Cruciferous (Brassicaceae) Vegetables

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INTRODUCTION

Brassicaceae, often called Cruciferae or mustard family, comprise many economically important species that are grown worldwide. They have been traditionally consumed in the human diet as fresh and preserved vegetables, vegetable oils, and condiments, from ancient times to the present time. They represent a monophyletic group distributed all over the world except Antarctica and contain approximately 338 genera and 3709 species (Al-Shehbaz et al., 2006). The Brassicaceae originated in the Eocene in the Irano-Turanian region from where they spread worldwide (Franzke et al., 2011). According Sanskrit records the use of cruciferous crops was documented in India as far back as 3000 BC, while some other ancestral data show that *Brassica* crops were grown along coastal Europe near to 8000 years ago (Al-Shehbaz, 2011). Nowadays Brassicaceae vegetables are highly diversified in Mediterranean Europe, Asia, and North America (Al-Shehbaz, 2011).

A key agricultural genus of the Brassicaceae family is the *Brassica* genus which contains oilseed (canola, mustard) and vegetable (cabbage, broccoli, bok choy) crops with a long history of agricultural usage on all the continents (Chen et al., 2011) (Table 3.11.1). The most grown and utilized *Brassica* vegetables include *Brassica oleracea* and *Brassica rapa* which are almost completely edible (leaves, inflorescence, root, stem, and seed), while the seeds of *Brassica nigra*, *Brassica carinata*, and *Brassica juncea* are also used as a condiment (Table 3.11.1.).

According to the FAOSTAT the *Brassica* oilseeds *Brassica napus*, *B. rapa*, *B. juncea* and *B. carinata* provide 12% of the world's supply of edible vegetable oil although some of them are used as leaf and root vegetables (http://faostat.fao.org/). The principal *Brassica* vegetable species is *B. oleracea* which includes different headed and nonheaded cabbages, kale, broccoli, Brussels sprouts, cauliflower, and others. The cultivars of *B. oleracea* are grouped, based on morphology and developmental forms, into seven major cultivar groups: *B. oleracea* "Capitata group" (different varieties of headed cabbage), *B. oleracea* "Acephala group" (kale and collard greens), *B. oleracea* "Alboglabra group" (Chinese broccoli or Kai-lan), *B. oleracea* "Botrytis group" (cauliflower, Romanesco broccoli, and broccoflower), *B. oleracea* "Italica group" (broccoli), *B. oleracea* "Gemmifera group" (Brussels sprouts), and *B. oleracea* "Gongylodes group" (kohlrabi) (Rakow, 2004). They may be used fresh as a salad, fresh or dried as a spice, cooked, fried, baked, or fermented.

Different Brassicaceae species, in addition to their culinary use, have been extensively used in traditional medicine from ancient times to the present day. In the last couple of decades epidemiological studies have provided evidence that diets rich in cruciferous vegetables are associated with a lower risk of several types of cancer (Liu et al., 2012; Liu and Lv, 2013; Liu et al., 2013a,b; Wu et al., 2013a,b,c). These findings allow the recognition of cruciferous vegetables as functional foods and numerous dietary supplements containing a variety of extracts or compounds isolated from cruciferous vegetables are already available on the market. The health benefits of *Brassica* plants are mainly associated with their sulfur-containing compounds known as glucosinolates and their hydrolysis products indoles (reviewed by Murillo and Mehta, 2001). In addition to glucosinolates, cruciferous vegetables are rich in other nonvitamin and nonmineral health-promoting compounds such as polyphenols and triterpenes (Jahangir et al., 2009; Šamec et al., 2017). All these phytochemicals have additive and synergistic effects that may contribute to anticancer, antioxidant, antiinflammatory, and cardioprotective activities associated recently with the consumption of cruciferous vegetables.

BIOLOGICAL ACTIVITIES

Anticancer Activity

Epidemiological studies on *Brassica* vegetables and their potential for cancer prevention have been the focus of scientific studies for almost half a century (Han et al., 2014; Higdonm et al., 2007; Kristal and Lampe, 2002; Latté et al., 2011;

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00027-8

TABLE 3.11.1 Some of the Most Important Crops From the Brassicaceae Family Forming Part of the Human Diet

Genus, species	Cultivar (group)	Common name	Edable part
B. oleracea	var. *capitata*	Cabbage (white, red, cone etc.)	Leaves
	var. *acephala*	Kale	Leaves
	var. *viridis*	Collard greens	leaves
	var. *alboglabra*	Chinese brocolli, Kai-lan	Leaves
	var. *gemmifera*	Brussels sprouts	Buds
	var. *gongylodes*	Kohlrabi	Stem
	var. *botrytis*	Cauliflower	Inflorescence
	var. *italica*	Broccoli	Inflorescence
	var. *botrytis*	Romanesco broccoli	Inflorescence
	var. *italica* × *alboglabra*	Brocollini, broccoflower	Inflorescence
B. rapa	ssp. *rapa*	Turnip	Root
	ssp. *pekinensis*	Chinese cabbage, napa cabbage	Leaves
	ssp. *narinosa* (or *rosularis*)	Asian greens	Leaves
	ssp. *chinensis*	Bok Choy, Pak Choy	Leaves
	ssp. *pervidis*	Komatsuna, Japanese mustard spinach	Leaves
	ssp. *nipposinica*	Mizuna	Leaves
	ssp. *parachinensis*	Rapini, broccoli rabe	Leaves, stem, flower buds
B. napus	var. *napobrassica*	Rutabaga (swede)	Root
	var. *pabularia*	Siberian kale	Leaves
	var. *oleifera*	Rapeseed	Seeds
B. juncea	var. *rugosa* (or *integrifolia*)	Mustard greens	Leaves
		Brown Indian mustard	Seeds
B. nigra		Black mustard	Seeds
B. carinata		Ethiopian mustard	Leaves, seeds
Brassica hirta		White mustard	Seeds
Amoracia rusticana		Horsradish	Root
Barbarea verna		Land cress	Leaves
Eruca vesicaria		Arugula (rocket)	Leaves, stems
Lepidium sativum		Garden cress	Leaves, stems
Nasturtium officinale		Watercress	Leaves, stems
Raphanus sativus		Radish	Root
	var. *longipinnatus*	Daikon	Root
Wasabia japonica		Wasabi	Root

Murillo and Mehta, 2001; Tse and Eslick, 2014; Verhoeven et al., 1996). Commonly, epidemiological studies have given inconsistent conclusions. In order to pool the relevant studies together and gain more clear conclusions, different meta-analyses were performed which demonstrated an inverse relationship between cruciferous vegetable consumption and the risk of breast cancer (Liu and Lv, 2013), ovarian cancer (Han et al., 2014), prostate cancer (Liu et al., 2012), gastric cancer (Wu et al., 2013c), colorectal cancer (Wu et al., 2013a), lung cancer (Wu et al., 2013b), bladder cancer (Liu et al., 2013a), and renal cell carcinoma (Liu et al., 2013b).

Mechanisms underlying the anticancer activity of *Brassica* vegetables have been attributed to the decomposition products of the abundant secondary metabolites, glucosinolates (Jahangir et al., 2009). Glucosinolates core structure contains a sulfated isothiocyanate group linked to thioglucose and side-chain groups. Modification of the chain group results in diverse chemical structures, covering around 200 aliphatic, aromatic, and indolic glucosinolates. Each type of cruciferous vegetable shows a characteristic glucosinolates profile. Usually, as many as 15 different glucosinolates have been found in different *Brassica* species/varieties, but only 3–4 predominate (Fahey et al., 2001). Glucosinolates are not bioactive until they have been enzymatically hydrolyzed, by the endogenous plant enzyme myrosinase, to various bioactive breakdown products (Cartea and Velasco, 2008). General glucosinolate breakdown products are isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidinethiones (Vaughn and Berhow, 2005). The hydrolysis product of glucosinolate glucobrassicin, indole-3-carbinol, has received considerable interest as a cancer chemoprotective agent and has been studied in vitro and in vivo. Indole-3-carbinol is unstable in the acidic conditions of the stomach and may rapidly be converted to a series of oligomeric products among which 3,3'-diindolylmethane is a major component (Fig. 3.11.1).

Both, indole-3-carbinol and its dimeric product 3,3'-diindolylmethane, have been shown to affect a number of cellular functions and appear to be responsible for many physiological effects. They target multiple aspects of cancer cell–cycle regulation and survival including Akt-NFjB signaling, caspase activation, cyclin-dependent kinase activities, estrogen metabolism, estrogen receptor signaling, endoplasmic reticulum stress, and BRCA (BReast CAncer) gene expression (Weng et al., 2008). Results from early phase clinical trials suggested that indole-3-carbinol is well tolerated at doses of 400 and 800 mg (Reed et al., 2005) and may be effective against precancerous cervical dysplasia (Bell et al., 2000) and vulvar intraepithelial neoplasia (Naik et al., 2006). Consequently, a lot of food supplements for women contain indole-3-carbinol as an active compound. However, additional studies are needed to determine under what conditions indole-3-carbinol could be a suitable chemopreventive agent in humans. Another indole-3-carbinol derivative with anticarcinogenic properties is ascorbigen (Fig. 3.11.1). In the presence of ascorbic acid, depending on the pH and temperature, indole-3-carbinol may be converted to ascorbigen (Wagner and Rimbach, 2009), the main glucosinolate hydrolysis product present in fermented cruciferous. Therefore, products such as sauerkraut (fermented white cabbage) are a good source of bioactive compounds including ascorbigen, especially during the winter period when the availability of fresh vegetables is limited (Martinez-Villaluenga et al., 2012). Evidence about anticarcinogenic effects of ascorbigen was mostly studied in vitro or in sporadic animal studies (Wagner and Rimbach, 2009). Further systematic clinical studies are necessary to obtain data regarding bioavailability and shed light on the possible bioactivity in vivo.

Apart from glucobrassicin and its derivatives, shown in Fig. 3.11.1, some evidence also suggests that anticarcinogenic activity of *Brassica* crops can be associated with glucosinolate sinirgin, flavonoids such as quercetin and kaempferol, triterpene lupeol, and α- and β-amyrins (Gibellini et al., 2011; Martelanc et al., 2007; Mazumder et al., 2016; Saleem, 2009).

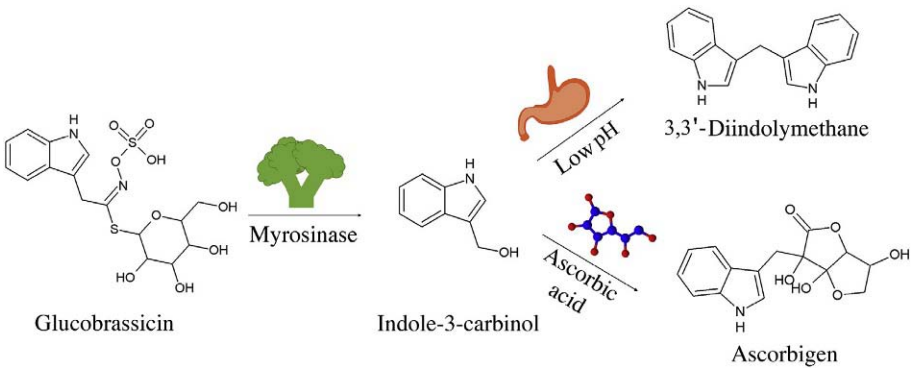

FIG. 3.11.1 Glucobrassicin and its hydrolysis product and derivatives.

Antiinflammatory Activitiy

Different *Brassicas* have been traditionally used for many years in different cultures around the world to prevent or treat various inflammations (Šamec et al., 2017). Traditional use of cabbage leaves by breastfeeding women as prevention against breast inflammation has been studied in humans, with conclusions that women who received cabbage leaf application during breastfeeding felt some benefits, although an application of cold cabbage leaves showed comparable effects to hot and cold compresses (Arora et al., 2008; Ayers, 2000). Preclinical data showed that daily intake of a blend containing 100 mg of ascorbigen and 400 mg broccoli powder may have a beneficial effect on people suffering from fibromyalgia syndrome, a rheumatologic condition characterized by chronic widespread pain with hyperalgesia and allodynia (Bramwell et al., 2000). However, it seems that more studies are needed to prove these findings.

Although some data gave evidence about *Brassica* antiinflammatory activity, the mechanism of such activity is still unknown and needs additional research to be explained. A Norwegian group (Westereng et al., 2006) indicated that polysaccharides with immunostimulatory effects may be responsible for the antiinflammatory activity of cabbage. Other authors linked *Brassica* antiinflammatory activity with the presence of polyphenols (Lin et al., 2008) and glucosinolates (Lippmann et al., 2014), particularly sinigrin (reviewed by Mazumder et al., 2016) and the glucoraphanin hydrolysis product sulforaphane (Folkard et al., 2015). Sulphoraphane can protect and repair cells of the gastric mucosa from oxidative injury and inflammation through stimulation of Nrf-2 gene-dependent antioxidant enzymes (Baenas et al., 2017). However, in a study which included pak choi and broccoli, Lippmann et al. (2014) showed that glucosinolates cannot be generally considered to act as antiinflammatories because their effects depend on the research model used, environmental conditions, habits of food intake, *Brassica* species, etc.

Effect on Gastrointestinal and Digestive System

Brassica vegetables are known for their use in traditional medicine treating different gastrointestinal and digestive ailments. Some legends say that the philosopher Aristotle ate cabbage before drinking alcohol to keep the wine "from fuddling his prudent academic head." Over the centuries cabbage juice was consumed in order to improve digestion and prevent scurvy, nowadays it is popular for treating a hangover. Cabbage juice was widely used in the treatment of peptic ulcers, an effect which has recently been confirmed in several animal studies (Šamec et al., 2017). Extract from fresh broccoli sprouts showed activity against *Helicobacter pylori*, a strain highly associated with a number of upper gastrointestinal tract diseases, including gastric inflammation, chronic superficial gastritis, duodenal and gastric ulcers, gastric adenocarcinoma, and non-Hodgkin's lymphomas of the stomach (Moon et al., 2010). Today, *Brassica* vegetables are included in many traditional and commercial weight-loss diets. In vivo studies have shown that they can improve the bioavailability of nonheme iron (Chiplonkar et al., 1999) and may have antiobesity, hypolipidemic, and hypoglycemic effect (reviewed by Latté et al., 2011; Šamec et al., 2017).

Some *Brassica* species are consumed after fermentation by starter or autochthonous lactic acid bacteria. Lactic acid bacteria can serve a dual function by acting as food-fermenting agents and probiotics, which have been found to demonstrate potential health benefits. Probiotics are a general name for the live microbial feed which is supplemented by food that beneficially affects the host by improving its intestinal balance (Beganović et al., 2013). Probiotic foods are a group of functional foods with a growing market share and large commercial interest. In fermented dairy products probiotics have been used for centuries. An increasing consumer demand for probiotic products and drinks which are not dairy based focused scientific interest on *Brassica* vegetables. Currently, there is significant ongoing research dealing with the modification of fermented *Brassica* products and probiotic-enriched beverages for lactose intolerant and vegetarian consumers (Jaiswal et al., 2012; Beganović et al., 2013).

Other Activities Demonstrating Health Benefits

Direct and indirect research evidence has demonstrated that the benefits of cruciferous vegetables may be also in the prevention of metabolic disorders, asthma, and Alzheimer's disease (Fibigr et al., 2014). Some of these effects could be attributed to the antioxidant activity of *Brassica* phytochemicals. The oxidative damage caused by reactive oxygen species, generated during cell aerobic respiration, on lipids, proteins, and nucleic acids may trigger various chronic diseases. Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status for single cells and whole organisms. Studies have shown that vegetables provide a good source of antioxidants and *Brassica* species are among the best sources (Podsedek, 2007; Šamec et al., 2011, 2014). Phenolic compounds and vitamin C are the major antioxidants of *Brassica* vegetables (Podsedek, 2007). Sinapinic acid is the main phenolic acid in cabbage. In addition to antioxidant

activity it shows antiinflammatory activity and can act as a neuroprotective agent by decreasing the levels of A_β and by protecting neuronal cell death. Recently, Baenas et al. (2017), in an animal study, gave evidence of the potential activity of broccoli sprouts in pain therapy without accompanying adverse effects as usually observed in analgesic drug therapy. The authors suggested that these effects might be associated with the presence of sulforaphane.

BIOAVAILABILITY AND SAFETY

The use of cruciferous vegetables and their phytochemicals as dietary supplements is a result of their use in traditional medicine and the above described scientific evidence which supports their health benefits. The most common compound, declared as an active compound in *Brassica*-based products and supplements, is indole-3-carbinol. However, nutraceuticals and food supplements being neither food nor pharmaceuticals often sit in a gray area for which control and regulation are difficult (Fibigr et al., 2014). Fibigr et al. (2014) reported a new high-performance liquid chromatography method for the determination and quantification of indole-3-carbinol in supplements. Their data showed a worrying outcome. Measured and declared values were comparable in just two out of six samples; in two samples the levels of indole-3-carbinol were lower, and in another two, completely absent. This suggested that the quality of nutraceuticals widely differs according to type and producer, and that regulatory agencies need to tighten rules in this area.

Another fact which needs to be taking into account when we think about the health benefits of some compounds, is bioavailability, i.e., the degree to which a substance is absorbed into a living system, or whether a particular substance can reach the targeted tissue after administration. For some compounds bioavailability is the main reason for great discrepancies between in vitro and in vivo data. Bioavailability of glucosinolates highly depends on the presence and activity of myrosinase which is, according to Martinez-Ballesta and Carvajal (2015), the most important issue for glucosinolate turnover. Consequently, method of preparation can make a large difference, both to the intake of glucosinolates and to the bioavailability of their breakdown products (Johnson, 2002). Maskell and Smithard (1994) suggested that about 60% of glucosinolates reach the colon unmodified where they may be hydrolyzed by colonic microflora (Verkerk et al., 2009). The in vivo bioavailability of the most studied glucosinolate hydrolysis product, indole-3-carbinol, is still not completely clear, due to its rapid acid oligomerization in the stomach after consumption, which consequently hinders its determination (Verkerk et al., 2009). After intake of *Brassica* vegetables which contain glucobrassicin, Fujioka et al. (2014) determined a higher level of 3,3-diindolylmethane in the urine of healthy, nonvegetarian, nonsmoking adults. Based on these results, the authors suggested that 3,3-diindolylmethane may be considered as a biomarker of glucobrassicin exposure and indole-3-carbinol uptake in humans. After consumption, the concentration of 3,3-diindolylmethane in an animal model was found to be highest in the liver, followed by lungs, kidneys, and heart and, and to a lesser extent, the brain and plasma (Anderton et al., 2004). In order to determine the quantitative relationship between glucobrassicin exposure and 3,3-diindolylmethane urinary level, Fujioka et al. (2016) performed a clinical trial where subjects consumed a mixture of Brussels sprouts and/or cabbage containing levels of glucobrassicin ranging from 25 to 500 mmol, once daily for two consecutive days. The authors found that consuming glucobrassicin in excess of 200 mmol, or about 100 g of raw Brussels sprouts, did not consistently lead to more urinary 3,3-diindolylmethane, suggesting that there may be a plateau in terms of its potential health effect. These findings bring new insight into defining a biologically relevant dose of *Brassica* vegetables, however, a lot of facts are still unclear.

The health benefits of drugs are dose dependent and positive or negative effects on human health depend on their concentrations. Compounds may have health benefits effective at lower concentrations while higher concentrations may be toxic. Good examples are plant extracts rich in polyphenols, which can act as antioxidants at lower doses, while at higher doses show prooxidant activity (Šamec et al., 2015a,b). Toxicity indicates the degree to which a substance is poisonous to cells and organisms, including humans. Cruciferous vegetables have been used in culinary and traditional medicine for many years and are considered generally safe. However a few experimental studies gave rise to the concern that they might also possess negative effects (Latté et al., 2011; Truong et al., 2010). Truong et al. (2010) suggested a positive association between the consumption of cruciferous vegetables and thyroid cancer among women with low iodine intakes. Contrary to this, Scott et al. (2012), who systematically reviewed 50 studies, found that adverse events presumably related to cruciferous plants have been reported in just 1335 out of 101,198 studied individuals. They concluded that *Brassica* vegetables are generally safe for human consumption and use, but individuals with known allergies/hypersensitivities to certain members of the *Brassica* genus, or those taking warfarin, should consult with their physician before consuming such vegetables. It is known that food matrix influences digestion, so although *Brassica* vegetables are safe, supplements which contain higher doses of specific compounds isolated from whole vegetables can have a different effect. Early clinical trials suggested that indole-3-carbinol is safe and well tolerated at doses of 400 and 800 mg (Reed et al., 2005), but the most recent findings by Fletcher et al. (2017) reported possible conflicting results. They fed supplemented immune-deficient mice diets containing

0–100 μmol indole-3-carbinol per gram of animal weight for 4 weeks and found intestinal damage occurred in animal models that received supplementation in levels as low as 10 μmol/g. The results of this study, although performed on an animal model, suggest caution is required for the use of indole-3-carbinol supplements in humans, especially in immune-deficient/compromised populations, such as cancer, transplant, and AIDS patients.

CONCLUSIONS

Epidemiological studies support the anticancer activity of cruciferous vegetables associated with the presence of secondary metabolites, glucosinolates, and their hydrolysis products. Additionally, there is some evidence that cruciferous vegetables may play a role in the prevention and treatment of inflammation, various gastrointestinal and digestive ailments, and chronic diseases. There is no doubt that cruciferous vegetables included in the diet can be a source of health-promoting compounds, however, their use in addition to consumption of compounds isolated from cruciferous plants as dietary supplements has not been sufficiently studied. More data about their bioavailability, effective concentration in tissues, mechanisms of action, and possible toxicity are needed.

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Chapter 3.12

Dandelion

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Taraxacum campylodes G.E. Haglund (syn. *Taraxacum officinale* (L.) Weber ex F.H. Wigg), commonly known as dandelion, is a cosmopolitan, perennial weed, of the Compositae family (Cichorioideae subfamily, Lactuceae tribe) commonly found in meadows, gardens, uncultivated areas, and roadsides. The scientific name comes from the Greek words "taraxis" and "akeomai," meaning "beneficial for inflammation." The name "dandelion" derives from the French term "dent-de-lion," alluding to the shape its leaves. It is also known under the French name "pissenlit" (bedwetter) alluding to its diuretic properties (Schütz et al., 2006). The plant stands up to 60 cm tall with a basal rosette of incised leaves and long pedunculated yellow capitula (2.5–4 cm), endowed with only ray florets. Fruits are ray-arranged cypselas equipped with pappus (Pignatti, 1982). The plant produces a stout taproot, which exudes white-colored latex having a bitter taste due to the presence of sesquiterpene lactones; it is also rich in nutrients such as sugars and minerals. According to European Pharmacopoeia (2005) and the Committee on Herbal Medicinal Products of the European Medicines Agency, the whole plant, including its roots, can be used for therapeutic purposes. Overall, dandelion is recommended as cholagogue–choleretic, diuretic, and appetizer.

Medical uses of dandelion can be traced back to Arab physicians (10th to 11th century AD) who used it to treat liver and spleen disorders (Bown, 2008). Aborigines of North America used to make infusions and decoction with the roots and aerial parts of the dandelion to cure heartburn and indigestion (Schütz et al., 2006). In traditional Chinese medicine, dandelion was used to treat affections of the respiratory tract (Schütz et al., 2006). Overall, dandelion was used to treat various disorders such as diseases of the liver and gallbladder, constipation, diabetes, eczema, arthritis, and rheumatic pains (Bisset and Wichtl, 1994; Newall et al., 1996; Önal et al., 2005; Racz-Kotilla et al., 1974). Besides its therapeutic uses, dandelion also enjoys a good reputation as a culinary plant, mainly used raw in salads but also cooked; in Italy it is also used to make liqueurs and marmalades (Martinez et al., 2015)

The bioactive constituents of dandelion extracts, obtained from both the roots and aerial parts of the plant, can be divided into four main groups, namely sesquiterpene lactones (e.g., tetrahydroridentin B, taraxacolide β-D-glucoside, ixerin D, 11β,13-dihydrolactucin), triterpenes (e.g., taraxasterol, arnidiol, α- and β-amyrin), phytosterols (e.g., β-sitosterol, stigmasterol), and phenolic compounds (e.g., caffeic acid, chicoric acid, quercetin glycosides, and luteolin glucosides) (Schütz et al., 2006). In addition, dandelion roots contain high levels of inulin (2%–40%) which is the marker storage carbohydrate of Compositae, along with other polysaccharides which are involved in ameliorating CCI4-induced hepatitis in rats by inhibition of the expression of TNF-α and IL-1β (Park et al., 2010). The main constituents found in the latex exuded by dandelion roots are phenolic inositol esters, sesquiterpene lactones, and triterpene acetates (Huber et al., 2015).

Several biological activities have been attributed to dandelion and its bioactive constituents, namely, antiinflammatory, anticarcinogenic, antiangiogenic, antirheumatic, antinociceptive, and hypoglycaemic activity (Park et al., 2011; Shidoji and Ogawa, 2004). In particular, methanolic and aqueous extracts of dandelion are able to inhibit oxidative stress and inflammatory response by inhibiting the NF-kB transcription factor and the expression of inducible NO synthase and increasing the activity of antioxidant defense enzymes (Park et al., 2011). Interestingly, two polysaccharides extracted from dandelion roots showed relevant antiinflammatory and Nrf-2-mediated antioxidant effects in RAW 264.7 cells through the inhibition of NF-kB and the modulation of PI3K/Akt pathways, respectively (Park et al., 2014). Leaf aqueous extract proved to exert antiinflammatory effects on endothelium during mastitis through the inhibition of TNF-α and ICAM-1 expression (Hu et al., 2017). Methanolic extract from the aerial parts of dandelion improved TRAIL (TNF–related apoptosis inducing ligand)-induced apoptosis in Huh 7 (hepatocellular carcinoma) cells (Yoon et al., 2016). Furthermore, the extract significantly inhibited the viral replication of HCV and gene expression of NS5B without having toxic effects on fibroblasts at the tested doses (Rehman et al., 2016). Administration of aqueous extract, obtained by decoction of the herb, in mice improved fatigue-related markers and immunological parameters (Lee et al., 2012). Dandelion extract showed antiinflammatory

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activity and protective effects against cholecystokinin-induced acute pancreatitis in rats (Seo et al., 2005). Rabbits fed with the roots and leaves of dandelion following a cholesterol-rich diet showed an improvement in the activities of antioxidant enzymes and a lowering of lipid profiles in their plasma (Choi et al., 2010). In 3T3L1 adipocytes, the extract of both leaves and roots of dandelion decreased the accumulation of lipid and triglyceride and modulated the expression of genes and noncoding RNAs involved in the regulation of adipogenesis, being potentially useful as a treatment for obesity (González-Castejón et al., 2014). In rats, administration of dandelion aqueous extract reduced male fertility by decreasing the functionality of sperm, altering sperm motility and morphology, and altering the morphology of the seminiferous tubules (Tahtamouni et al., 2011).

In conclusion, dandelion has enjoyed a long-standing use in traditional medicine, treating various ailments. Most therapeutic uses has been recently confirmed thanks to the development of advanced and reliable preclinical models. Further clinical studies on human subjects are, however, needed in the near future in order to support and improve the use of dandelion in the market of natural drugs.

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Chapter 3.13

Echinacea

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SOURCES AND AVAILABILITY OF *ECHINACEA*

The high costs of prescription drugs and their increased side effects have contributed to the popularity of herbal treatments. Currently, there is a cultural attraction towards a "natural" approach to medical care. However, the discovery of medicine is not new to nature. A large number of well-known drugs, like aspirin, digitalis, and quinine originated from plants. These drugs have been assessed for their efficacy and safety over the last few decades. *Echinacea* is commonly known as black sampson, black sampson coneflower, Kansas snakeroot, narrow-leaf coneflower, narrow-leaved coneflower, purple coneflower, roter sonnenhut, rudbeckia, snakeroot, Kansas, and zonnehoed. *Echinacea* comprises a group of herbaceous flowering plants of the daisy family with nine species, commonly named purple coneflowers. They are endemic in eastern and central North America, growing in moist to dry prairies and open wooded areas (Wichtl, 2004). The generic name comes from the Greek word ἐχιν&z.omicr;ζ (ekhinos), meaning "hedgehog," due to the plant having a spiny central disk. These flowering plants and their parts have different therapeutic uses (Table 3.13.1). Some species are grown in gardens for their showy flowers. *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*, are widely used for their therapeutic effects. Earlier in 1968, *E. pallida and E. angustifolia* were known as the same species of different varieties. There is now an interpretation that *E. purpurea* (L.) Moench is used inappropriately, and therefore, a taxonomic revision of the genus has been proposed that covers two subgenera with four species: *E. purpurea*, *E. pallida*, *Echinacea atrorubens*, and *Echinacea laevigata*, with *E. angustifolia* and *E. pallida* revised as *E. pallida* var. *angustifolia* (DC.) Cronq. and *E. pallida* var. *pallida* (Nutt.) Cronq (Binns et al., 2002a,b). A wide range of dosages for *Echinacea* products are available, which may contain one or more crude drugs from different geographical areas, such as tinctures, tablets, capsules, and parenteral products. The phytochemical diversity of *Echinacea* products proves its pharmacological and clinical research findings. *Echinacea* has been used to treat several physical abnormalities caused by infections, such as septic wounds and syphilis. It has also been used as an "antitoxin" for snakebites and blood poisoning (Hobbs, 1994). Traditionally, *Echinacea* was used for bacterial and viral infections, mild septicemia, furunculosis, and some skin problems, for example, boils, carbuncles, and abscesses (Tyler, 1993). *Echinacea* has also been used as a supportive treatment for influenza and recurrent infections of the respiratory tract and lower urinary tract and for poor healing of superficial wounds (British Herbal Medicine Association, 1990).

The rapid growth in research and information regarding *Echinacea* has revealed its attractiveness. The number of studies of *Echinacea* has increased from year to year (Wichtl, 2004; Yu, 2004). Different species of *Echinacea* are *E. angustifolia* (narrow-leaf coneflower), *E. atrorubens* (Topeka purple coneflower), *E. laevigata* (Smooth coneflower and/or smooth purple coneflower), *E. pallida* (pale purple coneflower), *Echinacea paradoxa* (yellow coneflower and/or Bush's purple coneflower), *E. purpurea* (purple coneflower and/or eastern purple coneflower), *Echinacea sanguinea* (sanguine purple coneflower), *Echinacea serotina* (narrow-leaved purple coneflower), *Echinacea simulata* (wavy leaf purple coneflower), and *Echinacea tennesseensis* (Tennessee coneflower). The morphological features of *Echinacea* are given in Table 3.13.2.

PHYTOCHEMISTRY OF *ECHINACEA*

There are differences in the constituents of *Echinacea* from species to species and between the different parts of the plant. The phytoconstituents that are responsible for showing the activity of *Echinacea* include alkamides, caffeic acid and its derivatives, polysaccharides, and alkenes (Matthias et al., 2004). The structure of echinacoside, which is caffeic acid glycoside, along with cichoric acid, echinaceine, and echinolone are shown in Fig. 3.13.1.

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TABLE 3.13.1 Some Traditional Uses of *Echinacea* Species

Species	Traditional use
E. atrorubens	Not frequently used
E. laevigata	
E. paradoxa	
E. sanguinea	
E. simulata	
E. tennesseensis	
E. purpurea	Respiratory tract infections: colds, flu, bronchitis, strep throat, and toothache Urinary tract infections: herpes sores and gonorrhea Skin disorders: *Staphylococcal* infections, cold sores, ulcers, wounds, burns, insect bites, eczema, and allergies Others: rheumatoid arthritis
E. angustifolia	
E. pallida	

TABLE 3.13.2 Morphological Characteristics of *Echinacea*

Feature	Description
Nature	Perennial herb, herbaceous
Height	Ranging from 10 to 60 cm
Adaptation	*Echinacea* plants are resilient and drought resistant but in such conditions their growth is slowed. They grow in moist to dry prairies and open wooded areas
Root	In contrast to *Echinacea* species the taproot of *Parthenium integrifolium* is short, only 3–8 cm long and 4 cm in diameter, bulbform, thickened, and black. The branches arise from the bottom part of the taproot. The lateral roots are partially shiny
Stem	The stem ascends either from a vertical taproot (*E. angustifolia*) or branched, fibrous roots (*E. purpurea*). *Echinacea* may have either simple or branched stems
Leaf	The leaf shape varies from lanceolate to ovate
Flower	Each "flower" or daisy-like head unit is actually a conglomeration of many tiny florets
Floral character	The inner (disk) florets end in spines, and are surrounded by droopy outer (ray) florets with teeth at their ends. The *Echinacea* genus is characterized by spiny flowering heads, with an elevated receptacle which forms the "cone"
Propagation	To grow *Echinacea* from seed, cut a stalk supporting a spent flower, enclose the flower in a paper bag, and hang the plant upside down. The plant will release the seeds into the bag when they are ready. Separate the seeds from the chaff, dry them for a few weeks, and then store them in a cool, dry place

About 20 alkamides including isobutylamides of long-chain fatty acids with olefinic bonds are present in *Echinacea* (Bauer et al., 1989; Lienert et al., 1998). Caffeic acid glycosides such as echinacoside (Table 3.13.3), verbascoside, and caffeoyl echinacoside, as well as caffeic acid esters of quinic acid (e.g., chlorogenic acid-5-caffeoylquinic acid, isochlorogenic acid-3,4- and 3,5-dicaffeoylquinic acid, cynarin-1,3-dicaffeoylquinic acid) and tartaric acid (e.g., caftaric acid-2-caffeoyltartaric acid, cichoric acid-2,3-dicaffeoyltartaricacid) have medical importance having different physiological activities (Pietta et al., 1998).

Two polysaccharides and a xyloglucan (molecular weight 79 kDa) were extracted from *E. purpurea*, the polysaccharides included PS1 (a methylglucuronoarabinoxylan, molecular weight 35 kD) and PS2 (an acidic rhamnoarabinogalactan, molecular weight 450 kDa) (Bauer, 1997). *E. pallida* root (0.2%–2.0%) has important constituents such as polyenes, polyalkenynes,

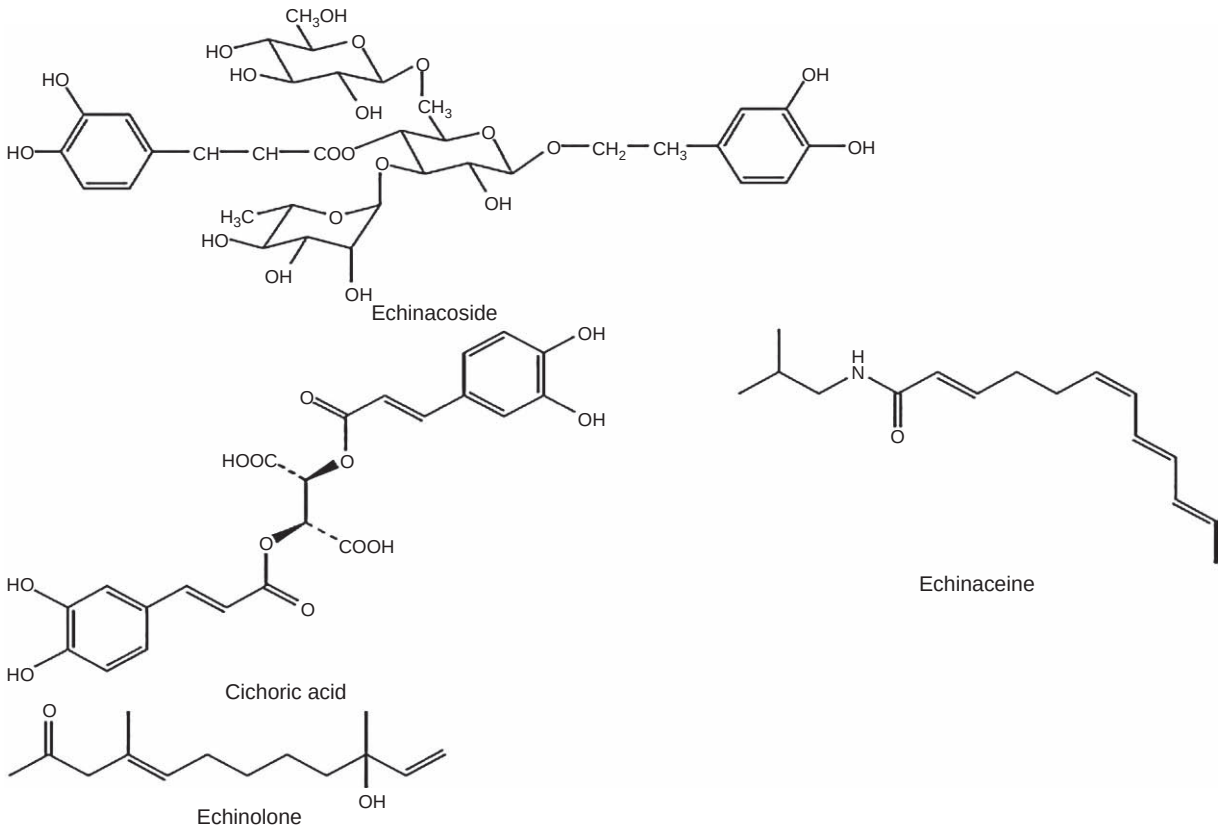

FIG. 3.13.1 Major phytoconstituents of *Echinacea* species.

TABLE 3.13.3 Major Constituents of *Echinacea* Species That Are Used Medicinally

Species	Plant part	Compound
E. pallida	Roots	Esters of caffeic acid (e.g., echinacoside), polysaccharides, and polyacetylenes
E. angustifolia	Roots and aerial parts	Esters of caffeic acid (e.g., echinacoside), polysaccharides, polyacetylenes, and alkamides
E. purpurea	Aerial parts	Alkamides, esters of caffeic acid (e.g., cichoric acid), polysaccharides, and polyacetylenes

along with carbonyl compounds (ketones), which include ketoalkenes, ketopolyacetylenes, and pentadeca-8Z-ene-2-one, pentadeca-8Z, 13Z-diene-11-yne-2-one, tetradeca-8Z-ene-11,13-diyne-2-one, among others (Bauer et al., 1988).

E. angustifolia and *E. purpurea* a have a wide range of constituents (alkaloids and flavonoids) including saturated pyrrolizidine-type alkaloids, tussilagine and isotussilagine (Röder et al., 1984), and flavonoids like quercetin, isorhamnetin, and kaempferol, along with their glycosides extracted from *E. purpurea* (0.48%) (Wichtl, 2004). Patuletin-3-rutinoside is extracted from the aerial parts of *E. angustifolia* (Bauer, 1998; Lin et al., 2002). A wide range of phenolic compounds have also been isolated from the aerial parts of *E. angustifolia* and *E. purpurea*, which include phenolic acids like protocatechuic acids, p-coumaric, and p-hydroxybenzoic (Glowniak et al., 1996).

HEALTH BENEFITS OF *ECHINACEA*

A vast amount of scientific literature on *Echinacea* species is available about its health benefits with special focus often on immunological effects based on in vitro and in vivo (animal) studies. *Echinacea* and its preparations exert immune stimulant activity through three mechanisms: activation of phagocytosis, stimulation of fibroblasts, and the enhancement

of respiratory activity that results in augmentation of leukocyte mobility. The production of cytokines (interleukin-1 (IL-1), IL-10) and tumor necrosis factor-α (TNF-α) is stimulated by *E. purpurea* (Burger et al., 1997).

Several in vitro studies have proved the antiviral activity of various different preparations of *Echinacea* (Bodinet and Beuscher, 1991). N-hexane extract from *E. purpurea* roots showed antifungal properties against several yeast strains such as *Saccharomyces cerevisiae* and *Candida albicans* (Binns et al., 2000). Again, the pure polyacetylenic compound (tridec-1-ene-3,5,7,9,11-pentayne) extracted from *E. purpurea* root has been found to provide inhibitory action against *S. cerevisiae* (Binns et al., 2000). The anticandida activity of *E. purpurea* extract has also been studied (Barrett, 2003). Some antibacterial activity of *E. purpurea* root extract has been found against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis* (Westendorf, 1982).

The antiinflammatory activity (in vivo) of *E. angustifolia* roots was observed through the carrageenan-induced rat paw edema test and in the croton oil-induced mouse ear test the polysaccharide fraction of *E. angustifolia* was found to be responsible for such antiinflammatory activity (Tubaro et al., 1987). Again in the croton oil-induced test aqueous extract of *E. angustifolia* roots was observed to be more effective than benzydamine (Tragni et al., 1985). Long-chain alkenes extracted from *E. angustifolia* possess in vivo antitumor activity. In rats, alkenes act by inhibiting the growth of Walker tumors and in mice alkenes act by inhibiting lymphocytic leukaemia (P388) (Voaden and Jacobson, 1972).

A number of alkamides (e.g., a mixture of dodeca-2E,4E,8Z,10E-tetraenoic acid isobutyl amide and dodeca-2E,4Z,8Z,10Z-tetraenoic acid isobutyl amide) isolated from dried *E. purpurea* roots exerted mosquitocidal activity (Clifford et al., 2002) as shown in Table 3.13.3.

The alcoholic compounds extracted from *E. purpurea*, *E. angustifolia*, and *E. pallida* have been observed to provide free radical removing activity (in vitro study) (Sloley et al., 2001). The anxiolytic activity of *Echinacea* was found to be positive in experimental animals with lower doses than those used in traditional indications. Alkamides of *Echinacea* have cannabinomimetic properties on both cannabinoid CB1 and CB2 receptors, having structural similarity to the endogenous cannabinoid receptor ligand anandamide (Haller et al., 2010).

POSSIBLE INTERACTION OF *ECHINACEA* WITH OTHER SUPPLEMENTS/DRUGS/FOODS

A drug interaction occurs when a drug affects the action of another drug due to the concomitant administration of both drugs. This action could be synergistic (increased drug effect) or antagonistic (decreased drug effect) or could even produce a new effect that neither of the drugs produces alone. Generally, interactions exist between drugs and drugs (drug–drug interaction), drugs and foods (drug–food interactions), as well as drugs and medicinal plants or herbs (drug–plant interactions). Here we discussed the major interaction between *Echinacea* and some selected drugs/foods/supplements (Table 3.13.4).

The antifungal activity of *Echinacea* has been evaluated and identified to reduce the symptoms of athlete's foot. *Echinacea* and econazole have an important interaction leading to the possible inhibition of yeast infections (Binns et al., 2000). *Echinacea* and immunosuppressants have synergistic effects which may harm normal cellular physiology, so the use of a combination of the two should be consciously prescribed (Binns et al., 2000). Specifically, the use of immunosuppressants should not be use along with *Echinacea* in organ transplant situations. This combination therapy has been mostly used for cancer treatment and to produce immune-suppressing effects. One study noted that caffeine when taken along with *Echinacea*, sustained caffeine's effects on the nervous system, with a special focus neurological

TABLE 3.13.4 Possible Interaction of *Echinacea* With Drugs/Food

Drugs	Effect	References
Echinacea and econazole	Reduces yeast infection rate	Binns et al. (2000)
Echinacea and immunosuppressants	Enhances immune function. Therefore, immunosuppressant should not be used with *Echinacea*	Binns et al. (2000)
Echinacea and caffeine	Increases the duration of caffeine in the body	Sloley et al. (2001)
Echinacea and midazolam	Increases the effects and side effects of midazolam	Barrett (2003)

effects (Sloley et al., 2001), as its breakdown caffeine and maintain in the blood plasma. When *Echinacea* interacts with midazolam it demonstrates side effects on cellular and molecular levels (Barrett, 2003), since its increases midazolam absorption. These interactions should be evaluated in this new era which has more drug–drug, drug–food, and drug–supplement interactions.

CONCLUSIONS

In conclusion, *Echinacea* plays many roles in biological systems, extending from being a structural component to having antiviral, antifungal, antibacterial, antiinflammatory, and mosquitocidal activities. Currently, in the treatment of a number of physiological conditions, *Echinacea* is widely used in both traditional and modern medicines, in addition to its special focus on cancers. It contains large amounts of compounds with diversified therapeutic properties. Further studies are warranted to discover its supreme potential in the field of medicinal and pharmaceutical sciences for innovative and productive uses. More research is needed to find out the diverse and protective effects of *Echinacea* against critical diseases like neurodegenerative disorders and cancers.

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Chapter 3.14

Elderberry (*Sambucus nigra* L.)

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INTRODUCTION

Nowadays, medicinal plants have an important role in preventing and treating many diseases. The etiology of lifestyle diseases is related to oxidative stress. There is an increase in the consumption of natural supplements because people are looking to increase the quality of their lives, prevent diseases rather than treat diseases, and feel drawn to nature, in terms of wanting to replace synthetic additives with natural components. Natural products rich in polyphenolics such as phenolic acids, flavonoids, tannins, *etc*., have received more and more attention in recent years as a result of their powerful antioxidant effects. Anthocyanins are a subgroup of flavonoids that contribute to the blue, purple, and red colors in many fruits and vegetables. With the technological development in the food industry, consumption of anthocyanin-rich berries in their frozen, fresh, or dried forms have increased. Berries are also offered to the markets in processed forms such as jams, jellies, ice creams, cakes, yoghurts, beverages, *etc*. These fruits are also used as natural colorants due to their attractive colors. The positive health benefits of anthocyanins and anthocyanin-rich fruits on human health, proved by different studies, have meant that it is becoming more and more attractive to the pharmaceutical industry (Senica et al., 2016). In recent years, one of the most attractive plants has been *Sambucus nigra* L. belonging to the Caprifoliaceae family. Its common names are "elder, common elder, black elder, European elder, and elderberry." It has also commonly used local names: sauco, sabugo, canillero (Spain), sambuco (Italy), zova (Serbia), sabugueiro (Portugol), sauco tilo (Ecuador), holunder, holler (Germany), and mürver (Turkey). *S. nigra* is a small tree or shrub, 1–8 m tall having a strong odor. The bark is brownish in color, with longitudinal fractures and deep grooves. The leaves are opposite, imparipennate, with 5–7 elliptic–lanceolate, dentate leaflets. The inflorescence is an umbel with many milky-white flowers. The fruit is a shiny black–purple, subspheric drupe (Pignatti, 1982). The plant is found in woods, clearings, and hedges from sea level to mountainous elevations (1400 m a.s.l.). The plant grows wild in Europe, Asia, North Africa, and North America (Senica et al., 2016). It is cultivated on a small scale in some northern European countries (Akbulut et al., 2009).

TRADITIONAL USES OF *SAMBUCUS NIGRA* L

Elderberry has a very long historical use as a medicinal plant. From the 5th century BC, data from Hippocrates, Dioscoridis, and Pliny described the use of medicines prepared from *S. nigra* (Mumcuoglu, 1998). *S. nigra* is widely used as both food and a medicinal plant in Europe. All parts of this plant have been used for the treatment of various ailments. Both elderberries and elderflowers are used traditionally to make alcoholic and nonalcoholic beverages, teas, ice creams, and yoghurts. A berry infusion is consumed to be diuretic, laxative, diaphoretic, and antiinflammatory. The plant's berries are used to treat flu and to stimulate the immune system. It has been suggested that drinking elderberry juice or tea several times a day has protective and therapeutic effects (Charlebois, 2007; Roxas and Jurenka, 2007; Vlachojannis et al., 2010; Vulic et al., 2008). Berries are best not eaten raw, as they are mildly poisonous, causing vomiting. Its mild toxicity is overcome by cooking. All green parts are poisonous and contain cyanogenic glycosides (Vulic et al., 2008). Elderflower is consumed as an herbal tea and gargling with it has benefits for respiratory tract illnesses such as coughs, influenza, and throat inflammations. Elderflowers are used for their diuretic and antidiabetic effects (Kaack, 2008).

PHYTOCHEMISTRY OF *SAMBUCUS NIGRA* L

Elderberries are rich in vitamins, organic acids, and a good source of major and trace elements. Vitamin B2 (65 mg), vitamin B6 (0.25 mg), vitamin C (18–26 mg), folic acid (17 mg), biotin (1.8 mg), β-carotene (0.36 mg), pantothenic acid

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(0.18 mg), nicotinamide (1.48 mg), potassium (288–305 mg), phosphor (49–57 mg), pectin (0.16%), and glucose and fructose (7.5%) are all contained in 100 g of fresh berries (Diviš et al., 2015). Elderberries are rich in anthocyanins, the majority of which includes cyanidin-3-glucoside and cyanidin-3-sambubioside, which are found in elderberry juice and polar extracts (Vlachojannis et al., 2015; Wu et al., 2004). In addition, elderberries are a rich source of flavanols, phenolic acids, and procyanidins. They also contain terpenes and lectins, free and conjugated forms of amino acids, proteins, fatty acids, and fiber (Glensk et al., 2014; Krüger et al., 2015; Salvador et al., 2015; Tejero et al., 2015). The characteristic aroma of elderberries is a result of (*E*)-*β*-damascenone, dihydroedulan, ethyl-9-decenoate, 2-phenyl ethanol, phenylacetaldehyde, and nonanal. Alcohols, esters, and aldehydes are frequently identified volatile groups in elderberries.

Elderflowers are particularly rich in flavonoids (up to 3%), such as kaempferol, astragalin, quercetin, quercetin-3-O-glucoside, rutin, isoquercitrin, and hyperoside (Krauze-Baranowska et al., 2009). Other major secondary metabolites comprise approximately 1% triterpenes (as α- and *β*-amyrin, ursolic acid, and oleanolic acid) and about 1% sterols (*β*-sitosterol, campesterol, and stigmasterol). In addition, pectins, tannins, and phenolic acids are found in the flowers (Ho et al., 2016, 2017). Elderflowers have a strong, flowery, pleasant odor, and an essential oil yield from the flowers of 0.03%–0.14%. The aroma composition of elderflowers includes aldehydes, ketones, alcohols, esters, oxides, terpenes, and free fatty acids (Jørgensen et al., 2000).

HEALTH BENEFITS OF *SAMBUCUS NIGRA* L

Elderberry products have been marked as antioxidant (Denev et al., 2010; Sun-Waterhouse et al., 2013; Topolska et al., 2015), antiviral (Kinoshita et al., 2012; Porter and Bode, 2017; Roschek et al., 2009), immunomodulatory (Frøkiær et al., 2012; Waknine-Grinberg et al., 2009), antiinflammatory (Burns et al., 2010; Olejnik et al., 2015), and antimicrobial (Arjoon et al., 2012). There are some reports on the anticonvulsant (Ataee et al., 2016) and antidepressant activities (Mahmoudi et al., 2014) of elderberry extracts. Elderberry food supplements, including those formed from parts of the plant or in extract form, as well as those formed from a combination of elderberry with other plants, have been produced for a long time. Commonly used combination products such as OptiBerry® IH141 (contains wild blueberry, strawberry, cranberry, wild bilberry, elderberry, and raspberry extracts); Sambucol® Active Defense (contains elderberry extract, vitamin C, zinc, *Echinacea angustifolia*, *Echinacea purpurea*, and propolis); Sambucol® Immune System (contains elderberry, *E. angustifolia* root, *E. purpurea*, propolis, vitamin C, and zinc); and Sambucol® for Kids (contains elderberry, *E. purpurea*, *E. angustifolia* root, and propolis) were analyzed for their potential health benefits (Ulbricht et al., 2014). Elderberries were found to have significant antiviral effect and immunomodulatory activity. Clinical trials indicated that Sambucol® is a very effective food supplement for relieving flu-like symptoms (Kong, 2009; Zakay-Rones et al., 1995, 2004). Sambucol® also showed prophylactic effects in chimpanzees. It also effectively reduced the duration of illness (Burge et al., 1999). An elderberry supplement including 300 mg of elderberry extract (22% polyphenols and 15% anthocyanins) showed significant effect on cold duration and cold-associated symptoms (Tiralongo et al., 2016). In other studies, elderberry extracts were determined for dose-dependent antiviral activity by *in vitro* methods. Elderberry extract inhibited the infectious bronchitis virus at an early point during replication (Chen et al., 2014). Sambucol® had a virucidal effect against H9N2 avian influenza (Karimi et al., 2014). Roschek et al. (2009) identified that flavonoid derivatives from elderberry extracts get bound to H1N1 virions and block the ability of the virus to attack host cells. Sambucol® and its formulations activate the healthy immune system by increasing inflammatory and antiinflammatory cytokine production. Therefore, it can be useful for healthy individuals as well as in patients suffering from cancer, influenza, and HIV infections (Barak et al., 2002; Waknine-Grinberg et al., 2009). There are no reported side effects or contraindications associated with its use. It is also safe for children to use. Sambucol® is currently marketed in the United States, Europe, Israel, and South Africa through health food stores and drugstores (Verrillo, 2012). It is estimated that there may be a beneficial interaction between elderberry preparations and decongestants and antibiotics. Consumption of elderberry extract has also been suggested for people with diabetic osteoporosis for improving lipid profile and reducing atherogenic risk and hyperglycemia (Badescu et al., 2012). Optiberry® was reported to have antiangiogenic, antioxidant, and anticarcinogenic effects (Baghci et al., 2004).

Elderflower is used as an antidiabetic drug used in traditional medicine. *In vitro* studies suggested that elderflower extracts stimulate insulin-dependent glucose uptake (Battacharya et al., 2013; Christensen et al., 2010; Gray et al., 2000). Also, elderflower extracts showed a natriuretic effect in rats and increased urine flow. These data show that elderflower can be regarded as a diuretic agent in both traditional medicine and conventional medicine (Beaux et al., 1999). Tea, containing a combination of *Pimpinella anisum* L. (fruits), *Foeniculum vulgare* Mill. (fruits), *Sambucus nigra* L. (flowers), and *Senna alexandrina* Mill. (flowers), has been commercially available in Brazil since 1926. In a randomized, crossover, placebo-controlled, single-blind trial, this tea combination proved an effective alternative treatment for chronic constipation (Picon et al., 2010).

Sinupret®, an herbal medicinal product (gentian root, primula flower, elderflower, sorrel herb, and verbena herb), is frequently used in the treatment of acute and chronic rhinosinusitis and respiratory viral infections such as the common cold. Sinupret® has been sold in the German and European market for more than 70 years. In Europe, Sinupret® preparations are prescribed by physicians for the treatment of sinusitis or acute and chronic bronchitis (Oliff and Blumenthal 2009). A study has demonstrated that Sinupret® shows a broad spectrum of antiviral activity *in vitro* against viruses commonly known to cause respiratory infections (Glatthaar-Saalmüller et al., 2011). Sinupret® can also be used in pregnancy. It was shown that the efficacy of Sinupret® was superior to that of ambroxol and *n*-acetyl cysteine (Ciuman, 2012).

CONCLUSIONS

S. nigra has been used as a medicinal plant in folk medicine for a long time throughout the world. Pharmacological studies of the flower's extracts correlate with their medicinal uses in folk medicine. Elderberries are becoming more popular day by day because of their anthocyanin-rich content. The berries are consumed as food in Europe. Elderberries have attracted researchers because of their high antioxidant activity, therefore, various biological activity studies on the berries have been conducted. The antiviral activity of berry extracts and commercial products such as Sambucol® have been studied *in vitro* and *in vivo*. These studies have yielded very important results, because lectins were found to bind to virus surfaces acting as antivirus agents. Quercetin-derived compounds were also identified as antivirals. Sambucol® products are very popular in Europe and they are consumed to help prevent viral infections and stimulate the immune system. However, it should be noticed that the berries are best not eaten raw, as they are mildly poisonous, causing vomiting. The mild toxicity is overcome by cooking. All green parts are poisonous, containing cyanogenic glycosides. Also, allergic reactions can be observed in some people sensitive to its protein content.

S. nigra is still an attractive plant to researchers and needs more *in vivo* and clinical studies. Therefore, the study of elderberry represents the development of modern medicines based on traditional information. The findings of this chapter indicate that historically people were wise to choose specific plants to relieve specific illnesses.

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Chapter 3.15

Fenugreek (*Trigonella foenum-graecum* L.) Seeds Used as Functional Food Supplements to Derive Diverse Health Benefits

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belonging to the family Fabaceae is a leguminous herb cultivated in India and North African countries. Fenugreek seeds have been used as spice in foods for thousands of years. Besides imparting a characteristic flavor, fenugreek can also modify the texture of food. The bitter seeds of fenugreek are traditionally used in India for seasoning, as a flavoring agent and to prepare soups and pancakes. The bulk of fenugreek seeds (50%) constitute dietary fiber composed of both insoluble (30%) and soluble (20%) fractions, corresponding mainly to galactomannan. Its bitterness is due to oils, steroidal saponins, and alkaloids. In indigenous Indian medicine, it is effective against anorexia and it is a gastric stimulant, carminative, and galactagogue (Chopra et al., 1986). Several physiological health benefits of fenugreek seeds have been experimentally validated in animal studies as well as human trials in recent decades. These include its action as a digestive stimulant and an antidiabetic, along with its hypocholesterolemic and hepatoprotective effects, among others (Fig. 3.15.1).

ANTIDIABETIC EFFECT

Soluble fiber-rich fenugreek seeds are understood to have an antidiabetic effect. Studies have revealed that dietary fiber of fenugreek (which constitutes nearly 52% of the seed) will delay gastric emptying and suppress the release of gastric inhibitory peptides and insulinotropic hormones (Srinivasan, 2005). Fenugreek seeds contain as much as 51.7% fiber comprising 19.2% mucilaginous fiber and 32.5% neutral fiber. A decoction of fenugreek seeds has been reported to improve diabetes and suppress glycosuria in mild diabetes as well as ameliorating the severity the diabetic condition (Srinivasan, 2005). Numerous animal studies on the potential antidiabetic effect of fenugreek seeds have employed diabetic rats, mice, rabbits, and dogs. Besides many animal studies, several human trials have unequivocally demonstrated the beneficial hypoglycemic potential of this spice in both type 1 and type 2 diabetes (Sharma et al., 1990; Sharma and Raghuram, 1990).

Defatted fenugreek (fiber-containing portion) or the soluble dietary fiber (SDF) fraction of fenugreek seeds has been shown to reduce postprandial increase of blood glucose in type 2 diabetic rats. Atherogenic lipids, that is, triglycerides, and low-density lipoprotein (LDL) cholesterol were found to decrease significantly in fenugreek fed rats. Thus, SDF has a beneficial effect on dyslipidemia associated with diabetes in diabetic rats. Increased serum insulin and stimulation of peripheral utilization of glucose are also inferred in addition to the effect at the pancreatic level. A single oral dose of fenugreek has also been reported to be beneficial to glycemic control in experimental animals.

There is much evidence to support that the hypoglycemic effect of fenugreek is attributable to fiber and gum, which constitutes as much as 52% of these seeds. The probable mechanism of hypoglycemic action is that dietary fenugreek delays gastric emptying by direct interference with glucose absorption. In addition, gel-forming dietary fiber reduces the release of insulinotropic hormones and gastric inhibitory polypeptides. It is also suggested that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the pancreatic beta cells of Langerhans and by increasing

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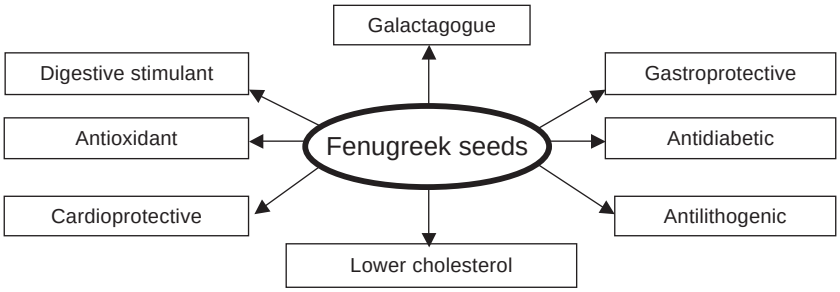

FIG. 3.15.1 The multiple health effects of fenugreek seeds.

the sensitivity of tissues to available insulin (Puri et al., 2002). The effectiveness of fenugreek seeds against type 1 diabetes has been attributed to the countering of changes in activities of enzymes involved in glycolysis and gluconeogenesis and also lipogenic enzymes in the liver and kidneys (Gupta et al., 1999; Raju et al., 2001).

The beneficial influence of dietary fenugreek seeds on hyperglycemia and its associated metabolic abnormalities is reported to be potentiated by simultaneous consumption of onion in streptozotocin-induced diabetic rats (Pradeep and Srinivasan, 2017a). These dietary interventions significantly countered hyperglycemia, partially improved peripheral insulin resistance, impaired insulin secretion, reduced β-cell mass, and markedly reversed the associated metabolic abnormalities such as glycated haemoglobin and the advanced glycation end products in diabetic rats. The beneficial influence of dietary fenugreek seeds on oxidative stress-mediated renal injury in streptozotocin-induced diabetic rats has revealed that dietary fenugreek countered nephromegaly, increase in glomerular filtration rate, and oxidative stress in renal tissue. The upregulation of the receptor for advanced glycation end products, inflammatory cytokines, and oxidative stress markers in the renal tissue of diabetic rats was effectively countered.

Several human studies have documented the beneficial blood glucose-lowering potential of orally administered fenugreek seeds (25 g/day) and improved glucose tolerance in type 2 diabetic patients (Sharma, 1986; Sharma et al., 1996). Sugar excretion, glycated haemoglobin and insulin levels, and serum cholesterol were also diminished. Diabetic symptoms like polyuria, polydypsia, and polyphagia were found to be under control. Further, in type 1 diabetic subjects, daily administration of 25 g of fenugreek seed significantly improved both their plasma glucose profile as well as glycosuria, and also reduced their insulin requirement. In a long-term trial, 100 g of fenugreek fed to both type 1 and type 2 diabetics reduced fasting blood glucose, urinary glucose excretion, serum cholesterol, and triglyceride levels (Sharma et al., 1990; Sharma and Raghuram, 1990).

The antidiabetic properties of subfractions of fenugreek seeds, namely, defatted fenugreek seeds, gum isolated from the seeds, and cooked fenugreek seeds have also been verified in healthy subjects for their effect in preventing an increase in plasma glucose after a glucose load has been introduced. The increase in blood glucose level was prevented by ingestion of fenugreek seeds. Serum insulin levels were also modified to a similar extent. The antidiabetic efficacy was in the order: whole seeds > gum isolated from the seeds > defatted seeds > cooked seeds. Serum cholesterol and triglycerides were also significantly lowered (Sharma and Raghuram, 1990).

Fenugreek generally has no effect on blood glucose and glucose tolerance in normal individuals. The effectiveness of debitterized fenugreek has also been validated by observing the beneficial reduction of postprandial glucose in type 2 diabetic subjects (Neeraja and Rajyalakshmi, 1996). The effects of fenugreek seeds on glycemic control and insulin resistance in a human type 2 diabetes mellitus, double-blind, placebo-controlled study has revealed that adjunct use of fenugreek seeds improves glycemic control and decreases insulin resistance in mild type 2 diabetic patients (Gupta et al., 2001).

In summary, fenugreek seeds, a common condiment in Indian homes has been found to diminish hyperglycemia in diabetic individuals. It has been possible to debitterize fenugreek seeds without compromising their hypoglycemic and hypocholesterolemic properties. Since fenugreek seeds are also a source of protein like pulses, they could replace pulses in the diets of diabetics. Fenugreek included in a daily diet (25–50 g) can be an effective supportive therapy in the management of diabetes.

HYPOLIPIDEMIC AND CARDIOPROTECTIVE EFFECTS

Fenugreek seeds have been exhaustively studied for their ability to influence cholesterol levels both in animal models and clinical trials. The efficacy of this spice tested at 15%, 30%, and 60% levels in the diet in rats fed on a 1% cholesterol-enriched diet revealed a marked reduction in the elevation of serum cholesterol levels (Sharma, 1984). Hepatic and serum

LDL cholesterol was significantly reduced. Fenugreek was shown to bring about the hypocholesterolemic effect through increased excretion of fecal bile acids and neutral sterols. Depletion of cholesterol stores in the liver was due to fenugreek's ability to stimulate the conversion of cholesterol to bile salts in the liver.

Among the different fractions of fenugreek seeds tested for their hypocholesterolemic effect, only the (defatted) fiber and saponin components exhibited a cholesterol-lowering activity. Hence, it is inferred that the cholesterol reducing property is associated with fiber and saponin portions of fenugreek seeds (Valette et al., 1984). It has also been inferred that saponins may be implicated, either alone or together with diosgenin, in the observed hypocholesterolemic effect of fenugreek seeds in diabetic dogs. The hypolipidemic effect of fenugreek characterized by significant lowering of blood cholesterol and triglycerides, has been demonstrated in both insulin-dependent and noninsulin-dependent diabetic subjects and in diabetic rats (Sharma et al., 1990; Sharma and Raghuram, 1990). Fenugreek improved insulin sensitivity in rats fed a high-fat, high-sucrose (HFS) diet, which was accompanied by distinct reductions of triglyceride and cholesterol levels in the blood and liver (Muraki et al., 2011). These results suggest that fenugreek enhanced insulin sensitivity at least partly by improving lipid metabolism disorders in the rats induced by the HFS diet. The hypolipidemic effect of fenugreek seeds is mediated through the inhibition of fat accumulation and LDL receptor upregulation (Vijayakumar et al., 2010).

The hypocholesterolemic influence of dietary fenugreek seeds when taken with a high-cholesterol diet (HCD) (at 10% level) was evidenced in Wistar rats (Mukthamba and Srinivasan, 2015a). Dietary fenugreek significantly countered hypercholesterolemia and elevated hepatic cholesterol brought about by HCD; the effect was potentiated by simultaneous intervention with garlic. The elevated cholesterol in the heart was also beneficially modulated by dietary fenugreek. Dietary fenugreek seeds also exerted significant lipid-lowering influence in rats fed with a high-fat diet (HFD) (Mukthamba and Srinivasan, 2016a). Increased serum triglycerides and LDL cholesterol caused by HFD was countered and so too was the increase in triglycerides and cholesterol: the phospholipid ratio in the heart tissue of HFD-fed rats.

The cardioprotective influence of dietary fiber–rich fenugreek seeds has been evaluated in induced myocardial infarcted rats. Pretreatment with dietary fenugreek was particularly beneficial under hypercholesterolemic conditions by their influence on the tissue lipid profile (Mukthamba and Srinivasan, 2015b). The results indicated that the hypercholesterolemic situation aggravated the myocardial damage during induction of myocardial infarction. Dietary fenugreek ameliorated the pathological changes in the heart tissue and lipid abnormalities in the serum and heart.

PREVENTION AND DISSOLUTION OF CHOLESTEROL GALLSTONES

Dietary fenugreek seed has been evaluated for its beneficial role in the prevention and treatment of cholesterol gallstones (CGS) in mice (Reddy and Srinivasan, 2009a,b). Dietary fenugreek (5%, 10%, and 15%) significantly lowered the incidence of CGS under a HCD-induced lithogenic condition; the incidence of CGS was brought down to 40 and 10% by dietary 10% and 15% fenugreek, respectively, as compared to 100% in the lithogenic control. The gallstone preventive influence of fenugreek is attributable to its hypocholesterolemic effect and reduction in cholesterol saturation index in the bile. While the majority of cholesterol is excreted from the body after being converted into bile acids in the liver and subsequently secreted into the bile, bile acids remained unaffected by dietary fenugreek under lithogenic conditions, and cholesterol, the bile acids ratio, was diminished due to lowered biliary cholesterol content. Fenugreek seeds also have the ability to dissolve preestablished CGSs (Reddy and Srinivasan, 2009b). After 10 weeks of dietary fenugreek seed intake the CGSs were dissolved by over 60% compared to a 10% regression in the basal control diet group. The beneficial influence of dietary fenugreek was accomplished through significant reductions in cholesterol concentrations in serum, liver, and bile, and in ratios of biliary cholesterol: phospholipids, cholesterol: bile acids, and cholesterol saturation index (Reddy and Srinivasan, 2009b). These findings are significant in the context of evolving a dietary strategy for the prevention of incidence of CGS, regression of existing CGS, as well as preventing their possible recurrence. Another study has indicated that the beneficial antilithogenic effect of fenugreek, which is primarily attributable to a reduction in the cholesterol content of the bile, is also complemented through a modulation of the nucleating and antinucleating proteins, which in turn affect the cholesterol crystallization (Reddy and Srinivasan, 2011).

ANTIOXIDANT EFFECTS

Oxidative stress plays a major role in the progression of diabetes and its complications. Dietary fenugreek seed is known to counter the surplus lipid peroxidation and alterations in the content of antioxidant molecules in circulation in diabetic rats (Ravikumar and Anuradha, 1999). The soluble portion of fenugreek seeds is associated with the antioxidant property. Fenugreek administration to diabetic animals reversed the disturbed antioxidant levels and activities of antioxidant enzymes, suggesting that fenugreek seeds exert a beneficial antioxidant effect that can be exploited for the treatment of

diabetic complications (Genet et al., 2002). Amelioration of diabetic hyperglycemia, and related metabolic abnormalities, by dietary fenugreek was potentiated by onion in experimental rats. It has also been inferred that alleviation of oxidative stress contributes to antidiabetic influence and this nutraceutical influence of fenugreek on diabetes-induced oxidative stress was higher when consumed along with onion (Pradeep and Srinivasan, 2017b).

The antioxidant influences of dietary fenugreek seeds, and potentiation of the same by simultaneous consumption of garlic along with an HCD has been evidenced in rats (Mukthamba and Srinivasan, 2015a). An increase in lipid peroxides was effectively countered which was accompanied by restoration of vitamin E in the liver and heart with the effect being highest with fenugreek and garlic consumption. The diminished activity of the antioxidant enzyme glutathione peroxidase in the serum, liver, and heart, along with that of catalase in the serum was effectively restored by these dietary interventions. Similarly, dietary fenugreek, and fenugreek along with garlic, improved the antioxidant status in HFD-fed rats which suggests that these nutraceuticals may have a higher cardioprotective influence when consumed together (Mukthamba and Srinivasan, 2016a).

Dietary fenugreek seeds have been observed to have a cardioprotective influence in experimentally induced myocardial infarction in rats. Elevated lipid peroxides accompanied with reduced antioxidant molecules caused by isoproterenol and altered activities of antioxidant enzymes in the serum and heart in induced myocardial necrosis were countered by dietary fenugreek. Dietary fenugreek seeds ameliorated the compromised antioxidant status, the cardioprotective effect being higher for the combination of fenugreek seeds and garlic. Since LDL oxidation is a key factor in the arteriosclerotic process, the potential of fenugreek in minimizing LDL oxidation has been examined in rats. The results suggest that dietary fenugreek is protective against LDL oxidation under normal situations as well as in a hypercholesterolemic situation (Mukthamba and Srinivasan, 2016b).

DIGESTIVE STIMULANT AND GASTROPROTECTIVE ACTION

Animal studies have evaluated the influence of dietary fenugreek seeds on the gastrointestinal system. Dietary fenugreek (at 2% for 8 weeks) significantly stimulated bile acid secretion in rats, which was associated with increased bile flow rates (Bhat et al., 1985). Dietary fenugreek significantly increased the activities of pancreatic lipase and chymotrypsin in experimental rats (Platel and Srinivasan, 2000). While dietary fenugreek had no beneficial influence on intestinal digestive enzymes, an appreciable increase in intestinal lipase and lactase activity was observed in animals given a single oral dose of fenugreek (Platel and Srinivasan, 1996). Thus, it is evidenced that the beneficial digestive stimulant action of dietary fenugreek seeds is mediated through the stimulation of the liver to produce and secrete more bile, enriched in bile acids, and an appropriate stimulation of activities of pancreatic lipase and chymotrypsin.

The gastroprotective effect of fenugreek seeds has been evidenced on ethanol-induced gastric ulcers (Pandian et al., 2002). The cytoprotective effect of the seeds seemed to be due to an antisecretory action and its effects on mucosal glycoproteins. Fenugreek seeds also alleviated lipid peroxidation induced by ethanol, presumably by enhancing the antioxidant potential of the gastric mucosa, thereby lowering mucosal injury.

OTHER HEALTH BENEFITS

Fenugreek seeds are reported to have a hepatoprotective effect and were found to alleviate lipid peroxidation in the liver and brain during experimental ethanol toxicity in rats (Thirunavukkarasu et al., 2003). An animal study has indicated that dietary fenugreek seeds inhibit colon carcinogenesis, by modulating the activities of β-glucuronidase and mucinase (Devasena and Menon, 2003). The immunomodulatory activity of fenugreek seeds has been evidenced in mice as indicated by their stimulatory effect on immune functions, relative thymus weight, cellularity of lymphoid organs, and delayed hypersensitivity response (Bin-Hafeez et al., 2003).

CONCLUSIONS

Fenugreek seeds form an important ingredient in several curries and specific dishes besides being a common spice used for seasoning. Several of the health benefits of fenugreek seeds have been experimentally evidenced in recent decades, having potential therapeutic applications. In view of these promising beneficial physiological effects, fenugreek deserves to be considered as a nutraceutical ingredient in our daily diet. The nonrestricted consumption of fenugreek is proved to be safe, and may be easily implemented to obtain putative health benefits through its high fiber, and other bioactive components, content.

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Chapter 3.16

Feverfew (*Tanacetum parthenium* (L.) Sch.Bip.)

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The *Tanacetum* L. genus (tansy), formerly *Pyrethrum* (Zinn), is a large genus of the Compositae (Asteraceae) family encompassing 154 species (Triana et al., 2013) distributed mainly in Europe and western Asia and worldwide used in food, cosmetics, and herbal drugs (Salamaci et al., 2007). *Tanacetum* is also a problematic genus due to its taxonomic complexity and polymorphism (Sonboli et al., 2011).

Within the genus, *Tanacetum parthenium* (L.) Sch.Bip., is one of the most important species from a pharmacological standpoint and the extract of its dried aerial parts is currently prescribed for the prophylaxis of migraine (Committee on Herbal Medicinal Products, 2010). This use was confirmed by several clinical trials (Murphy et al., 1988; Palevitch et al., 1997).

T. parthenium, commonly known as "feverfew," is a perennial herb occurring in the Caucasian area and southern Europe from which it has been introduced and naturalized elsewhere (e.g., North Africa, the remainder of Europe, and North and South America) (Euro+Med, 2006; Bremer and Humphries, 1993). It is also extensively cultivated for medicinal and ornamental purposes.

T. parthenium is also known under the scientific names *Chamaemelum parthenium* (L.) E.H.L. Krause, *Chrysanthemum parthenium* (L.) Bernh., *Pyrethrum parthenium* (L.) Sm., and *Matricaria parthenium* L., although they are currently recognized as synonyms (The Plant List, http://www.theplantlist.org).

The name "feverfew" comes from the English term "febrifuge," in turn derived from the Latin "febrifugia," alluding to its longstanding use in curing fever. Other common names used in Europe are "motherherb," "featherfoil," "flirtwort," and "bachelor's buttons." The scientific term "*parthenium*" probably derives from a Greek legend of the 5th century telling that the plant was used to cure a person injured working at the construction of the Parthenon in Athens. In addition, another explanation could be its origin from the Greek "*parthenios*" meaning "virgin," alluding to its old use in the treatment of womens' disorders (Heptinstall, 1988). On the other hand, the generic term "*Tanacetum*" comes from the Latin "tanazita" meaning "long," alluding to the period of blooming. Otherwise, the term can be related to the credence that its leaf infusion may provide eternal life (http://www.calflora.net/botanicalnames/index.html).

The plant, with a camphoraceous odor, grows on stony, ruderal, uncultivated places, often also in scrublands. It shows erect, striped, pubescent stems, up to 80 cm tall, branched in the upper part. Leaves are bipinnatisect, with ovate-oblong, dentate leaflets. Inflorescences are daisy-like capitula opening from June to September, 15–20 mm in diameter, with long stalks and clustered in corymbs. Fruits are achenes (Pignatti, 1982). The plant indumentum is characterized by biseriate glandular trichomes in which the feverfew bioactive constituents are synthesized and stored (Simmonds et al., 2002). In particular, trichomes are more concentrated in flowers and, to a lesser extent, in leaves and stems. As a consequence, feverfew bioactive constituents (sesquiterpene lactones) are more abundant in flowers than leaves (Majdi et al., 2013). Within capitula the major bioactive constituent, i.e., parthenolide, is more abundant in disk florets (Majdi et al., 2011).

Over the ages feverfew has been used worldwide in folk medicine to treat a wide range of diseases such as arthritis, migraine, helmintiasis, asthma, fever, menstrual disorders, stomach ache, toothache, and insect bites (Berry, 1984; Chaves and Da Costa, 2008; Murphy et al., 1988; Pavela et al., 2010). Its therapeutic virtues as an antipyretic can be found in the Materia Medica of Dioscorides (Heptinstall, 1988). It was also known as "medieval aspirin" (Pareek et al., 2011). Since then, several ethnopharmacological surveys attested its pharmacological importance. Also, in those areas where the plant was not native, feverfew was widely used in traditional medicine. As an example, in Mexico, the aerial parts are cooked, added to bathing water, and used to treat women in labor, or to make a tea used to cure gastrointestinal disorders and

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parasite infestations (Canales et al., 2005; Castillo-Juárez et al., 2009; Heinrich et al., 1992). It is also used to treat depressive disorders, anxiety, and emotional stress (Bourbonnais-Spear et al., 2007; Mendenhall et al., 2012). In Peru, an infusion made with leaves and flowers has been used as an antispasmodic (Hammond et al., 1998). Indians from the Andes used to utilize feverfew for colic, kidney pain, stomach ache, and morning sickness. In Costa Rica, a decoction of its aerial parts was used to aid digestion, as a cardiotonic, emmenagogue, and antihelminthic. In Venezuela, feverfew was used as a remedy against earache (Pareek et al., 2011). In Denmark, the aerial parts mixed with honey were assumed to treat epilepsy and used to induce sleep (Jäger et al., 2006, 2009). In Spain, feverfew has been used in veterinary medicine, most notably a tea prepared from its aerial parts mixed with bread, *Triticum aestivum* L., *Plantago lanceolata* L., and *Aloysia citriodora* Palau, is used as a postlabor antiseptic for cows (Bonet and Vallès, 2007). Fresh leaves, reduced to a poultice, have been used to loosen up stiff feet (Blanco et al., 1999).

In conclusion, feverfew is an old medicinal plant which is currently used in food supplements and phytotherapeutics to alleviate inflammatory states, notably in the case of migraine. Its therapeutic virtues are confirmed by reliable preclinical studies and clinical trials. No particular adverse side effects have emerged from the latter, making it a safe drug.

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Chapter 3.17

Garlic (*Allium sativum* L.)

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HISTORY OF GARLIC (*ALLIUM SATIVUM* L.)

Garlic (*Allium sativum* L.) is known as a prophylactic therapeutic medicinal plant. It plays an important dietary and medicinal role worldwide. This medicinal plant was noted in for the first time in Avesta, a collection of Zoroastrian holy writings that was likely compiled in the 6th century BC (Dannesteter, 2003). It should be noted that garlic was well known as a medicine among Sumerians and the ancient Egyptians. There is some evidence that shows that garlic was given to athletes in order to increase their stamina during the earliest Olympic Games in Greece. In ancient Chinese and Indian medicine, garlic is recommended to help respiration and digestion as well as to cure leprosy and parasitic infestations. It has played an important role in treating various diseases in the medieval period as well. In his well-known book, *Avicenna*, Al Qanoon Fil Tib (The Canon of Medicine) recommended garlic as a useful mixture to treat arthritis, toothache, chronic coughs, constipation, parasitic infestation, snake and insect bites, gynecological diseases, as well as in infectious diseases (being used as an antibiotic). During the Renaissance, particular attention was paid to the health benefits of garlic in Europe (Lawson and Bauer, 1998).

Modern medicine has focused special attention on garlic because of the widespread belief of its effects in maintaining good health.

Among leading prescription drugs, garlic products are widely sold in some Western countries. In addition, remarkable epidemiologic evidence has shown the preventive and therapeutic roles of garlic. Numerous experimental and clinical studies mention many desirable impacts of garlic. These impacts are mostly ascribed to the following: (1) antioxidant and antimicrobial effect; (2) risk reduction of cardiovascular diseases; (3) risk reduction of cancer; and (4) increased detoxification of foreign compounds and hepatoprotection (Aviello et al, 2009; Colín-González et al., 2012).

In this review, current experimental studies, as well as the clinical state of knowledge regarding the preventive and therapeutic effects of garlic against different diseases, will be addressed. Garlic is a bulbous plant that grows to 1.2 m in height. Garlic is easily grown and can be grown in mild climates. There are various types or subspecies of garlic, most notably hardneck garlic and softneck garlic. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the vital bioactive compound that is found in the aqueous extract of garlic or raw garlic homogenate. Therefore, the allinase enzyme is activated when garlic is chopped or crushed, and allicin is produced from alliin (present in intact garlic). The other important compounds present in garlic homogenate are 1-propenyl allyl thiosulfonate, allyl methyl thiosulfonate, (E, Z)-4,5,9-trithiadodecal,6,11-triene 9-oxide (ajoene), and γ-L-glutamyl-S-alkyl-L-cysteine. When the homogenate is incubated at room temperature for several hours, its adenosine concentration increases several-fold. Another widely studied garlic preparation is aged garlic extract. Sliced draw garlic stored in 20% ethanol for more than 1.5 years can be called aged garlic extract. This whole process is assumed to cause considerable loss of allicin and increased activity of certain newer compounds, such as S-allylcysteine, S-allylmercaptocysteine, allixin, N-0-(Ideoxy-D-fructos-1-yl)-L-arginine, and selenium which are both stable and significantly antioxidant. Garlic oil which has medical applications is mostly produced by steam distillation. Steam distilled garlic oil is composed of diallyl, allylmethyl, and dimethyl mono to hexa sulfides. *A. sativum* is a member of the Lillaceae family, together with onions, chives, and shallots (Iciek et al., 2009).

BENEFICIAL EFFECTS OF GARLIC ON CARDIOVASCULAR DISEASES

Garlic and its products have been extensively known as agents for cardiovascular disease prevention and treatment. Many scientific literature supports this assumption that garlic consumption has significant impact on the followings: (1) reducing

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serum cholesterol and triglyceride; (2) lowering blood pressure and preventing atherosclerosis; (3) inhibiting platelet aggregation; and (4) increasing fibrinolytic activity.

Both experimental and clinical studies on various garlic products have proved that these desirable cardiovascular effects do exist (Chan et al., 2013).

In vivo animal experiments, with intravenous administration of garlic extracts, resulted in significant reductions in both diastolic and systolic pressures. In addition, oral ingestion of garlic extract in hypertensive animals brought their blood pressure back to normal levels. In more than 80% of patients with high blood pressure, numerical clinical studies indicated that garlic reduced their blood pressure (Omar, 2013; Stabler et al., 2012). Reviewing the 47 hypertensive patients in one trial, it was found that garlic remarkably decreased the average systolic blood pressure by 12 mmHg and the average supine diastolic blood pressure by 9 mmHg vs. a placebo (Auer et al., 1989). The authors stated that there were no side effects taking garlic and no serious complications were reported. In another study, 200 mg of garlic powder was used 3 times daily, accompanied by hydrochlorothiazide-triamterene baseline therapy. This produced an average reduction of systolic blood pressure of 10–11 mmHg and of diastolic blood pressure reduction of 6–8 mmHg versus a placebo (Kandziora, 1988). However, more information is needed as to whether garlic provides a therapeutic advantage versus a placebo in terms of reduction of the cardiovascular morbidity risk in patients with hypertension. It has been found that the mechanism of garlic's antihypertensive activity is related to its prostaglandin-like effects, which result in a decrease in peripheral vascular resistance (Stabler et al., 2012). Aged garlic extract cause a decrease in the systolic blood pressure compared to a placebo (based on a dosage of 240–960 mg of aged garlic extract containing 0.6–2.4 of S-allylcysteine) by about 12 mmHg over 12 weeks (Ried et al., 2013). Garlic use in rats suffering from hypercholesterolemia induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride, and low-density lipoprotein (LDL), but did not affect serum high-density lipoprotein (HDL) (Kamanna and Chandrasekhara, 1982). In vitro studies showed that garlic suppresses LDL oxidation and increases HDL. In addition, this may be one of garlic's useful protective mechanisms for cardiovascular health (Milner, 2006). Long-term application of garlic and its products on experimental atherosclerosis, induced by a high-cholesterol diet, has indicated a 50% reduction in atheromatous lesions, especially in the aorta (Jain, 1977). Many human studies have shown that garlic's effects, and the effects of its products, are involved in the significant decrease in serum cholesterol and triglyceride. A metaanalysis was done on 39 primary trials considering the effects of a 2-month administration of garlic products on total cholesterol, LDL cholesterol, HDL, and triglycerides. The findings indicated that, in subjects with elevated total cholesterol levels, garlic plays a role in the reduction of total serum cholesterol by 17 ± 6 mg/dL and in LDL cholesterol by 9 ± 6 mg/dL (>200 mg/dL). A reduction in the concentration of serum total cholesterol of 8% magnitude results in a 38% reduction in the risk of coronary events at 50 years of age (Ried et al., 2013). HDL cholesterol levels were improved a little, and triglycerides were not influenced significantly. In all trials, garlic was highly tolerable and was accompanied by minimal side effects.

From this metaanalysis study, it was concluded that garlic should be considered as an alternative option with a higher safety profile than conventional cholesterol-lowering medications in patients with high cholesterol (Ried et al., 2013). However, a few studies have successfully shown a lipid-lowering side effect when using garlic powder (which has low allicin). It has been proposed that different people may show different responses to garlic (Zeng et al., 2013). Consequently, garlic may be more helpful for some specific groups.

The preventive effect of garlic on atherosclerosis has been ascribed to its capacity to reduce lipid content in arterial membranes. The active compounds responsible for its antiatherosclerotic effect include allicin, S-allyl cysteine, found in aged garlic extract, and diallyldisulfide, found in garlic oil. When this diet was improved by garlic use, the plasma fibrinolytic activity in animals, which was decreased on cholesterol feeding, was considerably increased (Yeh and Liu, 2001).

Human studies have shown that plasma fibrinolytic and its activity have increased by garlic among the healthy people and patients with acute myocardial infarction (Mirhadi et al., 1991). Of course, researche (pretreatment) has significantly displayed the inhibited intracellular Ca^{2+} mobilization, thromboxane-A2 (a potent platelet aggregator) synthesis, and protection against thrombocytopenia induced by collagen or arachidonate application in rabbits. From the observations, it is clear that garlic may be useful in preventing thrombosis. Garlic is involved in the inhibition of platelet adhesion or aggregation in human studies. It has been found that aged garlic extract prevents the binding of ADP-activated platelets to immobilized fibrinogen (Mirhadi et al., 1991).

It is implied that aged garlic extract inhibited platelet aggregation through the inhibition of the GPIIb/IIIa receptor and an increase in cAMP (Allison et al., 2012). Besides this, it was reported that garlic reduces the risk of peripheral arterial occlusive diseases, plasma viscosity, and unstable angina, and increases the elastic property of the blood vessels as well as capillary perfusion.

In one study, 78 patients aged 40–78, with peripheral arterial occlusive disease, were randomly given either 400 mg of garlic or a placebo twice daily. Both men and women aged 40–75 years were enrolled in the study. Whether receiving garlic

or the placebo, pain-free walking distance increased similarly after 12 weeks of treatment. In the same way, there were no differences between the groups in terms of blood pressure changes, heart rate, and ankle and brachial pressures. No severe side effects were shown, however, more people taking garlic (28%) than the placebo (12%) complained of a noticeable garlic smell. This suggests that any improvements in the symptoms of peripheral arterial occlusive disease due to garlic may require longer term treatment and follow-up times than occurred in this study (Jepson et al., 2000).

ANTITUMOR PROPERTIES OF GARLIC

Many in vitro and in vivo studies have suggested the possible cancer-preventive effects of garlic products and their respective compounds. It is clear that garlic has a large number of potent bioactive compounds with anticancer properties and plenty of allylsulfide derivatives. Different compounds have been reported in garlic which are involved in a number of molecular mechanisms in carcinogenesis, such as DNA adduct formation, mutagenesis, scavenging of free radicals, cell proliferation and differentiation, as well as angiogenesis. The growth rate of cancer cells is reduced by garlic, through the cell cycle blockade that occurs in the G2/M phase (Capasso, 2013). In 1990, the U.S. National Cancer Institute reported the Designer Food Program to determine which foods played an important role in inhibiting the development of chemically induced tumors in the liver, prostate (Hsing et al., 2002), bladder and mammary glands (Amagase and Milner, 1993), lung (Sparnins et al., 1986), skin (Nishino et al., 1989), and stomach (Wattenberg et al., 1989), in both rodents and human studies. It was found that garlic and its constituents.

An organosulfur compound isolated from garlic, diallyl trisulfide (DATS) has been shown in studies to have anticancer activity both in vitro and in vivo. As opposed to PC-3 cancer cells, the cytotoxicity of DATS to prostate epithelial cells was reduced (Borkowska et al., 2013).

Possible anticarcinogenic mechanisms of garlic and its constituents may comprise the inhibition of carcinogen activation, the enhancement of detoxification and excretion, and the protection of DNA from activated carcinogens. Furthermore, DATS caused the reduction of tumor mass and number of mitotic cells within tumors (Wallace et al., 2013). DATS reduced mitosis in tumors, decreased histone deacetylase activity, increased acetylation of H3 and H4, inhibited cell cycle progression, and decreased protumor markers (survivin, Bcl-2, c-Myc, mTOR, EGFR, and VEGF) (Wallace et al., 2013). It is proved that garlic components play a role in blocking the covalent binding of carcinogens to DNA, increase carcinogen degradation, are antioxidants, have free radical–scavenging properties, and regulate cell proliferation, apoptosis, and immune responses. Ajoene—garlic stable oil soluble sulfur's rich compound and garlic-derived natural compound—has been shown to induce apoptosis in leukemic cells in addition to other blood cells of patients with leukemia (Tsubura et al., 2011). Through the stimulation of peroxide production, activation of caspase-3-like and caspase-8 activity, ajoene-induced apoptosis in human leukemic cells. Garlic synergizes the effect of eicosapentaenoic acid, a breast cancer suppressor, and antagonizes the effect of linoleic acid, a breast cancer enhancer (Tsubura et al., 2011).

The antiproliferative activity of ajoene was shown against a panel of human tumor cell lines (Lin et al., 2002). Moreover, allicin was found to prevent the proliferation of human mammary, endometrial, and colon cancer cells (Finley, 2003). Growth inhibition is the result of an accumulation of cells in the WIG1 and G2lM phase of the cell cycle. Thus, allicin is also responsible for the antiproliferative effect of garlic derivatives. Diallyl sulfide and diallyl disulfide inhibit arylamine N-acetyltransferase activity and 2-aminofluorene-DNA in human promyelocytic leukemia cells (Lin et al., 2002).

Reduction of some malignancy risk due to the consumption of selenium enriched plants, such as garlic, was mentioned by Wang et al. (2012). Through enhancement of the levels of intracellular reactive oxygen species and DNA damage, and by inducing endoplasmic reticulum stress and mitochondria mediated apoptosis, DATS inhibited the cell growth of human melanoma A375 cells and basal cell carcinoma cells (Wang et al., 2012).

DIABETES MELLITUS

Experimental studies have shown garlic's clear hypoglycemic effect, though, the effect of garlic on human blood glucose remains controversial. Many studies indicate that garlic can reduce blood glucose level in diabetic animals. Garlic was effective in reducing blood glucose in streptozotocin-induced and alloxan-induced diabetes mellitus in rats and mice (Ohaeri, 2001). A short-term benefit of garlic was found against dyslipidemia in diabetic patients. Garlic significantly reduced total serum cholesterol and LDL cholesterol, in addition to moderately raising HDL cholesterol as compared to a placebo in diabetic patients. By preventing reactive oxygen species formation through modulation of NADPH oxidase subunit expression, S-allyl cysteine, a bioactive component derived from garlic, restored erectile function in diabetic rats (Yang et al., 2013).

In diabetic patients metformin and garlic treatment reduced fasting blood glucose (FBG) for 12 weeks, but the percentage of change in FBG was more substantial with metformin supplemented by garlic than metformin alone (Kumar et al., 2013). Chronic feeding of garlic extracts resulted in a significant decrease in blood glucose level. However, for some human studies no change in blood glucose level was noticed. Therefore, the role of garlic in diabetic patients needs to be further studied. The beneficial effect of garlic on diabetes mellitus is mainly ascribed to the presence of volatile sulfur compounds, such as alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene, and allyl mercaptan. It has been reported that garlic extracts could be effective in reducing insulin resistance (Padiya and Banerjee, 2013).

EFFECT OF GARLIC ON CHEMICALLY INDUCED HEPATOTOXICITY

Several studies have suggested that garlic can protect liver cells from some toxic agents.

In many countries, acetaminophen is the most important analgesic and antipyretic drug used. Overdose is known to cause hepatotoxicity and nephrotoxicity in humans and rodents. Although more than 90% of acetaminophen is converted into sulfate and glucouronide conjugates and excreted in the urine, a small portion is metabolized by different liver enzymes. This can arylate critical cell proteins and cause toxicity (Patten et al., 1993). It has been shown that garlic protects against acetaminophen-induced hepatotoxicity. In addition, gentamycin induces hepatic damage as revealed by elevation of liver damage marker enzymes (aspartate transaminase and alanine aminotransferase) and reduction in plasma albumin level. Dietary inclusion of garlic powder (1) protects rats against gentamycin-induced hepatotoxicity, (2) improves antioxidant status, and (3) modulates oxidative stress. Furthermore, garlic attenuated hepatotoxicity effect of nitrate in rats (El-Kott, 2012).

Garlic extract may decrease lipid peroxidation and enhance the antioxidant defense system (Ademiluyi et al., 2013).

ANTIMICROBIAL EFFECT OF GARLIC

In various societies, garlic has been used for centuries to treat infectious diseases. It is believed that Louis Pasteur historically described the antibacterial effect of garlic in 1858 for the first time, although no source of evidence of this is available. More recently, garlic has been proven to be effective against a plethora of gram-positive, gram-negative, and acid-fast bacteria. These comprise *Salmonella*, *Escherichia coli*, *Pseudomonas*, *Proteus*, *Staphylococcus aureus*, *Klebsiella*, *Micrococcus*, *Bacillus subtulis*, *Clostridium*, *Mycobacterium*, and *Helicobacter* (O'Gara et al., 2000). It has been documented that garlic establishes a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria. The antibacterial activity of garlic is widely ascribed to allicin. It is found that allicin has sulfhydryl-modifying activity and is able to inhibit sulfhydryl enzymes (Wills, 1956). Cysteine and glutathione counteract the thiolation activity of allicin. It is clear that garlic extract and allicin exert bacteriostatic effects on some vancomycin-resistant enterococci (Jonkers et al., 1999). Also, an inhibitory synergism was observed when used in combination with vancomycin. It is imagined that allicin modifies the sulfhydryl groups on the enzymes of the TN1546 transposon, which encodes vancomycin resistance, and enhances susceptibility to vancomycin.

In an in vitro study, the antibacterial effect of different concentrations of garlic extract against human dental plaque microbiota has been proven (Santhosha et al., 2013). A synergism between ciprofloxacin with garlic extract has been indicated, but not between ampicillin and garlic extracts (Zain al-abdeen et al., 2017). Cloves of garlic and the rhizomes of ginger, extracted with 95% ethanol, are said to have antibacterial activity against multidrug clinical pathogens and can be used to prevent drug-resistant microbial diseases. *Pseudomonas aeruginosa* was the most sensitive germ to the mixture (Karuppiah and Rajaram, 2012). Besides this, garlic is recommended as a treatment for multidrug-resistant tuberculosis (Dini et al., 2011).

ANTIPROTOZOAL PROPERTIES

Several studies have demonstrated that garlic extract is effective against a host of protozoa including *Candida albicans*, *Scedosporium prolificans*, *Tinea pedis*, *Opalina ranarum*, *Balantidium entozoon*, *Entamoeba histolytica*, Trypanosomes, *Leishmania*, *Leptomonas*, and *Crithidia* (Reuter et al., 1966). Garlic was suggested for the treatment of giardiasis due to the unpleasant side effects of, and *Giardia*'s increased resistance to, synthetic pharmaceuticals. Inhibitory activity of garlic on *Giardia* was recorded with a crude extract of 25 pg/mL and a lethal dosage set at approximately 50 pg/mL. A clinical trial encouraged by these results was carried out on patients with giardiasis (Soffar and Mokhtar, 1991). Garlic was set as an antigiardial, removing the symptoms from all patients within 24 h and completely removing any indication of giardiasis from stools within 72 h at a dosage of 1 mg/mL, twice daily, of aqueous extract or 0.6 mg/mL for commercially prepared

garlic capsules. In vitro assays were impossible, since was not possible to culture the protozoa in vitro. It was shown that allicin, ajoene, and organosulfides from garlic are effective antiprotozoal compounds.

ANTIFUNGAL PROPERTIES

Antifingal activity was studied in 1936 for the first time by Schmidt and Marquardt whilst working with epidermophyte cultures (Lemar et al., 2002). Many fungi, including *Candida*, *Torulopsis*, *Trichophyton*, *Cryptococcus*, *Aspergillus*, *Trichosporon*, and *Rhodotorula* are sensitive to garlic.

It is proved that garlic plays a role in the decrease of oxygen uptake, reduction of organism growth, inhibition of lipid, protein, and nucleic acid synthesis, and prevention of membrane damage (Yousuf et al., 2011). It was found that a sample of pure allicin could be used as an antifungal (Szymona, 1951). Removal of allicin from the reaction (via solvent extraction) decreased antifungal activity (Yousuf et al., 2011). It has also been observed with the garlic constituents, diallyl trisulfide, against *Cryptococcal meningitis*, ajoene, and against *Aspergillus* (Yoshida et al., 1987). In addition, thiol reduced this activity, suggesting that allicin blocks thiol oxidation. It is thought that inhibition of respiration is due to the control of succinate dehydrogenase. In the presence of garlic extract, the adhesion of *Candida* is also remarkably reduced (Ghannoum, 1988). Again, this effect is diminished by the addition of thiol compounds. Inhibition at concentrations lower than experienced with allicin is due to the addition of ajoene to some fungal growth mixtures, including *Aspergillus niger*, *C. albicans*, and *Paracoccidiodes*. Studies on aged garlic extract (with no allicin or allicin-derived constituents) showed no antifungal activity in vitro. However, the number of organisms that were seen was reduced by up to 80% when given to infected mice. It has been reported that garlic showed antifungal effects on two species, the airborne pathogen *Botrytis cinerea* as well as *Trichoderma harzianum*. Patients with denture stomatitis reported greater satisfaction using garlic than nystatin. Very few studies have been done to evaluate the antiviral properties of garlic in comparison to the antibacterial action of garlic. The few studies which do exist have suggested that garlic has an in vitro activity against influenza A and B, cytomegalovirus, rhinovirus, HIV, herpes simplex virus 1, herpes simplex virus 2, viral pneumonia, and rotavirus (Weber et al., 1992). Allicin, diallyl trisulfide, and ajoene have all proved to be active (Lanzotti et al., 2012).

In the case of HIV, it is believed that ajoene acts by inhibiting the integrin-dependent processes. Allyl alcohol and diallyl disulfide have also been shown to be effective against HIV infected cells (Tatarintsev et al., 1992). No activity has been observed with allicin or S-allylcysteine. Also, it seems that only allicin and allicin-derived substances are active (Shoji et al., 1993). It should be noted that the beneficial effects of garlic extract cause to be useful in medicine (Lissiman et al., 2012). There are insufficient clinical trials on the effects of garlic in order to prevent or treat the common cold. A single trial implied that garlic may prevent occurrences of the common cold, but more studies are needed in order to validate this finding. This trial randomly assigned 146 participants to either a daily garlic supplement (with 180 mg of allicin content) or a placebo for 12 weeks. The study showed that there were 24 occurrences of the common cold in the garlic group compared to 65 in the placebo group, resulting in fewer days of illness in the garlic group compared to the placebo group (Lissiman et al., 2012).

It appears that claims of effectiveness of garlic against the common cold rely largely on poor-quality evidence. Many countries use garlic extracts for clinical treatments, but the untoward action of garlic following long-term administration should be fully considered. Even though many studies on garlic and its derivatives have been performed, the exact biological mechanism of garlic extract remains yet to be elucidated (Bakhshi et al., 2012).

ANTIACNE ACTIVITY

The antimicrobial and antifungal properties of *Allium cepa* and *A. sativum* were found against *Malassezia furfur*, *C. albicans*, and some other *Candida* species, as well as some strains of dermatophytes and *Acne vulgaris* microbes. Accordingly, findings show that *A. cepa* and *A. sativum* might be promising in the treatment of bacterial and fungal-associated infections (Ravisankar et al., 2015).

IMMUNONUTRITION

Diet plays a vital role in maintaining health and the consumption of foods rich in phytochemicals helps to improve the immune system. The term “immune nutrition” refers to the intake of certain nutrients which play an important role in the maintenance of the human immune system (Lin and Karin, 2007).

The immune system prevents infections and diseases by warding off foreign cells. Garlic and its supplements were being consumed in many cultures as a means of enhancing the immune system in addition to its other beneficial effects.

In maintaining the functionality of the immune system, several compounds and classes have been identified which play an essential role. The effect of consumption of garlic and its components on immune stimulation is exhibited by means of an increase in total WBC count and improvement in bone marrow cellularity (Kuttan, 2000). The organosulfur (OSC) compound in garlic scavenges oxidizing agents and prevents the formation of proinflammatory messengers by interacting with sulfur-containing enzymes (Santhosha et al., 2013).

MEDICINE CONCENTRATION

Uses of garlic (*A. sativum*) include the treatment of respiratory infection and cardiovascular diseases. It seems that it has an antithrombotic activity (Bordia, 1978). Laboratory studies indicated that garlic controls CYP2C9, CYP3A, and CYP2D6. Researche on rats showed that garlic restrains CYP2E1 and increases CYP2C9. However, no effects of garlic on cytochrome P450 isoenzymes were shown in clinical studies (Baxter et al., 2013).

Reports have indicated that the use of garlic may lead to INR increasing and bleeding in patients taking warfarin. In patients taking warfarin, it has been proved that INR more than doubled and showed hematuria 8 weeks after taking garlic daily. When consumption of garlic was stopped this problem ceased (Baxter et al., 2013). On the other hand, a placebo-controlled study among 48 patients taking warfarin showed that there was no INR change among those who took 5 mL of garlic extract twice daily for 12 weeks (Macan et al., 2006). Likewise, initial reports of patients taking warfarin suggests that no significant increase was found in the risk of bleeding or INR among the patients taking garlic at the same time (Shalansky et al., 2007). Garlic leads to decreased platelet aggregation which may enhance the risk of bleeding. Nevertheless, it will not increase INR. In addition, reported cases have shown that its mechanism is unknown (Rahman and Billington, 2000; Steiner and Li, 2001). For garlic–warfarin interaction, clinical evidence is limited to this report. Also there seems to be no serious interaction found between warfarin and garlic. Due to the bleeding complications, it is wise not to take the garlic and warfarin simultaneously. In most studies, the interaction of garlic with medicines with antitumor, anticoagulatin, antiinflammatory, antiviral (Saquinavir which reduces its accumulation in plasma), and anticancer (such as Docetaxe which decreases its clearance) properties, as well as antidiabetic medicines such as Glibenclamide (which greater reduce blood glucose level in combination with garlic), are reported as antitumor medicines. Therefore, these undesirable effects are called nephrotoxic (for example, it makes the renal toxicity with Atorvastatin), digestive toxicity, blood clotting as well as medicine toxicity increases. In combination with garlic, Chlorzoxazone (which is muscle relaxant) leads to an increase in medicine plasma (Adhikari et al., 2015).

CONCLUSION

Garlic has been used against various types of diseases since ancient times. In various studies, the biological and medicinal properties of garlic and its compounds have been proved. In the meantime, in order to optimally open the active compounds existing in gastrointestinal part (which has the least enzymatic digestion, the most absorption and the best effect), this compounds should be appropriately packed (to sell this compound as drug by pharmaceutical companies). In addition, ingredient influencing every disease as well as effective dose of this substance on diseases or prevention should be fully determined in standard manner. For preparation of drug, it should be determined the following items: (1) how one can produce the most effect by pharmaceutical substance? (2) What items would break down the effective ingredient?

Food and drug interactions should be considered when using garlic, because herbs or pharmaceutical substance must be regarded as components of the diet—each component may affect the digestion and absorption the other components. Due to the low cost of garlic and its potential impacts on health, it provides consumers with many benefits without the side effects of chemical drugs. For each reported interaction with pharmaceuticals, further studies and researches is needed in order to determine the standard dose of garlic as well as its consumption type (i.e., cooked, intact or fresh, etc.).

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Chapter 3.18

Ginger (*Zingiber officinale* Roscoe)

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INTRODUCTION

Ginger, the rizhome of *Zingiber officinale* Roscoe (Zingiberaceae), is a plant originated in the Indo-Malayan region, and nowadays is a crop largely distributed across the tropics of Asia, Africa, America, and Australia (Kizhakkayil and Sasikumar, 2011). It has been used as a spice and medicine for over 2000 years (Bartley and Jacobs, 2000) and more recently as dietary supplement (Semwal et al., 2015; Yeh et al., 2014). The name *Z. officinale* is derived from the botanist William Roscoe (1807). "Zingiberis" is the Greek word derived from the Sanskrit "shringravera" meaning "shaped like a deer's antler's" (Elzebroek and Wind, 2008).

Ginger has a pungent flavor and odor due the presence of volatile oils, that are active ingredients which present analgesic, sedative, antipyretic, and antibacterial physiological effects (Rehman et al., 2011). Moreover, ginger is a rich source of phytochemicals, bioactive compounds, which have potentially protective effects against several diseases. Indeed, it is a strong antioxidant, avoiding the generation of free radicals. Therefore, it is a species which should be further investigated due its large use, low cost, and most importantly beneficial effects on human health.

In this chapter the antioxidant, antiinflammatory, antimicrobial, and other biological activities of ginger are highlighted vis a vis the scientific knowledge published recently.

PHYTOCHEMICAL AND NUTRITIONAL COMPOSITION

A great number of biologically active compounds in ginger are associated not only with this peculiar spice but also to its action in terms of health. There are a wide variety of constituents which are related to the origin of the ginger as being linked to whether the rhizomes are fresh or dry (Ali et al., 2008). *Z. officinale* has been characterized as a functional food according to its nutritional and phytochemical composition (Table 3.18.1). The nutritional composition of a powdered sample of ginger presents carbohydrates, protein, fat, dietary fiber, iron, calcium, vitamin C, and carotene (Kumari and Gupta, 2016) and its phytochemical profile reveals the presence of total polyphenols, flavonoids, and anthocyanidins (Trinidad et al., 2012). Indeed, the contents of organic acids and their varieties are of great importance for its application in novel functional foods. Five organic acids found in ginger rhizomes are citric, malic, oxalic, succinic, and tartaric (Yeh et al., 2014).

Chemical analysis of the phytochemical composition of ginger shows that it contains terpenes including zingiberene, beta-bisabolene, alpha-farnesene, beta-sesquiphellandrene, and alpha-curcumene, and phenolic compounds such gingerol, paradols, and shogaol (Grzanna et al., 2005). The bioactive compounds 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol have been characterized in the dietary supplements of ginger as major components (Schwertner and Rios, 2007). Zingiberene and bisabolene contribute to its aroma and the volatile oils, gingerols and shogaols, contribute to both its characteristic odor and pungent taste (Harold, 2004).

BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES

Ginger has been extensively studied due the presence of its active compounds which provide biological and therapeutic roles (Baliga et al., 2013). Fig. 3.18.1 shows ginger as well as its main biological properties. Regarding its beneficial health

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TABLE 3.18.1 Nutritional and Phytochemical Composition of Ginger

Composition of ginger root		Powder sample/100 g
Nutritional composition	Carbohydrate	39.35 g
	Protein	6.08 g
	Fat	3.6 g
	Dietary fiber	20.1 g
	Iron	9.8 mg
	Calcium	88.7 mg
	Vitamin C	9.2 mg
	Total carotene	76.7 µg
Phytochemical	Total polyphenols	55 mg
	Flavonoids	37 mg
	Anthocyanidins	22 mg

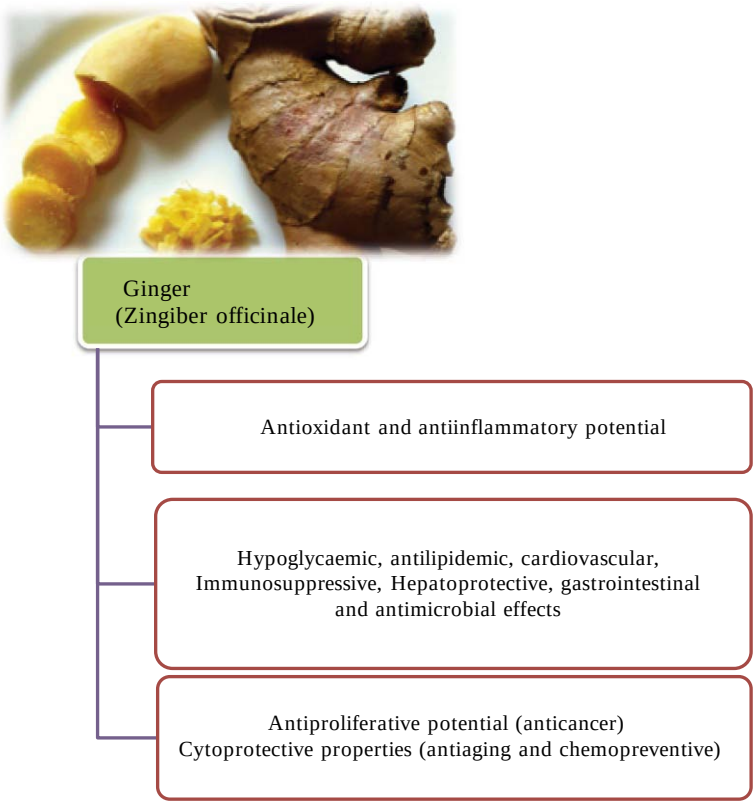

FIG. 3.18.1 Biological activities of ginger.

effects, as well as its identified bioactive compounds, extracts of ginger are commercially available as dietary supplements and pharmaceuticals products (Švarc-Gajić et al., 2016).

Yeh et al. (2014) compared the bioactive constituents and antioxidant activities of aqueous and ethanolic extracts from the roots of two varieties of ginger. They revealed that the antioxidant effect of the ethanolic extracts from ginger rhizomes was more effective than aqueous extracts. However, aqueous extracts were more effective in their scavenging and chelating abilities. These rhizomes could be used as a natural antioxidant.

A comprehensive evaluation of antioxidant capacity, cytotoxicity, and cytoprotective properties of purified ginger phenolic compounds (GPC), using a variety of in vitro assays, was carried out by Peng et al. (2012). In this study, 12 phenolics from fresh ginger were identified and classified into 3 classes to compare their activities. Isolated constituents showed potent antioxidant effects, cytotoxicity at low micromolar concentrations, moderate activity against xanthine oxidase, monoamine oxidase-A, and α-glucosidase, and the protection of rat pheochromocytoma PC12 cells and primary liver cells against H_2O_2-induced injury (Peng et al., 2012). The authors suggested that the biological activities of GPC occurred in diseases related to reactive oxygen species.

In a study carried out by Stoilova et al. (2007), CO_2 ginger extract showed a great deal of polyphenol which favors very good scavenging of the 2,2 diphenyl-1-picryl hydrazyl radical (DPPH). Moreover, the ginger extract also presented better lipid peroxidation inhibition, a higher chelating capacity, and better hydroxyl radical inhibition than quercetin which was the control. Indeed, several authors pointed out that ginger is a strong antioxidant substance that may either attenuate or avoid free radicals (Ali et al., 2008; Attia et al., 2013; Rehman et al., 2011; Stoilova et al., 2007).

It is well known that dietary ginger has been reported to decrease blood glucose level as well as enhance activities of intramitochondrial and extramitochondrial enzymes in diabetic rats (Ramudu et al., 2011). In addition, supplementation with 3 g of powdered ginger, in capsule form, daily for 3 months, improved glycemic indices, total antioxidant capacity, and serum paraoxonase-1 activity in patients with type 2 diabetes (Shidfar et al., 2015).

Furthermore, ginger essential oils (GEO) prevented chronic joint inflammation in rats in response to a streptococcal cell wall-induced arthritis model of rheumatoid arthritis that could be attributable to the combined effects of the pungent-tasting gingerols and the aromatic essential oils (Funk et al., 2016). Moreover, GEO exhibited hepatoprotective activity through its antioxidant potential against alcoholic fatty liver disease (Liu et al., 2013).

The preventive potential of ginger is also associated with obesity and cardiovascular diseases. It was demonstrated that dietary ginger extract increased the number of type I muscle fibers, decreased diet-induced obesity, and increased endurance capacity by stimulating fat utilization via enhanced peroxisome proliferator activated receptor δ signaling (Misawa et al., 2015). In addition, ginger extract lowered blood pressure by acting as a vasodilatator, the effect of which is attributed to cholinergic and calcium channel-blocking properties (Ghayur et al., 2005). A review carried out by Nicoll and Henein (2009) showed that doses of ginger (1–10 g) could exhibit safer therapeutic effects as an antiinflammatory agent than conventional cardiovascular drugs, having fewer side effects. Young et al. (2006) investigated the effect of ginger (1 g) and nifepidine (10 mg) on antiplatelet aggregation in humans. A synergic effect was identified between them, which could be advantageous to combat cardiovascular and cerebrovascular diseases in terms of platelet aggregation. Indeed, Nurtjahja-Tjendraputra et al. (2003) investigated the action mechanism of ginger phenolic compounds related to platelet aggregation and revealed that 8-gingerol, 8-shogaol, and 8-paradol exhibited more effective antiplatelet activities than aspirin. It was showed that 8-paradol was the most potent antiplatelet agent and COX-1 inhibitor.

More recently, Semwal et al. (2015) reviewed the biosynthesis, chemical synthesis, and biological activities of gingerols and shogaols, involved with several pharmacological effects including antiinflammatory, antioxidant, antidiabetic, and antiproliferative potential. In this review, Semwal et al. (2015) highlighted that the referred ginger biocompounds presented pharmacological significance against various types of cancer in vitro, including colon, lung, skin, and breast cancer. Karna et al. (2012) showed the anticancer activity of an extract of whole ginger (100 mg/kg of mouse body weight). They revealed a reduction in prostate cancer and did not detect any toxicity in normal tissues. Furthermore, Prakash and Srinivasan (2013) investigated the influence of ginger on the mineral uptake of a segment of intestine, revealing its positive effect on the intestinal absorption of micronutrient minerals.

In relation to the immune system, Lu et al. (2011) reported that 8-gingerol exhibited immunosuppression on the immune responses to ovalbumin (OVA) in mice by reducing OVA-specific immunoglobulin G (IgG) and also inhibiting lipopolysaccharide and concanavalin A-induced splenocyte proliferation. Moreover, 8-gingerol was able to decrease the percentage of $CD19^+$ B and $CD3^+$ T cells (Lu et al., 2011). These data suggested tumoral and cellular immune response inhibition.

Several ginger reports have shown that a wide range of ginger extracts are helpful to human health. The biocompounds 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol were highlighted as safe for human consumption to doses of 2000 mg (Zick et al., 2008). According Semwal et al. (2015) these indicated doses are lower than the recommended guidelines set by the US National Cancer Institute Common Toxicity Criteria. More recently, Shawahna and Taha (2017) carried out an extensive study in pregnant women in order to investigate the potential harmful and beneficial effects of ginger. It is well known that ginger is used as an antiemetic to alleviate nausea in pregnant women (Viljoen et al., 2014). However, it must be highlighted that ginger acts as an anticoagulant and as a hypotensive and hypoglycemic agent. Therefore, the coadministration of ginger with other drugs could generate adverse effects in response to drug interaction (Shawahna and Taha, 2017). In addition, the consumption of ginger by patients with gastrointestinal disorders should receive attention due the possible interactions of ginger with other drugs (Tsai et al., 2012). The effect of ginger on the pharmacokinetics

of metronidazole in rabbits showed a significant increase in the absorption and bioavailability of metronidazole (Okonta et al., 2008). Contrarily, it was showed that ginger coadministrated with warfarin did not affect the pharmacokinetics or pharmacodynamics of this anticoagulant (Jiang et al., 2006).

Nevertheless, the pharmacokinetics of ginger biocompounds in human biological trials is not well studied (Yu et al., 2011). On the other hand, ginger is a strong antioxidant, avoiding the generation of free radicals, and it is considered a safe medicine with only a few insignificant side effects (Al-Amin et al., 2006).

CONCLUSIONS

Z. oficinale has the potential to be a functional food and is considered a safe herbal medicine. Ginger is a good source of bioactive compounds and antioxidants. Food supplementation with ginger may be considered a novel nutritional approach to reduce chronic diseases. However, more clinical studies are required concerning safe doses for ginger supplements and their long-term effects.

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Chapter 3.19

Ginkgo biloba

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INTRODUCTION

Herbal medicines have been used for over 1000 years and they are one of the most promising sources of new medicines. One of these sources of newly emerging herbal medicines is *Ginkgo biloba* L., (of the Ginkgoaceae family; English name, maidenhair tree), a living fossil which has amazed scientists all over the world with its immense source of bioactive compounds and medicinal importance. The species is largely used in the treatment of central nervous system (CNS) disorders, such as Alzheimer's disease and cognitive deficits (Chan et al., 2007). The common name *Ginkgo* is a phonetic pronunciation of a Japanese name for the tree, while the species name "*biloba*" refers to the two distinct lobes, typical of the tree's leaves (Fig. 3.19.1). Ginkgo is a unique plant due to its distinctive classification in the plant kingdom, one of the oldest seed plants it is regarded as a "living fossil" (Mohanta et al., 2012). The Ginkgo tree flourished 150 million years ago during the Mesozoic era. It reached its greatest development during the Jurassic and Cretaceous periods (Kushwaha et al., 2014; Salvador, 1995). The Ginkgo tree is now cultivated extensively in Asia, Europe, North America, New Zealand, and Argentina (Huh and Staba, 1992). This tree has a long history of use in medicine by the Chinese, some 2000 years (Singh et al., 2008). Ginkgo leaf extracts are widely used in herbal medicinal products, food and dietary supplements, and botanical and complimentary medicines. A variety of bioactive compounds such as terpenoids (e.g., ginkgolides, bilobalide), flavonoids (e.g., kaempferol, quercetin, isorhamnetin), biflavonoids (e.g., sciadopitysin, ginkgetin, isoginkgetin), and organic acids (e.g., ginkgolic acid), among others, broaden its use in different biological systems (Chan et al., 2007). As such, the standard extract of *G. biloba* leaves (EGb 761) is widely used for treating neurological and cardiovascular disorders (Singh et al., 2008; Vellas et al., 2012) and thus is positioned as one of the most traded medicinal plants (van Beek, 2002; Nakanishi, 2005).

This chapter highlights the distribution of *G. biloba*, its trade and trends, classes of bioactive compounds, biological effects and possible molecular mechanisms, toxicity, and interactions with other drugs and food supplements.

GEOGRAPHICAL DISTRIBUTION

The *G. biloba* tree, which is native to China, Japan, and Korea, is distributed through cultivation in many parts of Europe, America, and the temperate regions of New Zealand, Argentina, and India (Table 3.19.1; Fig. 3.19.2). The last wild tree of *G. biloba* is reported in Zhejiang Province, China at an elevation of 1506 m a.s.l. (Singh et al., 2008). The wild populations of *G. biloba* have only few remaining trees, which place it in the endangered category according to the International Union for Conservation of Nature and Natural Resources (Endangered *B1+2c ver 2.3*. *Year* Published: 1998).

BIOACTIVE COMPOUNDS

The major bioactive compounds of *Ginkgo* are reported to be terpenoids, flavonoids, biflavonoids, organic acids, polyprenols, and many others (Table 3.19.2). Of these, ginkgolides and bilobalide are the major constituents of *G. biloba* that exhibit biological and/or pharmacological activities. Ginkgolides can be classified in five forms (A, B, C, J, and M), all having the same molecular geometrical skeleton but different numbers and geometric locations of hydroxyl functional groups (Fig. 3.19.3). The flavonoids like quercetin, kaempferol, and isorhamnetin are also the principal flavonoids occurring as glycoside derivatives in *G. biloba* (Fig. 3.19.3). A standardized leaf extract of *G. biloba*, known as EGb 761, contains 24% flavonoid glycosides, 6% terpenoids, 5%–10% organic acids, and other constituents, and are responsible for numerous health benefits (Chan et al., 2007; Salvador, 1995; Vasseur et al., 1994).

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00035-7

FIG. 3.19.1 Cultivated *Ginkgo biloba* tree at the G.B. Pant National Institute of Himalayan Environment and Sustainable Development campus, Almora, Uttarakhand, India.

TABLE 3.19.1 Native and Non-native Distribution of *Ginkgo biloba* Across the Globe

Country	Map Location	State/Region/Province	References
Native			
China	a–j	Zhejiang province, Guangxi, Guizhou, Sichuan province, Hubei, Chongqing, Henan, Shandong, Jiangsu, Fujian	Shen et al. (2005), Sun et al. (2003), Zhao et al. (2010)
Japan	k–o	Tsukuba, Ibaraki, Okayama, Tokyo, Fukuoka,	Zhao et al. (2010)
Korea	p–q	Seoul, Incheon	Zhao et al. (2010)
Non-native/cultivated			
Netherlands	1	Utrecht	Zhao et al. (2010)
Austria	2	Vienna University	Zhao et al. (2010)
France	3	Montpellier	Zhao et al. (2010)
Germany	4	Hannover	Zhao et al. (2010)
Italy	5	Padua	Zhao et al. (2010)
North America	6–7	Pennsylvania, MA	Zhao et al. (2010)
New Zealand	8	—	McWhannel (1981)
Argentina	9	—	Boelcke (1981)
India	10	Uttarakhand	Sati et al. (2013)

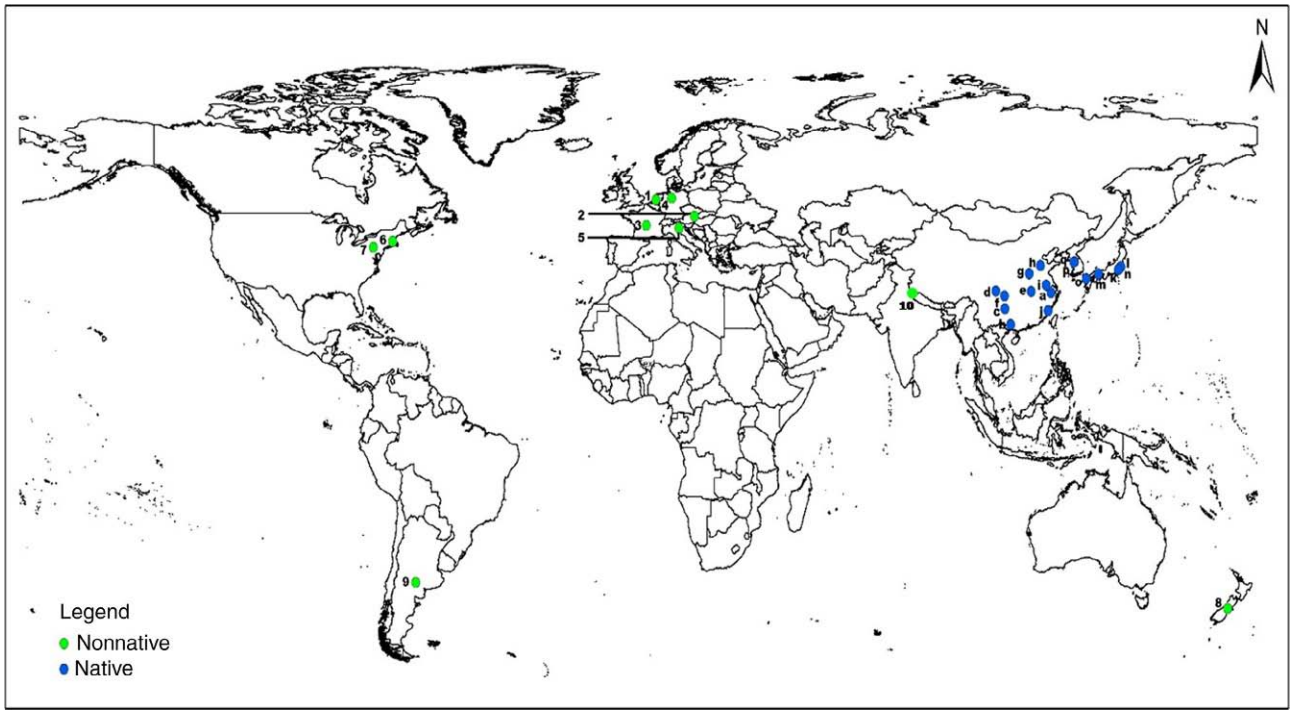


FIG. 3.19.2 Distribution of *Ginkgo biloba* showing native and nonnative occurrences across globe (*see also* Table 3.19.1).

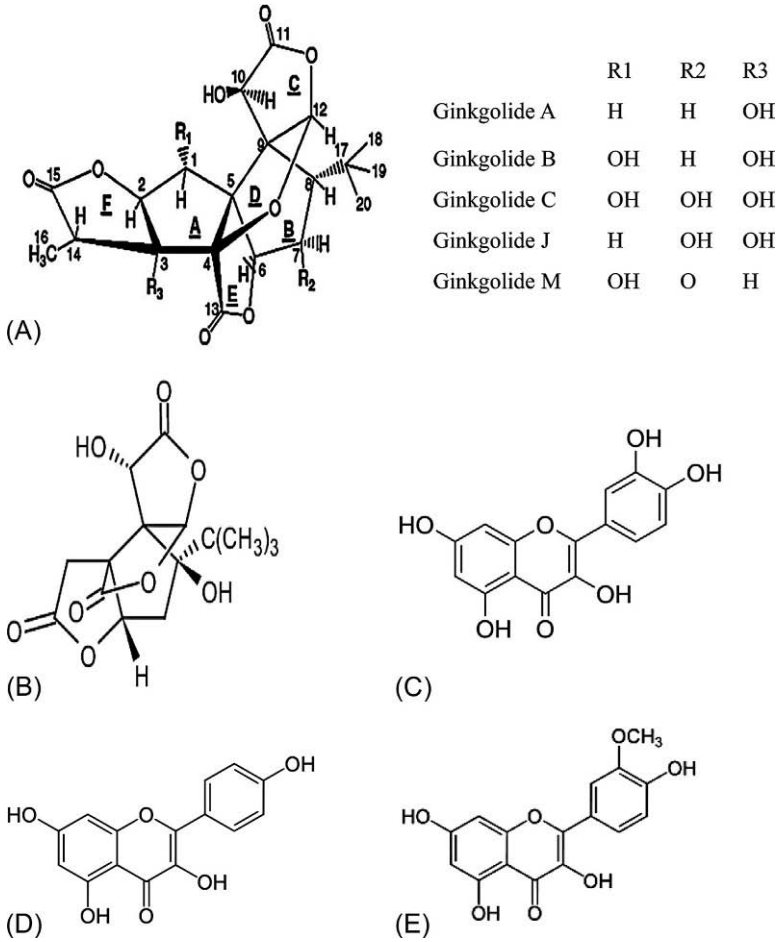
TABLE 3.19.2 Major Bioactive Components of *Ginkgo biloba*

Class	Plant parts	Major chemical constituents
Terpenoids	Leaf/root/bark	Diterpenes: ginkgolides A, B, C, and J (a)
	Root	Diterpenes: ginkgolides M (a)
	Leaf/bark	Sesquiterpene: bilobalide (b)
	Leaf/bark	Triterpenes: sterols
Flavonoids	Leaf	Quercetin (c), kaempferol (d), isorhamnetin (e), rutin, luteolin, delphidenon, myricetin
Biflavonoids	Leaf	Sciadopitysin, ginkgetin, isoginkgetin, amentoflavone, bilobetin, 5′-methoxybilobetin
Organic acids	Leaf	Benzoic acid derivatives (ginkgolic acid), *N*-containing acids
Polyprenols	Leaf	Di-*trans*-poly-*cis*-octadecaprenol
Others	Leaf	Waxes, steroids, 2-hexenal, cardanols, sugars, catechins, proanthocyanidins, phenols, aliphatic acids, rhamnose

Source: Modified from van Beek, T.A., Bombardelli, E., Morazzoni, P., Peterlongo, F. 1998. *Ginkgo biloba* L. Fitoterapia 69 (3), 195–244; van Beek, T. A. 2005. Ginkgolides and bilobalide: their physical, chromatographic and spectroscopic properties. Bioorg. Med. Chem. 13 (17), 5001–5012; DeFeudis, F.V., 1998. *Ginkgo biloba* extract (EGb 761): from chemistry to the clinic. Ullstein Medical, Wiesbaden, p. PP400.

THERAPEUTIC EFFECTS

G. biloba has been used in traditional Chinese medicine for many years for the treatment of asthma, bronchitis, tuberculosis, cognitive dysfunction, stomach pain, etc. (Almeida, 2009) and has been tested and clinically found effective as a dietary supplement and medication for the improvement of memory, treatment or prevent of Alzheimer's disease and other neurological disorders, and treatment of cardiovascular disorders through its neuroprotective, immunomodulatory, antiinflammatory, and antioxidant activities (Herrschaft et al., 2012; Kleijnen and Knipschild, 1992; Kanowski et al., 1997; Vellas et al., 2012). The molecular mechanism of the bioactive compounds of *G. biloba* for preventive actions have been well explored (Fig. 3.19.4) and some of these therapeutic effects are discussed in the following text.



	R1	R2	R3
Ginkgolide A	H	H	OH
Ginkgolide B	OH	H	OH
Ginkgolide C	OH	OH	OH
Ginkgolide J	H	OH	OH
Ginkgolide M	OH	O	H

FIG. 3.19.3 Chemical structures of the major bioactive compounds founds in *Ginkgo biloba*: (A) ginkgolide; (B) bilobalide; (C) quercetin; (D) kaempferol; (E) isorhamnetin. *Source: From Chan, P.C., Xia, Q., Fu, P.P. 2007. Ginkgo biloba leave extract: biological, medicinal, and toxicological effects. J. Environ. Sci. Health C 25(3), 211–244.*

Neuroprotective Effect

G. biloba extract prevents neurological damage and it is one of the most popular dietary supplements reported for enhancing memory (Ahlemeyer and Krieglstein, 2003a,b; Fitzpatrick et al., 2006; Santos-Neto et al., 2006; Ramassamy, 2006). The leaf extract EGb 761 has been reported to be effective against Alzheimer's at a dose of 240mg/kg/day (Ahlemeyer and Krieglstein, 2003b; Kleijnen and Knipscheld, 1992), which may be due to its antioxidant effect inhibiting Aβ-induced toxicity and cell death (Bastianetto and Quirion, 2002; Christen, 2000; Ponto and Schuitz, 2003). *G. biloba* leaf extract was found effective in reducing the behavioral deficit when tested against 6-hydroxydopamine-induced neurotoxicity in rats (Kim et al., 2004). The isolated compound sesquiterpene bilobalide at a dose of 3 and 6 mg/kg/day and EGb 761 at 25, 50, and 100 mg/kg/day, exert effect against gerbil global brain ischemia when administered orally for 7 weeks in rats (Chandrasekaran et al., 2002). The underlying mechanism for the neuroprotective effect was highlighted by CA1 neuron protection from death and downregulation of COX III mRNA encoded by mitochondrial DNA. The neuroprotective, as well as neurorestorative, effects of *G. biloba* extract were also studied in mice (Wu and Zhu, 1999). EGb 761 when administered at 20, 50, and 100 mg/kg per/day intraperitoneal (i.p) for 7 days before or after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment, protected nigrostriatal dopaminergic neurotoxicity along with a reduction in monoamine oxidase (MAO) activity in the brains of mice, suggesting one possible mechanism for the neuroprotective activity of *G. biloba*. Similarly, the isolated polysaccharides from *G. biloba* leaf extract were found to be effective against ischemia/reperfusion (I/R) injury in rat brains (Yang et al., 2013). Administration of a *G. biloba* supplement 7 days before I/R injury resulted in improvement in neurological deficits by a reduction in MDA content and proinflammatory cytokinins (TNF-α and IL-1β), while increased levels of antiinflammatory cytokinin (IL-10), superoxide dismutase (SOD) and myeloperoxidase (MPO) activity have also been recorded.

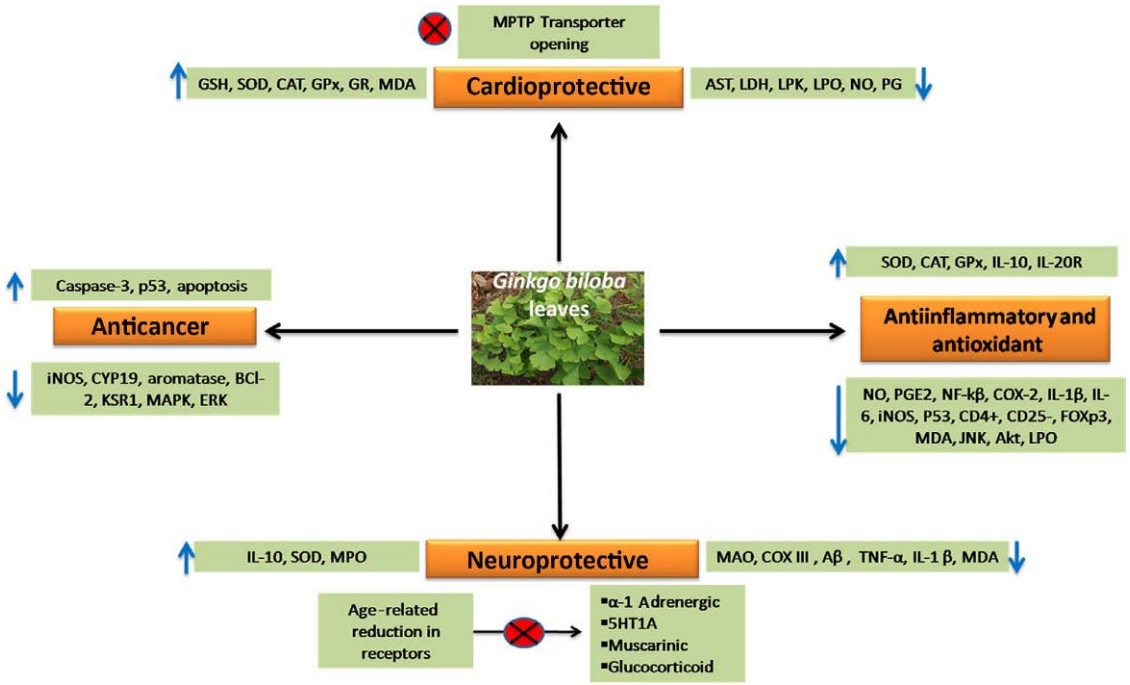


FIG. 3.19.4 Molecular mechanism underling different therapeutic effects of *Ginkgo biloba.* *Aβ*, Amyloid beta; *AST*, aminotransferase; *BCl-2*, B-cell lymphoma 2; *CAT*, catalase; *COX III*, cyclooxygenase 3; *CYP19*, aromatase gene; *ERK*, extracellular signal-regulated kinases; *GPx*, glutathione peroxidase; *GSH*, glutathione; *Il-1β,* interleukin 1 beta; *Il-10*, interleukin 10; *Il-20R*, interleukin 20R; *iNOS*, nitric oxide synthase; *KSR1*, kinase suppressor of Ras 1; *LDH*, lactate dehydrogenase; *LPK*, L-pyruvate kinase; *LPO*, lipid hydroperoxide; *MAO*, monoamino oxidase; *MAPK*, mitogen-activated protein kinase; *MDA*, malondialdehyde; *MPO*, myeloperoxidase; *MPTP*, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; *NO*, nitric oxide; *p53*, tumor protein p53; *PGE2*, prostaglandin E2; *SOD*, superoxide dismutase; *TNF-α,* tumor necrosis factor α.

G. biloba exerts its neuroprotective effect when coadministered with bone marrow derived mesenchymal stem cells (BMSCs) in an experimental autoimmune encephalomyelitis (EAE) rat model by inhibiting the secretion of proinflammatory cytokinins, demyelination, and protecting axons and neurons (Hao et al., 2016). *G. biloba* has been widely consumed to improve memory and learning power. As such, at 100mg/kg/day, orally administered *G. biloba* leaf extract, consumed for 4–8 weeks in mice, resulted in improved memory and learning during appetitive operant conditioning (Winter, 1991). In addition, a 40 mg/kg i.d dose for 1–3 weeks was found to enhance learning in young and aging mice (Cohen-Salmon et al., 1997). The flavonoids and bilobalide from *G. biloba* extract showed antioxidant and antiaging properties and exerted its action by scavenging free radicals and activating antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx), which protect against tissue injuries (Kim et al., 1997).

Antiinflammatory Effect

The antiinflammatory effect of *G. biloba* extract has been recorded with downregulation of nitric oxide (NO) and PGE2 production along with mRNA expression of iNOS and COX-2 enzymes and proinflammatory cytokinins (IL-1β, IL-6, and TNF-α) and upregulation of NF-kB factor (Mir and Albaradie, 2015). The effect of *G. biloba* leaf extract on the chronic inflammatory condition found in the colons of mice showed that the extract effectively suppresses the activation of macrophages and downregulates inflammation (iNOS, COX-2, and TNF-α) and inflammatory stress markers (p53 and p53-phospho-serine 15). Also the numbers of T cells ($CD4^+/CD25^-$/FOXp3) were reduced during this treatment (Kotakadi et al., 2008). In a similar study, *G. biloba* extract was found to be effective in helping rats recover from colitis by significantly reducing macroscopic and histological damage, elevating the activity of antioxidant enzymes, and reducing MDA content (Zhou et al., 2006). This colon tissue was also examined for inflammatory markers and revealed that *G. biloba* extract inhibited mRNA expression of TNF-α, NF-kBp65, and IL-6. Upregulation of antiinflammatory markers (IL-10 and IL-20R) in an atherosclerosis rat model was also recorded with administration of 100 mg/kg per/day of *G. biloba* leaf extract for 8 weeks along with a downregulation of the mRNA expression of IL-1β and TNF-α in comparison to a control group (Pietri et al., 1997).

Cardioprotective Effect

G. biloba extract was found to improve blood flow, prevent hypoxia and platelet aggregation, improve blood rheology, and reduce capillary permeability through the release of NO and prostaglandins (DeFeudis, 1998; Pietri et al., 1997). *G. biloba* leaf extract, terpenoids (ginkgolide A and B), and a terpene-free extract was examined for its cardioprotective activity on isolated ischemic and reperfused rat hearts. The result revealed that both *G. biloba* extract and isolated terpenoids delayed the onset of contracture during ischemia and postischemia and improved functional recovery (Liebgott et al., 2000). *G. biloba* extract also showed cardioprotective activity on a cardiac necrosis model of rats. A *G. biloba* phytosome (GBP) formulation was administered orally for 21 days at 100 and 200 mg/kg per day which significantly reduced the level of marker enzymes (AST, LDH, LPK, and lipid peroxidase) and increased GSH, SOD, CAT, GPx, and GR antioxidant enzymes, as well as playing a preventive role against myocardial necrosis (Panda and Naik, 2008). Hyperglycemia is a key initiating factor in diabetes-associated diseases. Diabetic cardiomyopathy is one factor which is induced by diabetic oxidative stress resulting in an opening of MPTP, leading to disfunctioning myocardium. *G. biloba* extract attenuates the oxidative stress and improves antioxidant enzyme levels, acting as a blocker of MPTP in animal models. When coadministered with atractyloside (an mPTP opener), the preventive effect is reversed (Saini et al., 2014).

Anticancer Activity

The antitumogenic effect of EGb 761 using an in vitro cell model and an in vivo xenograft model has been investigated (Park et al., 2016) and the extract was found effective as an antitumor agent by inhibiting aromatase activity in MCF-7 cells. In addition, CYP19 mRNA and CYP19 promoter 1.3 and PII expression was decreased in the treated cell model. In an in vivo experiment, aromatase overexpressing MCF-7 cells were implanted in BALB/c nude mice and given oral EGb 761 treatment for 3 weeks. Their tumor sizes were found to significantly decrease along with the downregulation of CYP19 mRNA expression. *G. biloba* extract was also found effective at preventing gastric cancer cell proliferation and at inducing apoptosis with a significant increase in caspase 3 and P53 and a decrease in antiapoptotic Bcl-2 levels (Bai et al., 2015). It has been well documented that the kinase suppressor of Ras1 (KSR1) is responsible for activation of oncogenic mitogen activated protein kinase (MAPK) and the extracellular signal-related kinase (ERK) signaling pathway, which contributes to the tumerogenesis and chemoresistance of human gastric cells (Roberts and Der, 2007). EGb 761 was found to be effective at increasing the sensitivity of chemotherapy and reversing chemoresistance by inhibiting the KSR1-mediated ERK1/2 pathway (Liu et al., 2015). *G. biloba* flavanoid compound, kaempferol, was also tested for cell proliferative and apoptosis activity against pancreatic cancer cells. This flavonoid, at 70 μM concentration for 4 days, was found to significantly inhibit the proliferation of cancer cells. When coadministered with the anticancer drug, 5-fluorouracil, a synergistic effect was recorded with increased apoptic cell concentrations. The role of NO in cancer cell proliferation is also downregulated because of alteration of the NO synthase enzyme expression by *G. biloba* extract (DeFeudis et al., 2003).

G. biloba also overcomes the toxic side effects of anticancer drugs. As such, when *G. biloba* extract is coadministered with cisplatin (an anticancer drug), no significant auditory brainstem response (ABR) threshold shift is recorded. Similarly, endocochlear potentials (EPs) decreased less than 20% for *G. biloba* coadministration compared to 50% using cisplatin alone. Hair cells in both groups remained intact in rats treated with *G. biloba* extract in combination with cisplatin as compared to hair loss in rats treated with cisplatin alone (Huang et al., 2007).

Other Effects

Ginkgo has also been used to treat brain function impairment and inner ear disorders such as hearing loss, vertigo, and tinnitus (Hilton and Stuart, 2004; Salvador, 1995). Diabetic cataracts are one of the earliest secondary complications of diabetes, eventually leading to loss of vision. The pharmacological effects of *G. biloba* extract (EGb 761) for prevention of diabetic-induced cataract conditions in rat lenses, cultured in high-glucose conditions, have been reported (Lu et al., 2014; Pollreisz and Schmidt–Erfurth, 2010). EGb 761 was found effective in the prevention of pathological changes of high glucose–induced lens epithelial cells and ameliorated lens opacity with decreased intensity of oxidative stress, aldose reductase activation, and levels of advanced glycosylation end products. It also was found to suppress transforming growth factor β2 or Smad pathway activation, increase E-cadherin, and decreases α smooth muscle actin expression, all of which makes *G. biloba* an potential drug candidate for the prevention of diabetes-induced cataracts.

INTERACTIONS AND TOXICITY

G. biloba extract was found to increased bleeding and coagulation time (Kohler et al., 2004) and when coadministered with antiplatelet and anticoagulant drugs the antiplatelet activity was found to increase (Bent, 2008; Koch, 2005; Ryu et al., 2009). Interaction of *G. biloba* extract with drug-metabolizing enzymes has been well explored. As such, nicardipine (a calcium channel blocker), an antihypertensive drug when coadministered with EGb 761, causes a decrease in the rate of drug metabolism due to an inhibition of CYP3A which decreases the hypotensive action of nicardipine (Yoshioka et al., 2004a, b). Similarly, another calcium channel blocker drug, talinolol, which is a substrate drug for PGAP transporter in humans was found ineffective when coadministered with *G. biloba* extract (Fan et al., 2009a, b). Clinical studies suggest that drugs which are commonly metabolized by CYP3A4, that is, diltiazem, midazolam, fexofenadine, valproic acid, propanol, omeprazole, theophylline, and human immunodeficiency virus (HIV) protease inhibitor, when coadministered with *G. biloba* extract have their bioavailability affected with concurrent effects (Deng et al., 2008; Numa et al., 2007; Ohnishi et al., 2003; Robertson et al., 2008; Tang et al., 2007; Yin et al., 2004; Zhao et al., 2006). Interaction between CYP and *G. biloba* results in molecular changes. As such, in a study by Yeung et al. (2008), rat hepatocytes culture cells when treated with *G. biloba* extract, had their mRNA expression of the CYP3A23 gene upregulated. This is a target gene for the rat pregnane X receptor (transcription factor, role in drug metabolism, and transport). Similarly, ginkgolides A and B and flavonoids activated the pregnane X receptor and resulted in a change in the activity of hepatic drug–metabolizing enzymes (Li et al., 2009). Also, the pregnane X receptor, which constitutes androstane and aryl hydrocarbon receptors, activated by ginkgolides A and B and flavonoids, resulted in change in the activity of hepatic drug–metabolizing enzymes and transporters (Li et al., 2009). All this clinical evidence shows that drugs which are metabolized mainly by CYP enzymes need to be avoided or taken with precaution along with *G. biloba* extract. However, no side effects have been seen when *G. biloba* leaf extract is administered alone for 1–3 months at a dose of 120–160 mg/day (Kleijnen and Knipschild, 1992). In a similar study, when *Ginkgo* extract was administered at 120 mg/day for 52 weeks, gastrointestinal complications were reported (Le Bars et al., 2007). 4-O-methylpyridoxin (ginkgotoxin), a toxic chemical compound found in *G. biloba* leaf extract was reported to interfere with pyridoxine (vitamin B6) metabolism, leading to neurotoxicity, seizures, and loss of consciousness (Arenz et al., 1996). According to a recent study by the National Institute of Health, under the National Toxicology Program, *G. biloba* leaf extract exerts potential toxic and cancer-related consequences such as lesions including hypertrophy in the liver and thyroid gland, liver hyperplasia, hyperkeratosis, and stomach ulcers (Rider et al., 2014).

TRENDS IN TRADE

Trends in using plant-based products are shifting back to their roots with over 20% of the population using herbal cosmetics, dietary supplements, and medicines (Bent, 2008). In this context, the medicinal properties of *G. biloba* have attracted a global market for its potential applications in health, food, and supplements. The leaf extract of *Ginkgo* contains pharmaceutically imperative flavonoids, glycosides, and ginkgolides along with other bioactive compounds which have wider applications (Table 3.19.3). The standard leaf extract of *Ginkgo biloba* is EGb 761, which is one of the most commonly used herbal dietary remedies in many countries, including China, the United States, France, and Germany. EGb 761, also called Tebonin, Tanakan, Rokan, or Kaveri, is marketed in Europe as a medicine for cardiovascular disease. In the United States, Nature's Way, Inc., USA has exclusive distribution rights for EGb 761 and markets this product as a dietary supplement under the trade name Ginkgold (Huh and Staba, 1992; Salvador, 1995). The use of *G. biloba* has been growing at a very rapid rate in the open world's commercial markets and some of them are mentioned in Table 3.19.3.

CONCLUSIONS

The use of plant-based food and dietary supplements, botanicals and complementary medicine, cosmetics, and other products are gaining popularity all across the globe. *G. biloba*, considered by some as a living fossil, plays a role in the three "cals," that is, cosmeceuti*cals*, nutraceuti*cals*, and pharmaceuti*cals* (CNP). The reported uses and therapeutic potential of this tree positions it at a "wonder tree with multifarious uses." The presence of ginkgolides and other bioactive contents in the tree, particularly its leaves, has shown its effectiveness in neuroprotection, cardioprotection, and cancer protection; however, its long-term use and side effects are yet to be investigated. Therefore, knowledge of the long-term use of these bioactives, particularly ginkgolides, will be useful in understanding its mechanisms of protection and side effects, if any exist. Since the number of Alzheimer's patients is increasing along with patients with other brain-related problems, the use of *G. biloba* to overcome these health-related problems will be very useful. However, its availability in the wild is negligible and only commercially planted trees are available. In such circumstances, it is important to reintroduce the species in its natural habitat so that conservation of this species can be ensured together with its beneficial utilization.

TABLE 3.19.3 List of Commercially Available *Ginkgo biloba* Products

Product name	Manufacture	Uses	Approximate price ($)
Zenith Nutrition *Ginkgo biloba* 60 mg	Zenith Nutrition Pvt. Ltd., India	Improving blood circulation	7.32
Vista Nutritions *Ginkgo biloba* 60 mg	Vista Nutrition's Pvt. Ltd., India	Improving memory and mental alertness	6.57
NUTRILITE Siberian Ginseng with *Ginkgo Biloba* (500 gm)	Amway, USA	Endurance and mental sharpness	34.63
Doctor's Best, Extra Strength Ginkgo, 120 mg	Doctor's Best Inc., USA	Promote mental function and alertness	21.22
Goicoechea Lotion, *Gingkobiloba*, 13.5 Fluid Ounce	Goicoechea, Argentina	Nourishing and refreshing skin	52.42
Antioxidant Vitamin C Creme with *Gingkobiloba*	Pree Cosmetics Inc., USA	Neutralize free radicals, skin moisture	61.12
Nature Made *Ginkgo biloba* 30 mg	Nature Made Inc., USA	Herbal supplement	127.95
Nature's Way, Ginkgold Eyes, 60 Tablets	Nature's WayInc., USA	Improving visual Function and optimal night vision	35.04
Solgar, Bilberry *Ginkgo* Eyebright Complex Plus Lutein, 60 Veggie Caps	Solgar Inc., USA	Eye health, antioxidant support	23.32
Nature's bounty *Ginkgo biloba* 120 mg 100 capsules	Nature's Bounty Inc., USA	Supports healthy brain functions and circulation	33.68
Healthvit *Ginkgo biloba* (60 mg), capsule	Healthvit Ltd., India	Promote healthy brain function, supports sharpened alertness	11.43
Forever ginkgo plus 60 tablets	Forever Living Inc., USA	Helps support circulation, energy level booster	19.15
Puritans Pride *Ginkgobiloba* 120 mg-100 Capsules	Puritans Pride Inc., USA	Supports healthy brain functioning	24.64
Ginkgo biloba powder (100 g)	Raw Living Ltd., England	Reduces the stickiness of platelets	6.10
Qi Teas Organic Fairtrade Green Tea with *Ginkgo*	Holland and Barrett Retail Ltd., UK	Helps in uplifting of body and mind	2.45
Tebonin Egb 761	Dr. Schwabe, Germany	Symptomatic relief and management of Tinnitus and Vertigo	40.69

Source: amazon.in, amazon.com, iherb.com, healthcart.com.

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Chapter 3.20

Panax ginseng: More Than an Adaptogen Remedy

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INTRODUCTION

Ginseng is considered one of the oldest remedies for improving human health. Its uses and "magical" effects in China have been known for 5000 years (Nag et al., 2012). Ginseng includes numerous plant species belonging to the *Panax* species, such as *Panax ginseng* C.A. Meyer and *Panax quinquefolius*, L. *P. ginseng*, the most popular, can be found in the vast territory between China, Russia, Korea, and Mongolia. Its botanical name expresses its beneficial properties; in fact, ginseng derives from the Chinese words (jen shen) that mean human-like form, while panax (Latin word for panacea) means "cures all diseases" (Anonymous, 2009). Several active components are present in the *Panax* genus, including ginsenosides, polysaccharides (Ru et al., 2015), and polyacetylenes (Knispel et al., 2013). Ginsenosides are the major components possessing biological activities with differences regarding their aglycone moieties, as showed in Fig. 3.20.1. There are several ginseng products on the market, with Korean red ginseng (KRG) undoubtedly the most popular. KRG is obtained by steaming, using conventional methods, while white ginseng is obtained by drying the fresh plant. KRG produces new constituents, not present in white ginseng, such as Rg3, Rg5, and RK1 (Kim et al., 2007; Fig. 3.20.1). When ginseng is eaten, the ginsenosides present undergo a chemical transformation, which limits their bioavailability. In the gut, Rb1 is transformed into compound K, by intestinal microbiota, operating progressive deglycosylation (Wang et al., 2011). This chemical modification is an important process aimed to increase the bioavailability of ginsenoside metabolites and to enhance their biological effects (Qian et al., 2006). In fact, compound K, but not Rb1, possesses chemopreventive effects (Wang et al., 2012; Yang Hsu et al., 2013). However, the bioavailability of ginsenoside metabolites is limited, because of extensive biliary excretion (Liu et al., 2009) or efflux processes regulated by P-glycoprotein (Xie et al., 2005). Even diet, influencing the intestinal microbiota, causes changes in the levels of ginsenoside metabolites (Wan et al., 2017).

BIOLOGICAL EFFECTS

Several biological functions have been associated with *P. ginseng* consumption. In the following paragraphs, we will briefly review some of its principal biological effects, such as capacity to behave as an adaptogen, cardioprotective and anticancer agent, along with its ability to interact with conventional drugs. Finally, we will comment on clinical trials involving *P. ginseng*.

Adaptogen Effect of *Panax ginseng*

P. ginseng is an effective adaptogen, able to help calm stressful conditions and restore normal functions in humans. An adaptogen can operate on endocrine, immune, and nervous system levels, influencing the hypothalamic–pituitary–adrenal (HPA) system and the sympathetic-adrenal system (Panossian and Wagner, 2005). Stress produces hormonal HPA changes that determine cortisol increase (Hasegawa et al., 2013). The adaptogenic functions of *P. ginseng* largely depend upon the presence of ginsenosides and protopanaxatriol-derived M4 which was found to induce, in vitro, an inhibitory production of corticosteroid in bovine adrenal fasciculata cells in the presence of adrenocorticotrope hormone (Hasegawa et al., 2013).

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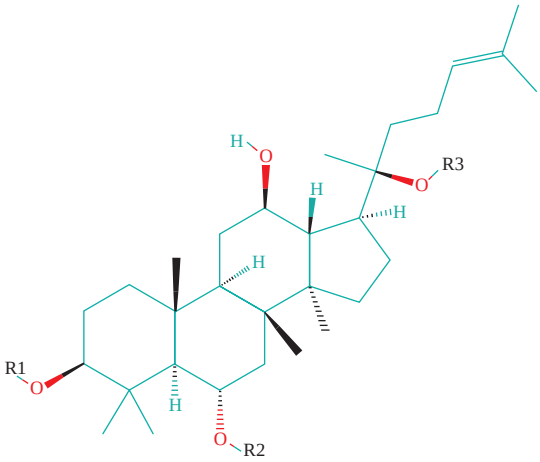

Ginsenoside	R1	R2	R3
(20S)-Protopanaxadiol	O-H	H	O-H
Rb1	O-*G*-*G*	H	O-*G*-*G*
Rb2	O-*G*-*G*	H	O-*G*-*Ap*
Rb3	O-*G*-*G*	H	O-*G*-X
Rc	O-*G*-*G*	H	O-*G*-*Af*
Rd	O-*G*-*G*	H	O-*G*
Rg3	O-*G*-*G*	H	O-H
Compound K	O-H	H	O-*G*
(20S)-Protopanaxatriol	O-H	O-H	O-H
Re	O-H	O-*G*-*R*	O-*G*
Rf	O-H	O-*G*-*G*	O-H
Rg1	O-H	O-*G*	O-*G*
Rg2	O-H	O-*G*-*R*	O-H
Rh1	O-H	O-*G*	O-H

FIG. 3.20.1 Chemical structures of protopanaxadiol and protopanaxatriol ginsenosides. *Af*, α-L-arabinofuranosyl; *Ap*, α-L-arabinopyranosyl; *G*, β-D-glucopyranosyl; *R*, α-L-rhamnopyranosyl; *X*, β-D-xylopyranosyl.

Cardiovascular Effects of *Panax ginseng*

Cardiovascular diseases are the leading cause of deaths worldwide (Lee and Kim, 2014) and include a variety of illnesses affecting the heart and/or blood vessels. *P. ginseng* is widely used in people with cardiovascular problems, such as in the treatment of hypertension and hypercholesterolemia (Lee et al., 2016; Saba et al., 2016). Hypertension is a major risk factor for the development of cardiovascular disease (Mallat et al., 2016) and vascular endothelial cells, localized in the intima, are directly involved (Bochenek et al., 2016). These cells play a fundamental role in the regulation of vascular tone, through multiple factors, including nitric oxide (Siasos et al., 2015). RG3-enriched KRG, isolated from KRG with high Rg3 content, improved vascular tone in spontaneous hypertensive rats, compared to a control group, and increased nitric oxide levels in serum after a 6-week treatment with RG3-enriched KRG (Park et al., 2014). Compound K showed a potent hypoglycemic effect, inhibiting hepatic gluconeogenesis in a mouse model developing type 2 diabetes, activating AMP-activated protein kinase (AMPK), and inhibiting the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, at a dose of 30 mg/kg body weight per day for 4 weeks (Wei et al., 2015).

Anticancer Effects of *Panax ginseng*

Cancer morbidity and mortality worldwide represents a constant health concern (Wong et al., 2015). Chemotherapy is one of the most effective approaches to cure cancer, but its toxicity and the development of drug resistance represents important limits in determining its efficacy. Therefore, in the last decades, it has been mandatory to develop alternative approaches for the prevention and treatment of cancer. The use of alternative medicine, working alongside regular chemotherapy, may represent a desirable advance. Studies showed that regular consumption of ginseng has beneficial effects against specific forms of cancer (Wong et al., 2015). The potential anticancer activity of ginseng is associated with cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis (Choi et al., 2013). Ginsenosides act by inhibiting cell proliferation and inducing apoptosis in cancer cells, via an intrinsic pathway. Proapoptotic proteins Bak and Bim were activated when human breast cells (MCF-7 and MDA-MB-231) were incubated with Rh2 (Choi et al., 2011) and a formulation of red ginseng,

containing Rk1, Rg3, and Rg5, upregulated the mitochondrial accumulation of Bax, Bak, and caspase-3 activation in the human adenocarcinoma cell line (HeLa), as well as in human colon cancer cell lines (SW111C, SW480) (Lin et al., 2015). Vascular endothelial growth factor (VEGF) is a growth factor which promotes angiogenesis (Kerbel, 2008) and is induced in cells, without an adequate support of oxygen (Holmes et al., 2007), by the hypoxia inducible factor (HIF) α/β complex (Maxwell et al., 2001). Ginsenoside Rg3 inhibited the expression of VEGF and HIFα in acute leukemia patients, downregulating the phosphorylation of Akt and ERK 1/2 (Zeng et al., 2014) and reduced significantly VEGF mRNA, induced under hypoxic conditions, in human esophageal carcinoma cells (Chen et al., 2010). Matrix metalloproteinases (MMPs) play a critical role in metastasis (Deryugina and Quigley, 2006). Ginsenoside Rh2 inhibited proliferation, migration, and invasion of pancreatic cell line Bxcp-3, downregulating MMP-9 at the level of mRNA and protein expression (Tang et al., 2013), while ginsenoside Rg3 inhibited angiogenesis and metastasis in malignant ovarian cancer cells (SKOV-3), through the inhibition of MMP-9 expression (Xu et al., 2008).

Interaction of *Panax ginseng* With Drugs

Ginseng contains several chemical compounds able to interact with conventional drugs, determining a modulation of their therapeutic efficacy (Qi et al., 2011). As an example, warfarin, used under anticoagulant therapy, is present in two enatiomeric forms, S and R, recognized by different cytochrome P450 (CYP) enzymes (Wadelius et al., 2007), responsible for the metabolism (degradation and elimination) of drugs. The activity of CYP can be influenced by phytochemicals (Wanwimolruk and Prachayasittikul, 2014), such as ginsenosides. However, the competitive inhibition of CYP activity by ginseng occurs in in vitro assays only at very high concentrations, unlikely to be achieved in vivo following moderate ginseng intake (Wanwimolruk et al., 2014). In fact, *P. ginseng* does not affect the pharmacokinetics of warfarin enantiomers in healthy subjects (25 mg single dose). In this study, it was also observed that the intake of *P. ginseng* did not alter blood coagulation values and platelet aggregation (Jiang et al., 2004). In addition, no significant effect on CPY activity was found when *P. ginseng* (1.5 g/day standardized to 5% ginsenosides) was given to young and elderly subjects for one month with or without midazolam, caffeine, chlorzoxazone, and debrisoquine drugs, metabolized by CYP3A4, CYP1A2, CYP2E1, and CYP2D6 enzymes, respectively (Gurley et al., 2002, 2005).

Clinical Studies

Medicinal plants, including ginseng, have been employed for centuries to cure diseases and other illnesses in humans, but controlled clinical studies are limited. In many cases, the safety and efficacy of medicinal plants have been passed down from one generation to the next by traditional tales from healers in the absence of a consistent, scientific documentation. Fortunately, in the case of *P. ginseng*, clinical studies continue to support its beneficial role in healthy subjects and in several clinical disorders. As shown in Table 3.20.1, Jovanovski et al. (2014) reported that in healthy individuals 400 mg of Rg3-KRG supplement improved indices of vascular function, with blood pressure at normal values, as well as KRG ameliorating the pigmentation and erythema in patients affected by melasma, a skin problem common in Asian countries (Song et al., 2011) and having a beneficial effect on alopecia (in fact, when the KRG was combined with 3% of minoxidil, hair density was ameliorated with respect to patient groups without KRG treatment (Ryu et al., 2014)). In male subjects affected by varicocele, the administration of 1.5 g of KRG daily for 12 weeks, improved sperm concentration and motility, but without significant differences, with respect to untreated patients, in testosterone and luteinizing hormone levels (Park et al., 2016). In 45 male patients with erectile dysfunction, a dose of 900 mg of KRG, 3 times daily, had a beneficial effect in 60% of them (Hong et al., 2002). When KRG was given at a dose of 2.7 g/day for 2 weeks in healthy women, gynecologic complaints, such as menstrual pain and constipation, were alleviated (Yang et al., 2014). Behavior in children with hyperactivity signs was significantly improved by KRG intake, without any difference in salivary cortisol and dehydroepiandrosterone levels, with respect to a control group (Ko et al., 2014). In addition, the ginseng extract reduced depressive symptoms when administered to 35 female patients at doses of 3 g/day (Jeong et al., 2015). Finally, in patients affected by chronic hepatitis B, the administration of KRG, in association with antiviral agents, improved fibrosis serologic markers, in particular TGF-β and hyaluronic acid, but not type IV collagen (Choi et al., 2016). In association with antiretroviral therapy, KRG administered to 23 patients, infected with immunodeficiency virus type 1 (with a dose range of 2.7–5.4 g/day), improved CD4 T-cell counts, supporting the opportunity to use ginseng as an antiretroviral supplement (Sung et al., 2009) (Table 3.20.1).

CONCLUSIONS

Overall, ginseng is safe (Lee et al., 2012; Paik and Lee, 2015), but some recommendations are necessary. Ginseng is a dietary supplement with sometimes unknown mechanisms of action at a molecular level. It should not be taken

TABLE 3.20.1 Ginseng Effects in Selected Clinical Studies

Subjects	Drug/other	Ginseng effects	References
Healthy	NA	Lowering central and peripheral arterial pressure	Jovanovski et al. (2014)
Healthy	Bisphenol A	Improvement of menstrual irregularity and menstrual pain	Yang et al. (2014)
Melasma	NA	Improvement of melasma	Song et al. (2011)
Alopecia	Minoxidil	Improvement of hair density and thickness	Ryu et al. (2014)
Erectile dysfunction	NA	Improvement of erectile dysfunction	Hong et al. (2002)
Male infertility with varicocele	NA	Improvement of spermatogenesis	Park et al. (2016)
Attention-deficit/hyperactivity disorder	NA	Decreasing inattention/hyperactivity scores and QEEG TBR	Ko et al. (2014)
Residual depression symptoms	NA	Decreasing depressive symptoms	Jeong et al. (2015)
Chronic hepatitis B	Antiviral agents	Decreasing fibrosis serologic markers	Choi et al. (2016)
HIV-1	Antiretroviral therapy	Increasing CD4 T-cell count	Sung et al. (2009)

by those who are allergic to any plant components. Also, as ginsenosides can alter platelet function (Stanger et al., 2012), it is necessary to stop ginseng intake if undergoing surgery, or in the case of any bleeding. Diabetes mellitus patients should control ginseng intake, as this supplement may interfere with blood glucose level (Gui et al., 2016). Ginseng is not recommended for children and should not be taken by pregnant or breast-feeding women. It may cause sleep disorders (Paik and Lee, 2015). Other side effects, such as nausea, diarrhea, and headache have been observed (Anonymous, 2009) and theratogenicity has been reported in a rat embryo model; however, in this study the ginsenoside concentrations employed were significantly higher compared to those present in ginseng for human consumption (Chan et al., 2003).

In conclusion, current studies provide supporting evidence that *P. ginseng* may be beneficial in several biological activities mainly attributed to ginsenosides. Human studies have yielded positive evidence, suggesting the need for further clinical trials, that is using various dosages, before any conclusions with respect to its therapeutic use can be made.

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Chapter 3.21

Goji Berry (*Lycium barbarum*)—A Superfood

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SOURCES AND AVAILABILITY OF *LYCIUM BARBARUM*

Lycium barbarum has different local names, for example, the Chinese know it as Chinese wolfberry, barbary wolfberry, boxthorn, Chinese boxthorn, marriage vine, Chinese ritule vine; the Japanese as kuko, red medlar, and the Duke of Argyll's tea tree; Mandarin-speaking Chinese people call it gou qi while Cantonese-speaking Chinese know it askeitze; Korean locals know it as gugija; Vietnamese call it củkhởi; Thai people know it as gǎogèe; and Tibetan people have named it dretsherma. Its fruit is called lycium fruit, fructuslycii ("product of lycium"), lycii berries, lyciifructus, lycii organic product, dried wolfberries, gou qi zi (Chinese), gouqizi (Chinese), goji, Tibetan goji berry, goji berry, and goji juice. Its dried root has names including di gu pi and digupi (Bryan et al., 2008). The Chinese name for the *Lycium* plant is gouqi and for its natural product, gouqizi. "Zi" can be translated as "little organic product" or "fruit." The other part of the name, "gou," means wolf and is related to its common name "wolfberry." The name, ritual vine is also given to its prickly form for reasons unknown. Carolus Linnaeus, a Swedish botanist, gave the scientific name *L. barbarum* to this plant in 1753. Later in 1768, another botanist Philip Miller, named it *Lycium chinense* (Dharmananda, 2007). Recent studies on extracts from *L. barbarum* fruit and its active compound, *L. barbarum* polysaccharide (LBP), indicate a wide range of biological activities. The most important of which include slowing of the aging process, neuroprotection, metabolism enhancement, along with antidiabetic, antiglaucoma, anticancer, and cytoprotective activities, as well as antioxidant, and immunomodulatory properties (Amagase and Nance, 2011; Bensky et al., 1993; Bryan et al., 2008; Chang et al., 1986; Potterat, 2010; Zhu, 1998).

L. barbarum is native to southeastern Europe and Asia and is well known in traditional Chinese medicine. In temperate to subtropical areas of Australia, Eurasia, southern Africa, North America and South America, around 70 different types of *Lycium* can be grown (Fukuda et al., 2001). *Lycium* spp. belong to the Solanaceae family which includes peppers, tomatoes, eggplants, and potatoes (Bryan et al., 2008). It normally grows in Asia, fundamentally in northwest China (Bensky et al., 1993; Wu, 2005; Zhu, 1998). Goji berries are gathered in the mid-year harvest and are then processed by first drying in the shade until the skin recoils and then being exposed to the sun until the external skin ends up dry and hard, with their centers remaining soft (Zhu, 1998). China is the primary producer of *L. barbarum*/wolfberry products worldwide.

TRADITIONAL USES OF *LYCIUM BARBARUM*

L. barbarum fruits are consumed as both fresh and dried berries. They are also used as ingredients to make *Lycium* alcohol and beverages by mixing concentrated extracts and infusions of the berries in alcohol. As therapeutic nourishment, *Lycium* are made into a sauce to be added to steamed rice. Young leaves from the plant can be directly used as sustenance. The dried natural product is an incredibly popular pharmaceutical in Asian countries such as Tibet, Thailand, Vietnam, Japan, Korea, and China, which have special names for goji as mentioned earlier in the text. It has been broadly used among these nations for therapeutic purposes and as a functional food for over 4500 years (Bensky et al., 1993; Chang et al., 1986; Wang, 2006; Zhu, 1998). *L. barbarum* nourishes the liver, kidneys, and the eyes, as recorded by ancient ethnobotanists. LiShi-zen classified *L. barbarum* in his book, "*Compendium of Medica*," as one of the top grade medicinal plants that can nourish the liver and kidneys, as well as playing a vital role in improving eyesight. Another herbalist, Shennong, also explained

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that long-term use of goji berries can contribute to slowing the process of aging. Another well-known Chinese herbalist also praised the use of goji berries by saying goji can improve energy levels, blood, maintain a favorable internal temperature, and resist wind and humidity. According to some ethnobotanists, *Lycium* is still used by healers in Israel (Dafni and Yaniv, 1994).

HEALTH BENEFITS OF *LYCIUM BARBARUM*

Regular intake of standardized *L. barbarum* fruit juice (GoChi^{TM} 120 ml, equivalent to 150 g of fresh fruit) for 14–30 days enhances the efficacy of the cardiovascular system, joint and muscle function, gastrointestinal tract (GIT) regularity, general behavior, and neurological/psychological traits, and was recently proved to do so according to five randomized studies conducted in the United States and China (Amagase and Hsu, 2009; Amagase and Nance, 2008a,b; Amagase and Nance, 2009; Amagase et al., 2009a,b). These five studies showed that *L. barbarum* caused a significant increase in the energy levels of individuals, increased stamina, improved sleep quality, sharpness of mind, mental keenness, serenity, feeling of wellbeing, satisfaction, and joy, while also reducing fatigue, stress, weakness, sorrow, spinal pain, and physical and mental discomfort associated with menstruation. Conversely, placebo groups showed no improvement in sleep quality in any of the clinical studies (Amagase and Hsu, 2009; Amagase and Nance, 2008a). Improved mental well-being during menstruation was demonstrated in women in a randomized trial after administration of a standardized *L. barbarum* organic juice product. Conversely, the placebo treatment demonstrated no noteworthy changes (Amagase and Nance, 2008b). Neuroprotective effects against toxins and antiaging effects are known to be exhibited by *L. barbarum* and LBPs in age-related neurodegenerative diseases (Chang and So, 2008).

LBP-standardized *L. barbarum* fruit juice considerably amplified postprandial energy expenditure in randomized clinical studies (Amagase, 2010). Consumption of *L. barbarum* on a regular basis reduced the waist circumference of individuals, and also reduced the risks of metabolic syndrome (Amagase and Nance, 2009). Regular intake of *L. barbarum* in the form of fruit juice causes changes in waist circumference, possibly due to the fact that *L. barbarum* may stimulate metabolic rate through adrenocortical hormone control (Amagase, 2010). In vivo experiments were conducted on weanling mice. In which after 21 days of oral consumption of Lycium barbarum at doses of 5, 10, and 20 mg/kg/day, it was revealed that their LBP composed of six kinds of monosaccharides enhanced food conversion rate and the body content on zinc and iron, and reduced their body weights (Zhang et al., 2002). In another in vivo experiment, administration of LBP to mice by stomach perfusion for seven consecutive days resulted in the reduction of endoplasmic reticulum damage, promotion of protein synthesis and detoxification, restoration of the normal function of hepatic cells, and promotion of a regeneration of hepatic cells (Bian et al., 1996). Alcoholic fatty livers in rats were also prevented effectively by LBP (Gu et al., 2007). These effects were attributed to the inhibition of hepatocyte cytochrome P450 enzyme 2E1 (CYP2E1) expression, and prevention of lipid peroxidation. Significant reduction of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), liver malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and CYP2E1 gene and protein expressions were observed, and an increase in the activity of liver superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) content were found (Gu et al., 2007).

Experiments conducted on 26-month old mice and rats showed a significant increase in the maximum combination capacity of cardiac muscle β-receptors with the administration of *L. Barbarum* (Liu et al., 1996; Shi et al., 1998). In another experiment, a decoction of *L. barbarum* was made and administered to rats via stomach perfusion. The decoction was administered in doses ranging from 1 to 4 g/kg for 10 days consecutively. Serum samples showed a decrease in low-density lipoprotein (LDL) cholesterol levels and a decrease in the levels of total cholesterol and triglyceride in both the serum and the liver (Wang et al., 1998). An increase in high-density lipoprotein (HDL) cholesterol levels, as well as a reduction in serum total cholesterol and triglyceride concentrations, was also observed in other experiments with rabbits over 10 days (Luo et al., 2004).

L. barbarum, already well known in traditional Chinese herbal medicine for its use in diabetes, is also reported by several clinical and experimental studies for its use as an antidiabetic agent. *L. barbarum* was also found to be effective in the reduction of oxidative stress in patients with retinopathy (Li et al., 2000). In a randomized diabetic retinopathy examination, after administration of *L. barbarum* for 3 months, serum lipid peroxidation reduced by around 20%, and vitamin C and SOD increased by 61% and 87%, respectively, in relation to controls (He et al., 1998). Patients suffering with diabetic retinopathy had improved immunological function of red blood cells due to *L. barbarum* intake by any means (He et al., 1998).

The loss of vision in elderly people is often due to a common disease called age-related macular degeneration (AMD) and it is one of the major neurological disorders of the eye. Zeaxanthin (Fig. 3.21.1) is one of the carotenoids found in *L. barbarum* that is effective in preventing AMD. In addition, the fruits and seeds of *L. barbarum* contain β-carotene, β-cryptoxanthin, (Fig. 3.21.1) and other carotenoids in small amounts (Potterat, 2010). Zeaxanthin and lutein are oxygenated carotenoids with antioxidant and blue light-absorbing properties which accumulate in the macula preventing AMD.

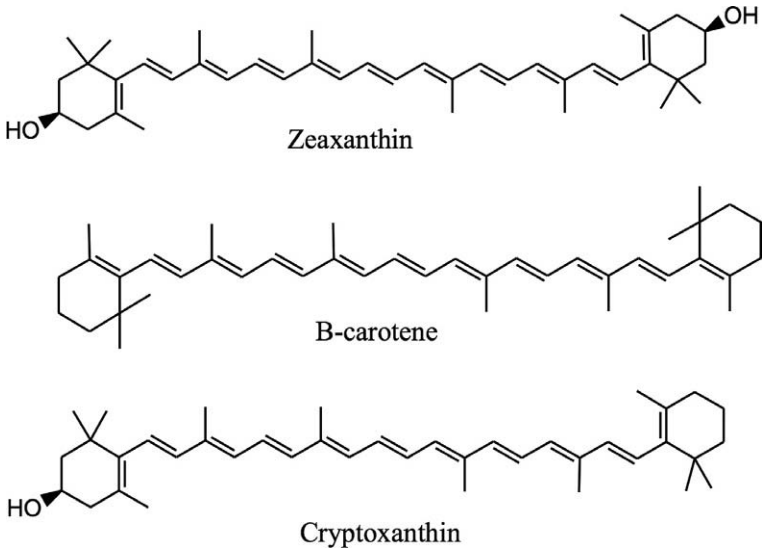

FIG. 3.21.1 Carotenoid constituents of the fruits and seeds of *Lycium barbarum*.

Progressive loss of retinal ganglion cells in the retina is caused by the increase in intraocular pressure in glaucoma. *L. barbarum*, when administered orally, reduced the loss of retinal ganglion cells, but the increased intraocular pressure was not altered considerably. Hence, from these experiments it was clear that *L. barbarum* demonstrates therapeutic activities against neurodegeneration in the retina of rats caused by ocular hypertension, and *L. barbarum* could be considered as a prospective agent for the development of neuroprotective drugs against the loss of retinal ganglion cells in glaucoma (Li, 2007). Further animal experiments also proved that *L. barbarum* may be used to protect subjects from light damage in the retinal pyramid, rod cell layer, outer nuclear layer, and retinal pigmented epithelium (Na et al., 1995). One experiment was also conducted on humans, it was a single-blind, placebo-controlled, and parallel design aimed to provide data on how fasting plasma zeaxanthin concentration changed as a result of dietary supplementation with whole *L. barbarum* (15 g/day *L. barbarum*, estimated to contain almost 3 mg of zeaxanthin) over 28 days. A 2.5-fold significant increase in plasma zeaxanthin was seen after supplementation, however, the placebo group did not show significant changes. Such an increase in plasma zeaxanthin levels may benefit the eye (Cheng et al., 2005).

The efficacy of *L. barbarum* and LBPs as antioxidants was proved by several clinical studies, by protecting against various peroxidation related conditions (Gong et al., 2005; Huang et al., 1999; Huang et al., 2001; Huang et al., 2003; Huang et al., 1998; Li et al., 2007b; Li et al., 2002; Luo et al., 2006; Ni et al., 2004; Reeve et al., 2010; Ren et al., 1995; Sui et al., 1996; Wang et al., 2002; Wu et al., 2004; Zhang et al., 1997; Zhang, 1993; Zhao et al., 2005). The oral consumption of *L. barbarum* had intrinsic antioxidant effects (Amagase et al., 2009a). Various diseases and symptoms may be caused by free radical oxidations (Borek, 2004). Therefore, *L. barbarum* may be useful in protecting against these diseases and symptoms. Also, an animal trial on the antioxidative effect of *L. barbarum* combined with doxorubicin chemotherapy was reported (Xin et al., 2007). *L. barbarum* demonstrated skin protection abilities against immune suppression and oxidative stress by solar simulated ultraviolet (SSUV) radiation (Reeve et al., 2010). Himalayan goji juice, along with apple and pear juice was tested against hypersensitivity reactions and it was found that goji berry juice was helpful against hypersensitivity reactions and presented good results against it (Reeve et al., 2010). Drinking goji juice demonstrated photoprotective effects via inducing the endogenous cutaneous antioxidant HO-1, and might protect humans against actinic skin damage leading to cancers (Reeve et al., 2010).

LBPs provided a protective effect against testicular tissue damage induced by 43°C heat exposure through improved SOD activity (Luo et al., 2006). A rise in sexual hormone levels and improvement in copulatory performance and reproductive function of hemicastrated male rats was observed (Luo et al., 2006). These findings support the reputation of *L. barbarum* fruits as an aphrodisiac and fertility facilitating agent as well as the extensive use of *L. barbarum* fruits as a traditional remedy for male infertility in China (Luo et al., 2006).

Many studies have revealed that *L. barbarum* and LBPs have a widespread diversity of immunomodulatory roles in the initiation of various immune cells (Xu et al., 2000). Cell-mediated and humoral immune responses are enhanced through the use of LBPs, revealed through a review of research on *Lycium* fruit (Zhou, 1991). Daily usage of LBP at 5–10 mg/ kg for 1 week on laboratory animals amplified the activity of T-cells cytotoxic, natural killer (NK) cells, and T-cells.

L. barbarum extract inhibited the cyclophosphamide-induced decrease in white blood cell count, and promoted its recovery, significantly delaying death (Pu, 1998). Expression of interlukin-2 receptors was increased with the use of *L. barbarum* extract at concentrations between 1.9 and 7.8 mg/ml (Du and Qian, 1995). *L. barbarum* tempered the binding-caused reductions in body weight and TNF-α activity in rats (Li et al., 2007a). Phagocytic action and phagocytic index rate was enhanced by isolated and purified LBP, also lymphocyte translation and acceleration in the production of serum hemolysin was promoted. LBP was proved to be a sort of homogeneous glycoconjugate with immune activity and antioxidant activity (Peng and Tian, 2001). LBP enhanced innate immunity by activating macrophages. A trial, with administration of LBP to mice through stomach perfusion for seven consecutive days, increased the weight of the immune organs, and significantly increased the reticuloendothelial system's phagocytotic capacity on Indian ink (Sui et al., 1996). Effects of pure and crude LBP on immunological activity were compared. The pure LBP, with lower doses (5–20 mg/kg/day), showed a remarkable impact on immunological improvement.

In particular, 10 mg/kg/day, presented extremely substantial alteration associated with crude LBP on immune indices in mice (Luo et al., 1999). The sulfated alteration of LBP expressively endorsed lymphocyte proliferation and enhanced serum antibody titer both in vitro and in vivo and was more effective than LBP (Wang et al., 2010a,b). Recently, a clinical study was conducted in the United States and China to examine the immunomodulatory properties of orally consumed *L. barbarum* fruit in a form of LBP-standardized juice (GoChi^{TM}) provided to healthy adults. In this experiment lymphocytes increased by 27%, IL-2 by 58%, and IgG levels by 19% compared to the group treated with *L. barbarum*, whereas no significant changes were seen in the number of CD4, CD8, and NK cells, and IL-4 and IgA levels. In contrast, changes in visual acuity, pulse rate, urine composition, body weight, stool, blood pressure, or blood biochemistry were not seen during the experiment. The placebo group had no significant developments in any of the symptoms. Thus, a regular intake of *L. barbarum* amplified numerous actions of immunological functions without any antagonistic responses (Amagase and Nance, 2009).

According to a preliminary in vitro study, *L. barbarum* extract has been reported to have antibacterial effects on 17 bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Salmonella paratyphi A-C*, *Salmonella typhimurium*, *Bacillus subtilis*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Bacillus dysenteriae* (*Shigella dysenteriae*), *Escherichia coli*, *Candida albicans*, and *Typhoid bacillus* (Jin et al., 1995).

Experiments conducted on animals suggested that LBPs have anticancer effects through their enhancement of the immune system. The increased number of CD4(+) and CD8(+) T-cells by LBPs may cause an antitumor effect and may also enhance the antitumor function of the immune system (He et al., 2005). Experiments have proved LBP is significantly effective against human prostate cancer cell growth in mice (Luo et al., 2009). Both tumor volume and weight in the LBP-treated group were considerably lower than in the control group (Luo et al., 2009).

Various in vitro experiments were carried out on *L. barbarum* and LBP to evaluate their mechanism of action. *L. barbarum* proved to be effective against human breast cancer, inhibiting the growth of estrogen receptor positive human breast cancer cells by favorably altering estradiol metabolism (Li et al., 2009). LBP was seen to have markedly increased induced prostate cancer cell apoptosis, with the highest apoptosis rates at around 40% (Luo et al., 2009). LBP also induced apoptosis of human leukemia cells (Gan et al., 2001).

Natural products made from goji berries are regularly fused into complex herb formulae in customary pharmaceuticals, equivalent to 6–18 g of dried goji material. For a decoction, the NHI monograph specifies 5–15 g of *Lycium* (goji berries) are required, equivalent to 25–120 g of fresh berries. Meanwhile, studies are using *Lycium* organic products as solitary herbs or as a noteworthy component in a formula. For people suffering from atrophic gastritis or diabetes, 10 g of *Lycium* organic product, taken a few times every day, is recommended (Wang and Dong, 1990; Xu, 1989). As a treatment for fortifying the elderly or impaired, *Lycium* is cooked with lean pork, bamboo shoots, and traditional Chinese flavorings, the daily dosage being 15–30 g (Amagase and Nance, 2011). A dosage of 15 g/day for eye treatment was considered valuable in providing satisfactory zeaxanthin (Cheng et al., 2005). A daily dose of 20 g of *Lycium* tea is considered helpful for improving impaired vision (Zong and Liscum, 1996). In a clinical trial with 79 severe cancer patients, a combined medication of lymphokine-activated killer (KAL)/IL and LBPs was administered and the results from 75 patients showed that regression was achieved. These results were observed in patients with renal cell carcinoma, lung cancer, malignant melanoma, nasopharyngeal carcinoma, colorectal carcinoma, and malignant hydrothorax. This Chinese clinical tumor study, comprising 79 advanced cancer patients, treated with LAK/IL-2 combined with LBPs, was seen to be effective (Cao et al., 1994). In view of this data, it is suggested that 30 ml of *Lycium*, four times every day (up to a total of 120 ml/day), which is proportional to roughly 150 g of fresh berries, should be consumed. It was found that servings could be consolidated (Amagase, 2010; Amagase and Handel, 2008; Amagase and Hsu, 2009; Amagase and Nance, 2008a,b; Amagase and Nance, 2009; Amagase et al., 2009a,b).

INTERACTION OF *LYCIUM BARBARUM* WITH OTHER DRUGS/FOODS/SUPPLEMENTS

The fact that *L. barbarum* has been used as a traditional Chinese medicine (TCM) or functional food in Asia for some time suggests that the herb is nontoxic and safe to take with other products. Although potential drug interactions of *L. barbarum* are yet to be studied, several preparations of the herb are shown to inhibit CYP450 and FMO3 mediated metabolism (Rui et al., 2016). Warfarin is marketed as a racemic mixture of *R*- and *S*-warfarin enantiomers. *S*-warfarin is metabolized by CYP2C9 and has a much more potent anticoagulant activity than *R*-warfarin, which is metabolized mainly by CYP1A2 and CYP3A4 (Lam et al., 2001). In their study Zhang et al. (2015) confirmed the anticoagulant effect of warfarin is enhanced by *L. barbarum* and may result in bleeding. However, further investigation is required to study the exact underlying mechanism of the interactions. These may include the identification and composition of the plant, further in vitro studies to classify mechanisms of interactions such as pharmacokinetic considerations including P450 enzyme study, or pharmacodynamic interactions (Leung et al., 2008). Close monitoring of widely available plant-based drugs and dietary supplements is important. Besides which, product labeling of *L. barbarum* regarding its content and concurrent use of medical products will help mitigate the problem (Rivera et al., 2012).

CONCLUSIONS

In conclusion, as proven by various clinical trials both in vivo and in vitro, *L. barbarum* possesses a wide variety of biological effects. *L. barbarum* plays a multipurpose role in biological systems from being a structural component to a precursor of GHS. Studies have revealed that *L. barbarum* may palliate the symptoms of many disorders like autoimmune disorder, type 2 diabetes, pesticide and fungicide use, and various cancers including lung cancer. More studies need to be conducted to find out the diverse biological activities of *L. barbarum* in critical neuroinflammatory diseases like neurodegenerative disorders, cancers, and HIV/AIDS. Also, the food and drug interactions of *L. barbarum*, including its pharmacokinetic and pharmacodynamic properties, should be studied in greater detail.

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AUTHOR CONTRIBUTIONS

All authors have directly participated in the planning or drafting of the manuscript and read and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 3.22

Gotu Kola (*Centella asiatica*)

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INTRODUCTION

Plants as a source of food, herbs, and medicines have been used for thousands of years in many traditional medicine systems. Growth in research on plant-based herbal products has increased exponentially both in developed and developing nations (Gohil et al., 2010; Vaidya, 1997). Possible reasons for this could include its therapeutically safe and effective action against mild and chronic diseases, and also its increased use as a dietary supplement (Gohil, 2011).

Centella asiatica L. is a perennial herb that belongs to the Apiaceae family. The species is commonly known as "mandukaparni" in Sanskrit, "brahmi" in Hindi, "Indian pennywort" in English, and "gotu kola" in many others parts of the world. The plant is tasteless, odorless, and is mainly found in and around water (Fig. 3.22.1). It has been used by many ancient cultures and tribal groups of various countries in their traditional medicinal systems to cure various types of ailments such as anemia, epistaxis, and hepatitis amongst others, and it is most popular for its use as a "brain tonic" agent, according to the World Health Organization (WHO). It has been used for hundreds of years in Indian Ayurveda, Malaysian, and Chinese herbal medicine, as well as being used in other parts of Asia (Brinkhaus et al., 2000). It also helps improve memory and treat mental fatigue, anxiety, and eczema (Gupta et al., 2003; Hamid et al., 2002; Kartnig, 1988). The species is currently being cultivated in many parts of the world due to its high medicinal value and wide range of uses (Imada, 2012; Matsuda et al., 2001). This chapter discuss detailed information regarding *C. asiatica* distribution, bioactive compounds and bio-activity, interaction with other known drugs, toxicity studies, as well as listing *C. asiatica* major products dealing with wide therapeutic activity.

DISTRIBUTION

C. asiatica is distributed throughout tropical and subtropical countries from 200 to 2100 m a.s.l. (Hedge and Lamond, 1992; Samant and Pant, 2006) (Table 3.22.1). It is native to Asia, Africa, America, and Oceania as shown in Fig. 3.22.2.

BIOACTIVE COMPOUNDS

C. asiatica has been extensively studied for identification of its bioactive compounds. It is a rich source of amino acids (e.g., alanine, serine, aspartate, and glutamate), phenols (e.g., kaempferol and quercetin), terpenoids (e.g., asiaticoside, centelloside, madecassoside, and brahmoside), and carbohydrates (e.g., glucose, mesoinositol, and centellose) among others (Table 3.22.2), which have found wider applications as both health and food supplements.

THERAPEUTIC ACTIVITY

C. asiatica was known as "brain food," due to its various well-known neuroprotective activities. It has also been used as an antiinflammatory (Somchit et al., 2004), an antipsoriatic (Sampson et al., 2001), and antiulcer treatment (Cheng et al., 2004; Cheng and Koo, 2000), as a hepatoprotective treatment (Antony et al., 2006), an anticonvulsant (Visweswari et al., 2010), a sedative (Wijeweera et al., 2006), an immunostimulant (Wang et al., 2003), as a cardioprotective treatment (Gnanapragasam et al., 2004), an antidiabetic treatment (Mutayabarwa et al., 2003), as a cytotoxic and antitumor treatment (Bunpo et al., 2004; Xu et al., 2012), an antiviral (Yoosook et al., 2000), an antibacterial (Oyedeji and Afolayan, 2005), an

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FIG. 3.22.1 *Centella asiatica* **in its natural habitat at Surya Kunj (GBPNIHESD, Kosi-Katarmal, Almora, Uttarakhand).**

TABLE 3.22.1 Distribution of *Centella asiatica* Across the World

S. No.	Region	Country	References
1.	Africa	Angola, Madagascar, Zambia, Zimbabwe, Senegal, Sudan, Mali, Tanzania, Somalia, Nigeria, Mozambique, Mauritius, Congo, Kenya, Republic, Botswana, Malawi, Cameroon, South Africa, Central Africa	Biswas and Mukherjee (2003); Caldas and Machado (2004); Jamil et al. (2007); Zainol et al. (2003); Singh et al. (2010); Jana et al. (2010) and Gupta (2013)
2.	North America	United States, Mexico	Jamil et al. (2007); Gupta (2013)
3.	Asia	Vietnam, Taiwan, Pakistan, Nepal, Malaysia, China, Japan, Korea, Thailand, Bhutan, Indonesia, Yemen, Bangladesh, India, Myanmar, Saudi Arabia, Sri Lanka	Biswas and Mukherjee (2003); Jamil et al. (2007); Jana et al. (2010); Singh et al. (2010) and Gupta (2013)
4.	Australia		Gupta (2013)
5.	South America	Venezuela, Brazil, Colombia, Eastern South America	Caldas and Machado (2004); Jamil et al. (2007)
6.	Europe	France	Caldas and Machado (2004)

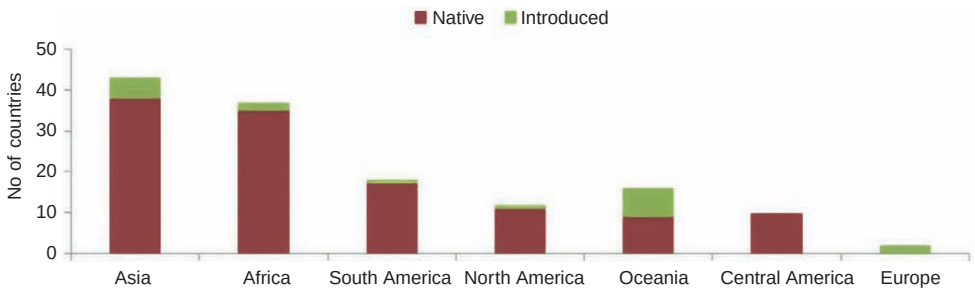

FIG. 3.22.2 Native and nonnative distribution of *Centella asiatica* **among within different continents.** *(Data taken from: www.cabi.org/isc/datasheet/12048).*

insecticidal (Rajkumar and Jebanesan, 2005), an antifungal (Dash et al., 2011), an antioxidant (Hamid et al., 2002), and a venous deficiency treatment (Cesarone et al., 2001; Pointel et al., 1987). These activities have been tested in both in vitro and in vivo models and are discussed in Table 3.22.3.

TOXICITY AND INTERACTIONS

Today, millions of people use herbs either as food or medicines along with prescription and nonprescription medications. These herbs are examined for their toxicity and interaction with a wide range of drugs and foods. As such, *C. asiatica* has been examined for toxicity and interactions, which are discussed in this section.

In an earlier paper from 1969, the saponoside fraction of the plant extract containing brahmic acid and its derivatives were found to cause infertility in both human and rat sperm (Singh and Rastogi, 1969). In 2010, Oruganti carried out a

TABLE 3.22.2 Bioactive Compounds Isolated From *Centella asiatica*

Class	Compound name	References
Triterpene acid	asiatic acid, asiaticoside, madecassic acid, madecassoside, terminolic, centic, centellic, centoic acid, indocentoic acid, isobrahmic, brahmic, betulic, and madasiatic acid	Rastogi et al. (1960); Singh and Rastogi (1969); Asolkar et al. (1992) and Schaneberg et al. (2003)
Polyphenolic compounds	quercetin, quercitrin, kaempferol, luteolin, chlorogenic acid	Bhandari et al. (2007)
Alkaloids	hydrocotylin	Chopra et al. (1956)
Volatiles and fatty acids	glycerides of oleic, palmitic, stearic, lignoceric, linoleic, and linolenic acids	Chopra et al. (1956)
Glycosides	asiaticoside A, asiaticoside B, madecassoside, centelloside, brahmoside, brahminoside, thankuniside, glycoside D, and glycoside E	Datta and Basu (1962); Singh and Rastogi (1969); Chopra et al. (1992) and Schaneberg et al. (2003)
Others	iligosaccharide centellose, stigmasterol, sitosterol, campsterol, polyacetylenes, carotenoids, vitamin B and C, vellarine, pectic acid, tannins, sugars, inorganic acid, and resins	Chopra et al. (1956); Singh and Rastogi (1969) and Kapoor (2005)

TABLE 3.22.3 List of Some of the Pharmacological Activities of *Centella asiatica*

Pharmacological/ therapeutic activity	Type of study and model used	Results	Mechanism	References
Antiproliferative	Effects of water extract from *C. asiatica* on the mortality of human lung cancer cells (A549) with the use of novel 3-D scaffolds infused with CMC hydrogel.	*C. asiatica* extract showed antiproliferative activity against A549 and there were no cytotoxic effects on human normal fibroblast cells IMR90.	*C. asiatica* extract induces apoptosis and mortality in A549 cells.	Aizad et al. (2015)
Cognitive functions (memory enhancer, etc.)	Protective effect of *C. asiatica* fresh leaf aqueous extract on learning and memory of albino rats.	Revitalize the brain and nervous system thus exhibit significant effect on learning and memory process.	Decreasing level of norepinephrine, dopamine, and 5- HT in the brain.	Nalini et al. (1992)
	Aqueous extract of *C. asiatica* tested against streptozotocin-induced cognitive impairment and oxidative stress in rats.	Cognitive enhancing and antioxidant properties.		Veerendra and Gupta (2002, 2003)
	Effect of different extracts (aqueous, methanolic and chloroform extracts) of *C. asiatica* on cognition and markers of oxidative stress in rats.	Improved learning and memory and increased antioxidant property.	Decreasing lipid peroxidation and augmenting the endogenous antioxidant enzymes in brain.	Kumar and Gupta (2002)

(Continued)

TABLE 3.22.3 List of Some of the Pharmacological Activities of *Centella asiatica*—cont'd

Pharmacological/ therapeutic activity	Type of study and model used	Results	Mechanism	References
Antiinflammatory	Effect of asiatic acid and asiaticoside isolated from the leaves of *C. asiatica* on LPS-induced NO and PGE (2) production in macrophage cells.	Positive antiinflammatory effect.	Inhibition of enzyme (iNOS, cyclooxygenase-2 (COX-2), interleukins (IL-6, IL-1β), cytokine tumor necrosis factor (TNF-α)) expression through the downregulation of NF-κB activation.	Yun et al. (2008)
	Antinociceptive and antiinflammatory activity of *C. asiatica* aqueous extract tested in acetic acid–induced writhing and hot-plate method in mice.	Significant antinociceptive and antiinflammatory activity in both models.		Somchit et al. (2004)
	Antirheumatoid arthritic activity of madecassoside tested in type II collagen-induced arthritis (CIA) in mice models.	Madecassoside substantially prevented CIA in mice.	Reduced serum level of anti-CIIIgG, suppressed delayed type hypersensitivity, and moderately suppressed CII-stimulated proliferation of lymphocytes.	Liu et al. (2008)
Anticancer	Effects of *C. asiatica* leaf extract on decreasing the number of benzo(a) pyrene induced lung tumor nodules and determining the histopathological features of mice.	Extract of *C. asiatica* leaves in some doses could decrease the number of lung tumor nodules in mice induced by benzo(a)pyrene.	*C. asiatica* increased the phosphorylation of cyclic AMP response element binding protein (CREB) in neuroblastoma cultured cells that expressed beta amyloid 1-42(A beta). Thus, was preventing cell proliferation toward malignancy	Hamid et al. (2016); Gohil et al. (2010)
	U-87 MG human glioblastoma cell death induced by asiatic acid (AA) from *C. asiatica*.	AA induces cell death by both apoptosis and necrosis, with Ca^{2+}-mediated necrotic cell death predominating.	AA-induced glioblastoma cell death is associated with decreased mitochondrial membrane potential, activation of caspase-9 and caspase-3, and increased intracellular free Ca^{2+}.	Cho et al. (2006)
	Effect of *C. asiatica* extract and its purified fractions on tumor-bearing mice.	Retards the development of tumors and increases the life span of mice.	Cytotoxic and antitumor effect involve direct action on DNA synthesis.	Xu et al. (2012)
	Effect of *C. asiatica* extract and its purified fractions on solid and Ehrlich Ascites tumor-bearing mice.	Retards the development of tumors and increases the life span of mice.	Induces apoptosis.	Babu et al. (1995); Babu and Paddikkala (1993)

TABLE 3.22.3 List of Some of the Pharmacological Activities of *Centella asiatica*—cont'd

Pharmacological/ therapeutic activity	Type of study and model used	Results	Mechanism	References
Antioxidant	Cytoprotective effect of asiatic acid (AA) against oxidative stress by the t-BHP-induced model in HepG2 cells.	AA has a cytoprotective effect against t-BHP-induced cell damage via suppressing cytotoxicity, ROS generation, and apoptosis.	AA activates Nrf2 signal in HepG2 cells. It is well known that Nrf2 promotes transcriptional activation of a variety of antioxidant genes through binding to ARE, such as HO-1, NQO-1, GCLC, and GCLM.	Zhimin et al. (2017)
	Effect of *C. asiatica* methanolic extract against oxidative stress in lymphoma-bearing mice models.	Prevents oxidative stress.	Increased antioxidant enzymes (SOD, CAT, GSHPx).	Jayashree et al. (2003); Gohil et al. (2010)
	Kaempferol and quercetin from *C. asiatica* tested for in vitro DPPH activity.	DPPH activity as IC50 vales of 9.64 and 11.97 μg/ml for kaempferol and quercetin, respectively.	Scavenging DPPH radical.	Dewi and Maryani (2015)
Neuroprotective	Effect of *C. asiatica* leaf extract on hippocampal CA3 neurons.	Protects the hippocampal CA3 neurons from degeneration in stressed mice.	In the present study the cytoprotective and antioxidant property of CA may be responsible for the neuroprotection against cell death and the deleterious effects of stress and hence increased dendritic arborization.	Hemamalini and Rao. (2016)
	Asiatic acid neuroprotective activity tested on cultured cortical cells by exposure to excess glutamate.	Asiatic acid proves to be effective in protecting neurons.	Enhanced cellular oxidative defense mechanism	Kumar et al. (1998)
	Effect of *C. asiatica* extract against rotenone-induced Zebrafish model.	Maintain neuronal motility.	Increasing dopamine level.	Khotimah et al. (2015)
	Effect of asiaticoside derivatives against beta-amyloid induced neurotoxicity on B103 cell culture and hippocampal slices.	Protect neurons from beta-amyloid toxicity.	Strong inhibition of beta-amyloid and free radical–induced cell death.	Mook-Jung et al. (1999); Gohil et al. (2010)

(Continued)

TABLE 3.22.3 List of Some of the Pharmacological Activities of *Centella asiatica*—cont'd

Pharmacological/ therapeutic activity	Type of study and model used	Results	Mechanism	References
Antiulcer	Antiulcerogenic activity of ethanol extract of *C. asiatica* against ethanol-induced gastric mucosal injury in rats.	Inhibited significantly gastric ulceration induced by cold and restraint stress in Charles-Foster rats.	Reduction of ulcer areas in the gastric wall as well as the reduction or inhibition of edema and leucocyte infiltration of submucosal layers.	Abdulla et al. (2010)
	Effect of ethanolic extract of *Tinospora cordifolia* and *C. asiatica* on animal model.	A dose of 100 mg/kg per day produced a protective effect against stress-induced ulceration.	Strengthened mucosal barrier and reduced damaging effect of free radicals.	Sarma et al. (1995); Cheng and Koo (2000)
	Fresh juice of *C. asiatica* tested against experimental ulcer models.	Significant protection against ulcer.	Strengthening of mucosal defensive factors.	Sairam et al. (2001)
Wound healing	Animal model	Asiaticoside from *C. asiatica* facilitates wound healing.	Increase in peptidic hydroxyproline content, tensile strength, collagen synthesis, angiogenesis, and epitheliazation.	Bonte et al. (1994); Shukla et al. (1999)
	Animal model	Asiatic acid and madecassic acid facilitates wound healing.	Increase in peptidic hydroxyproline and an increased remodeling of collagen synthesis.	Bonte et al. (1994); Maquart et al. (1999)
	Aqueous *C. asiatica* extract tested on open wounds in rats.	The treated wounds recover faster compared to untreated wounds.	Increased cellular proliferation and collagen synthesis resulted in increased tensile strength.	Gohil et al. (2010); Kumar et al. (1998)
	Activity of asiaticoside was studied in normal as well as delayed (diabetic)-type wound healing in rats.	Both types of wound healing activity have been recorded under treatment.	Increase in tensile strength, collagen content, hydroxyproline content, and better epithelisation.	Suguna et al. (1996); Gohil et al. (2010)

study to assess safety levels for oral doses of *C. asiatica* given to albino rats. After an extensive study for 30 days, a dose-dependent increase in serum biomarkers was reported. A dose of 1000 mg/kg increased the weight of the spleen and caused a high level of apoptosis in hepatic and renal tissues (Oruganti et al., 2010). In another chronic toxicity study on Wistar rats (male and female) receiving 20 mg/kg per day, 200 mg/kg per day, 600 mg/kg per day, and 1200 mg/kg per day of *C. asiatica* for 6 months, the rats displayed no sign of a significant alteration in body weight, blood chemistry, clinical chemistry, or histopathology when compared to a control group (Chivapat et al., 2004). In 2011, the same author carried out another toxicity study using ECa 233 (a standardized extract of *C. asiatica*) on male and female Wistar rats. After a study period of 14 days no lethality was observed at a dose of 10 g/kg along with an absence of any form of toxic damage to any organs. Subchronic toxicity of the standardized extract on a group of 24 Wistar rats led to no difference in their average weight, nor did they show any signs of abnormal behavior. On autopsy of the study animals, it was found that all the ECa 233 treated animals displayed no damage to their organs and there was no difference in their organ weights, except for the male rat group that was given ECa 233 (10 mg/kg per day), this group showed lower relative right adrenal weight when compared to the control group (Chivapat et al., 2011). In 1991, Dandekar et al. carried out studies on the interaction between phenytoin (an antiepileptic drug) and an Ayurvedic formulation (sankhapushpi) in which *C. asiatica* is the second most abundant component. The study was carried out on random-bred Sprague-Dawley rats of both sexes, with weights

ranging between 100 and 150 g. The study showed that plasma phenytoin levels lowered significantly when sankhapushpi was coadministered with phenytoin orally, twice daily, for 5 days, hence the combination was not recommended (Dandekar et al.,1992). However, when *C. asiatica* extract was administered in mice along with phenytoin (13 mg/kg), valproate (104 mg/kg), and gabapentin (310 mg/kg) in combination, the anticonvulsant activity increased with a significant decrease in the effective dose of drugs of 62%, 72%, and 75%, respectively (Vattanajun et al., 2005). In a case study carried by Izu et al. (1992), the occurrence of allergic contact dermatitis with topical application of creams containing *C. asiatica* extracts was observed. Also, *C. asiatica* was postulated to interfere with blood glucose level when coadministered with hypoglycemic therapy (Gohil et al., 2010). The excessive amont of *C. asiatica* consumed when taken orally can cause headaches and transient unconsciousness. Moreover, the continuous use of *C. asiatica* for more than 6 weeks can cause spontaneous abortion in women, an is therefore not recommended (Gohil et al., 2010).

TRADE AND TRENDS

C. asiatica has great market potential attributed to its rich medicinal properties. Due to its high medicinal value, it has been among the 25 top selling medicinal herbs in the United States (Randriamampionona et al., 2007). In traditional as well as commercial medicinal products, leaves, stems, and whole plants have been used (Jamil et al., 2007; Samant and Pant, 2006). Many scientific studies on *C. asiatica* have demonstrated its different pharmacological and therapeutic properties, that is, as an antioxidant, an antibacterial, an antifungal, an antiviral, as an antiulcer and antidiabetic treatment, as an antiinflammatory, as a cytotoxic, demonstrating protection of the skin as well as being a cardioprotetive, radioprotective, and neuroprotective, having immunomodulatory properties, enhacing memory, and being able to heal wounds. In light of this, various market products having *C. asiatica* as an active ingredient have been introduced, some of them are listed in Table 3.22.4.

TABLE 3.22.4 List of Commercial Products Having *Centella asiatica* as an Active Ingredient

S. No.	Product name	Uses	Approximate price (US$)	Manufacturing company
1	Way 4 Organic Pure *Centella asiatica* raw powder	Enhances the overall immune system	2.95/100 g	Genius Nature Herbs Pvt. Ltd, India
2	Vallarai (*Centella asiatica*) powder	Memory enhancer, used to cure hair, skin, and stomach problems as well as curing stress and depression	7.4/100 g	Neotea DCBS ideas, India
3	Gotu Kola	Balances the effects of aging, increases healing power for longevity, and is a neuroprotective	7.59/500 mg	Morpheme Remedies, India
4	Organic India Organic Brahmi capsules	Used to cure venous insufficency, leg edema, stress, lack of concentration, memory decline	2.60/60 g	Shop Organikos, Punjab, India
5	Skin 1004 *Centella asiatica* ampoule	Skin nourishment	80.13/454 g	Skin Industries, Korean cosmetics, Korean beauty
6	Xhekpon Face Neck & Decolleté Anti-ageing Cream	Skin nourishment	52.86/40 g	XHEKPON, Spain
7	*Centella asiatica* facial cleaning gel	Skin nourishment	75.60/150 g	Thai herb, Thailand
8	Richelth capsules	Rich antioxidant support for health and longevity	4.78/100 g	CharakPharma Pvt. Ltd, India

(Continued)

TABLE 3.22.4 List of Commercial Products Having *Centella asiatica* as an Active Ingredient—cont'd

S. No.	Product name	Uses	Approximate price (US$)	Manufacturing company
9	Green Tea Brahmi	Promotes Relaxation. Maintains mental health	4.15/50 g	Biosap, Rajasthan, India
10	Contorno Occhi (eye gel)	Antioxidant as well as an antiinflammatory agent	117.14/15 ml	L'ERBOLARIO, Italy
11	Patanjali Sharbat Brahmi	Effective in the treatment of edema, urinary disorders, anaemia, and fever	1.48/750 ml	Patanjali Ayurved, India
12	Gotu Kola extract	Dietary supplement	29.75/120 ml	Hawaii Pharm LLC., Honolulu, Hawaii, USA
13	Capisol	Dietary supplement	29.45/380 mg	R.I. Group srl, Cornuda (TV), Italy

(Data taken from: amazon.in, amazon.com, renacoitalia.net)

CONCLUSIONS

C. asiatica is one of the most potent herbal supplements for treating central nervous system (CNS)-related disorders. It has a balancing effect on mood and also increases concentration. Clinical evidence suggests that it can be used to treat venous and arterial problems, as it showed beneficial effects in strengthening vascular systems and connective tissues. Further studies on clinical activities need to be carried out on *C. asiatica* to explore its wider possibilities in treating disease conditions.

Due to its diverse potential health applications, *C. asiatica* is being exploited at a faster rate than previously thought, and as a result is listed as a highly threatened plant species by the International Union for Conservation of Nature. There is a need for continuous production of *C. asiatica* on a large scale, grown in vitro, to meet the demands of the herbal industry. Further investigations on the effect of environmental and bioprocess factors on the accumulation of secondary metabolites in *C. asiatica* would strengthen its utilization for industrial purposes. Also, genetic characterization, by means of genetic fingerprinting, is needed to further understand the diversity seen in *C. asiatica*. This will involve understanding the genetic variability and heritability of *C. asiatica*, thereby helping to improve the component yield of the plant.

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Chapter 3.23

Arctium lappa

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INTRODUCTION

Arctium lappa, commonly called greater burdock, gobō, edible burdock, lappa, thorny burr, beggar's buttons or happy major is a perennial herb belonging to family of Asteraceae (Compositae) is a rich source of many bioactive molecules reportedly involved in modulation of wide ranging biological mechanisms. *Arctium lappa* has been extensively studied because of its health promoting and pharmacological properties. Arctigenin, tannins, arctiin, caffeic acid, beta-eudesmol, inulin, trachelogenin-4, sitosterol-beta-D-glucopyranoside, diarctigenin, lappaol and chlorogenic acid are major active ingredients isolated from *Arctium lappa*. In this chapter we have attempted to provide an overview of the health promoting effects of *Arctium lappa*. We will first focus on the tumor suppressing properties of *Arctium lappa* and how it modulates protein network in cancer cells to inhibit cancer progression.

TUMOR SUPPRESSING EFFECTS OF BIOACTIVE MOLECULES ISOLATED FROM *ARCTIUM LAPPA*

Cancer is a multifaceted and therapeutically challenging disease and increasingly it is being realized that intra- and intertumor heterogeneity make it difficult to target. Because of interdisciplinary approaches, researchers are now focusing on natural products to find bioactive molecules having significant clinical outcome and minimum off-target effects. *Arctium lappa* and its bioactive molecules have reported anticancer activities in different cancer cell lines and cancer models.

Dichloromethane (CH2Cl2)-soluble extract of *Arctium lappa* at a concentration 50 μg/mL was preferentially cytotoxic against nutrient-deprived cells (Awale et al, 2006). Arctigenin showed concentration- and time-dependent preferential cytotoxicity against nutrient-deprived cells. Pancreatic cancer PANC-1 cells had significantly higher resistance to extreme nutrient deprivation and survived under these circumstances for >48 hours (Awale et al, 2006). Arctigenin (0.01 μg/mL) treated cells demonstrated 100% apoptosis within 24 hours of starvation. Furthermore, sensitivity of nutrients deprived and starved cancer cells was notably higher when 1 μg/mL of arctigenin was used which induced 100% cell death within 12 hours (Awale et al, 2006). Mechanistically it was shown that arctigenin (0.1 μg/mL) completely inhibited phosphorylation of Akt (Protein kinase B) stimulated by glucose deprivation. Furthermore, arctigenin also dose-dependently suppressed Akt activation stimulated by insulin and insulin-like growth factor-I (IGF-I). Arctigenin considerably inhibited tumor growth in mice xenografted with 5×10^6 PANC-1 cells (Awale et al, 2006).

Arctigenin treatment increased the proportion of cells in the G0/G1 phase from 55.8% to 81.4% in lung adenocarcinoma A549 cells. Arctigenin (6g/ml) dramatically reduced level of the proteins involved in the G1/S checkpoint signaling, including CDK7, CDK2, cyclin E, cyclin H, NPAT and p-CDK (Susanti et al, 2013).

Lappaol F was found to significantly increase 4N G_2- (or M) phase population or 2N G_1 phase population in treated cell lines. HeLa and MCF-7 cells were mainly arrested in 2N G_1 phase, while colorectal cancer (RKO) cells and breast cancer (MDA-MB-231) cells were mainly arrested in 4N G_2 (Sun et al, 2014). Data clearly suggested that an increase in 4N population in Lappaol F treated MDA-MB-231 and RKO cells was indicative of G2 instead of mitotically arrested cells. Additionally, Lappaol F–treated cells had bigger nuclei. CDK inhibitors (p27, p21) were significantly enhanced in Lappaol F treated cells (Sun et al, 2014). On the contrary, cyclin B1, CDK2, CDK1 were noted to be considerably repressed.

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00039-4

Activation of both extrinsic and intrinsic pathways was observed in Lappaol F treated MDA-MB-231 cells. Lappaol F significantly reduced the growth of tumor by 54% (5 mg/kg/day) and 64% (10 mg/kg/day) (Sun et al, 2014).

Matrix metallo-proteinases (MMPs), a family of zinc-dependent enzymes played central role in the metastatic spread. Arctigenin treated breast cancer MDA-MB-231 cells demonstrated significant inhibition of the activities of both MMP-2 and MMP-9. Treatment of cells with different concentrations of arctigenin (20 μM, 40 μM) exerted inhibitory effects on mean activity of MMPs. There was a reduction in activity of MMP-9 to 43.05 ± 4.70 and 25.05 ± 4.11%, and activity of MMP-2 reduced to 58.89 ± 9.11 and 19.06 ± 5.10% correspondingly. Significantly downregulated expression levels of MMP-9 and MMP-2 were noted in arctigenin treated breast cancer cells (Lou et al, 2017).

INHIBITION OF JAK-STAT SIGNALING

JAK-STAT has emerged as a central communication node for the immune systems as evidenced by immunological phenotypes observed in humans and experimental mice bearing gain-or loss- of-function mutations of genes which encode JAK–STAT components.

Arctigenin markedly inhibited both IL-6-induced and constitutively activated STAT3 phosphorylation and nuclear accumulation of phosphorylated STAT3. Arctigenin exerted inhibitory effects on STAT3 tyrosine phosphorylation through suppression of JAK1, JAK2 and Src (Yao et al, 2011). Whereas ERK mediated phosphorylation of STAT3 at serine residue was also inhibited by arctigenin. Arctigenin substantially enhanced cisplatin-mediated apoptosis in cancer cells which indicated that arctigenin made cancer cells sensitive to cisplatin mainly via suppression of STAT3 (Yao et al, 2011).

In a recent study it was reported that arctigenin inhibited STAT3 triggered transcriptional regulation of target genes by repressing the loading of STAT3 to binding sites present with promoter region of genes (Feng et al, 2017). It had been noted that STAT3-Y705 phosphorylation existed in 2 protonation states either Y1P or Y2P. STAT3-Y2P had considerably higher affiliation for DNA and the free binding energy of STAT3-Y2P to DNA increased by 88.12 kcal/mol which was higher than STAT3-Y1P-DNA complex. Data clearly indicated that Y705P played central role in STAT3-DNA binding which was blocked by binding of arctigenin to the SH2 domain of STAT3-Y705P. Arctigenin substantially reduced growth of the tumor in mice inoculated subcutaneously with MDA-MB-231 cells (Feng et al, 2017).

Although much attention is being devoted to tumor suppressing properties of *Arctium lappa* however, we still do not have sufficient knowledge related to different signaling cascades which are targeted by bioactive components of *Arctium lappa*. Fig. 3.23.1

ANTI-DIABETIC EFFECTS

Hydro-alcoholic root extract of *Arctium lappa* was effective against nicotinamide-streptozotocin induced type 2 diabetes model (Ahangarpour et al, 2017). Administration of different doses of the extract considerably reduced levels of very low density-lipoprotein (VLDL), alkaline phosphatase, glucose and triglycerides in diabetic mice. Markedly enahnced levels of insulin were noted in diabetic mice treated with 200 mg/kg. Both Leptin and HDL were also found to be increased in diabetic mice treated with 300 mg/kg of the extract (Ahangarpour et al, 2017).

ANTI-OBESITY EFFECTS

Arctium lappa is reportedly involved in significantly reducing weight gains and reduction in accumulation of white adipose tissues in high-fat diet induced obese mice (Han et al, 2016). LDL-cholesterol and triglycerides were reduced but a concomitant increase in the level of HDL-cholesterol was noticed in the treated group. In 3T3-L1 cells, water extract and 70% EtOH extract of *Arctium lappa* considerably downregulated adipogenesis and repressed PPARγ (peroxisome proliferator activated receptor γ) and CEBP (CCAAT/enhancer binding protein α) (Han et al, 2016). Data clearly suggested that *Arctium lappa* prevented obesity development by inhibiting differentiation of white adipocytes and activation of brown adipocyte differentiation.

ANTI-ALLERGIC ACTIVITIES

Mast cells have a dramatic role in IgE-associated immune responses both at early as well as later phases of allergic reactions. Allergens and IgE acted as triggers for activation of mast cells which consequently underwent IgE-dependent maturation and secreted allergy related mediators such as β- hexosaminidase, tryptase and histamine (Yang et al 2016). Antigenic crosslinking of IgE receptor (FcεRI) on mast cells resulted in an immediate secretion of histamine and induced leukotrienes, prostaglandins

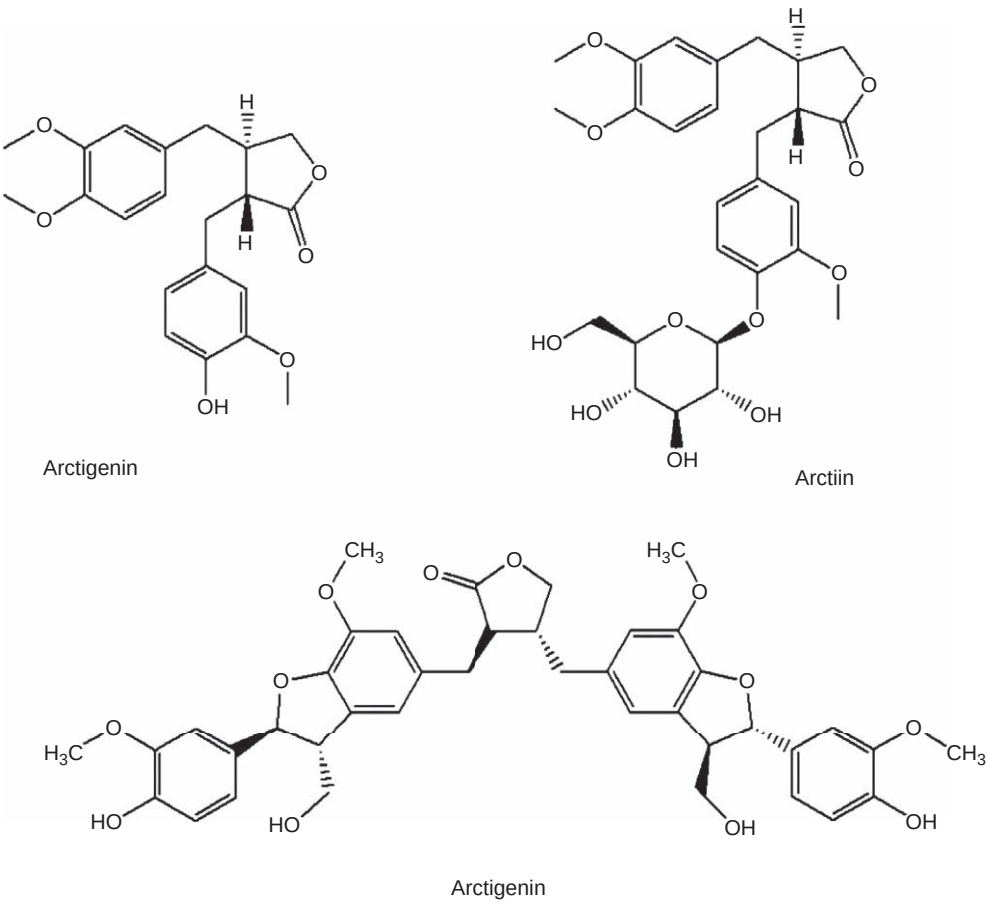

FIG. 3.23.1 shows chemical structures of the constituents present in *Arctium lappa*

and inflammation associated cytokines which played instrumental role at later phase of an allergic reaction. Oleamide, a bioactive molecule isolated from *Arctium lappa* suppressed FcεRI-tyrosine kinase Lyn-mediated pathway, p38 mitogen activated protein kinase (p38MAPK) and c-Jun N terminal kinases (JNK/SAPK). Oleamide at 100 μg/mL markedly reduced TNF-α (Tumor necrosis factor-α) and IL-4 production in A23187/PMA-stimulated RBL-2H3 cells (Yang et al 2016).

ANTIMICROBIAL ACTIVITIES

Root extract of *Arctium lappa* and its component chlorogenic acid had shown significant efficacy against Klebsiella pneumoniae. Root extract of *Arctium lappa* and its component chlorogenic acid demonstrated potent β-lactamase inhibitory activity (Rajasekharan et al, 2017). Chlorogenic acid strongly interacted with active sites of sulfhydryl-variable-1 β-lactamase. Additionally, significant downregulation of 2 biofilm-associated genes (trehalose-6-phosphate hydrolase treC and type 3 fimbriae mrkD) was found in chlorogenic acid and extract-treated samples. Moreover, chlorogenic acid inhibited biofilm formation by *Candida albicans* and *Escherichia coli* (Rajasekharan et al, 2017).

ULCERATIVE COLITIS

Wealth of information suggested that T helper 1 (Th1), T helper 17 (Th17) cells and related cytokines were contributory in the pathogenesis of ulcerative colitis. Arctigenin (1-100 μM) concentration dependently inhibited proliferation of T cells induced by concanavalin A or anti-CD3/CD28 (Wu et al, 2015). Arctigenin considerably downregulated RORγt and T-bet (transcription factors belonging to lineage of Th17 and Th1, respectively). Arctigenin substantially repressed the absolute number and percentage of IL-17 + CD4 + T cells. Arctigenin markedly reduced expression levels of IL-17F, IL-17A, IL-22, IL-21, RORγt and IL-23R (interleukin receptor) in CD4+ T cells. Arctigenin and rapamycin (mTORC1 inhibitor) repressed phosphorylated levels of p70S6K (p70S6 kinase) and RPS6 (ribosomal protein S6) in T cells cultured

under Th17 or Th0 -polarizing condition. PI3K (Phosphoinositide 3-kinase) /AKT and ERK are upstream signals which regulated mTORC1 activation (Wu et al, 2015). Arctigenin concentration-dependently reduced phosphorylated levels of AKT and ERK. TSC2 (Tuberous sclerosis complex 2) acted as central controller of mTORC1 activity and an inhibitor of differentiation of Th1 and Th17 cells. Arctigenin exerted inhibitory effects on T cell differentiation were impaired because of overactivation of mTORC1 via TSC2 knockdown (Wu et al, 2015). Data clearly suggested that arctigenin was effective against ulcerative colitis and inhibited Th1 and Th17 differentiation.

There is evidence of Arctigenin mediated significant reduction in the subarachnoid hemorrhage induced vasospasm in animal models (Chang et al, 2015). Increased expression of Akt was noted in the cerebral arteries of subarachnoid hemorrhage (SAH) animals. Arctigenin triggered PI3K/Akt signaling axis and 450μM of Arctigenin increased the expression of eNOS (endothelial nitric oxide synthetase) upto 1.4-folds (Chang et al, 2015).

EFFECTIVE AGAINST OSTEOARTHRITIS

Arctium lappa root tea considerably reduced serum levels of IL-6,malondialdehyde and high sensitivity C-reactive protein (hs-CRP), whereas serum levels of total antioxidants capacity (TAC) and superoxide dismutase activities were found to be significantly enhanced (Maghsoumi-Norouzabad et al, 2016).

In depth analysis of pathological changes which occur in a young healthy joint and alter its signaling during ageing will be helpful in the treatment of osteoarthritic joints.

REGULATION OF BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS

Blood pressure was noted to be reduced in spontaneously hypertensive rats administered with arctigenin. Arctigenin significantly improved level of serum nitric oxide (Liu et al, 2015). Arctigenin remarkably repressed generation of vascular superoxide anions in rats. It was concluded that arctigenin improved nitric oxide levels by enhancing phosphorylated levels of Akt and eNOS and inhibited NADPH oxidase expression (Liu et al, 2015).

GASTRO-PROTECTIVE ACTIVITY

1,3-dicaffeoylquinic acid isolated from ethanol soluble fraction demonstrated gastro-protective activity in rats (Carlotto et al, 2015).

CLINICAL TRIALS

Japanese investigators tested tolerance toxicity and safety profile of GBS-01 (orally administered drug which contained higher percentage of arctigenin). Pancreatic cancer patients who showed resistance against gemcitabine were given arctigenin (bioactive molecule) in cohorts of 3g to 12g/day. In first 4 weeks, primary endpoints used for analysis were hematologic toxicity grade 4, dose limited toxicities and non-hematological grade 3-4 toxicities (Strimpakos and Saif, 2013). Most importantly, dose limited toxicities were not recorded in 15 patients in all cohorts. Some of adverse side-effects noted during administration of GBS-01 were slightly increased gamma-glutamyl transferase, glucose serum levels and total bilirubin. Hence, phase II dose (recommended dose) was set at 12g/day. Although secondary end-point was observed in 1 out of 15 patients while 4 patients had stable disease. Duration of median overall-survival was noted to be over 5 months in pre-treated patient group (Strimpakos and Saif, 2013).

Drug interactions with *Arctium lappa*:

Although some information is available on interactions of *Arctium lappa* with different drugs however, we still have insufficient understanding of the exact mechanisms through which *Arctium lappa* enhances or opposes the action of different drugs/supplements. *Arctium lappa* has been noted to potentialize the effects exerted by warfarin (Leite et al, 2016).

CONCLUSION

Arctium lappa is highly appreciated for its health promoting activities. Confluence of information suggested that *Arctium lappa* was effective against different diseases. Anti-diabetic effects of *Arctium lappa* are quite encouraging but we still need to know the underlying mechanisms which are regulated by *Arctium lappa* or its bioactive ingredients. *Arctium lappa* has also shown potential in reducing the obesity and improving lipid profile in animal models. Emerging evidence has highlighted role of *Arctium lappa* in suppressing tumor in cancer cell lines and xenografted mice. However, it will not be wrong

to say that we have just started to scratch the surface and future high-quality research will unravel additional modes of action through which *Arctium lappa* exerted its tumor suppressive effects. Hopefully, well designed preclinical and phase-1 clinical trials will help us to develop a better understanding of some other roles played by *Arctium lappa* in prevention and treatment of human diseases.

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Chapter 3.24

Guarana

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INTRODUCTION

Guarana was known before the conquest of America and was domesticated in the Brazilian Amazon (Smith and Atroch, 2010). The Brazilian Maués Indians discovered it and were the first to use it as a beverage (Kuri, 2008). The fruits of guarana, orange-red capsules containing black seeds partially covered by white arils, have the appearance of eyeballs, thus giving credence to some legends (Beck, 2005). The first written description was made by a Jesuit, Johannes Philippus Bettendorf, in 1669. Ever since, many features of this plant have been explored, accompanied by a great deal of research into its chemical composition (Henman, 1982). Because of the widespread use of guarana, mainly as a result of its stimulating effect on the central nervous system, it was officially described in the *Brazilian Pharmacopoeia* of 1977 (Brasil, 1977). Risks related to concentration, composition, individual contaminants, and chemical interactions between different supplements are a growing concern in public health (Petroczi et al., 2011).

Guarana seeds are commonly used by dissolving the powder of toasted and ground seed in water, either alone or in combination with other herbal drugs that are commercially available. Due to the great variety of its chemistry, it can be used both as a supplement and in energy drinks. Different procedures can select for bioactive compounds (Marques et al., 2016), allowing a variety of pharmacological effects for various therapeutic purposes.

CHARACTERISTICS AND CHEMICAL COMPOSITION OF GUARANA

Some authors have studied the chemical composition of guarana and reported the principal constituents as: caffeine 2.41%–5.07% (Baumann et al., 1995; Bittencourt et al., 2014; Henman, 1982; Spoladore et al., 1987; Zeidan-Chulia et al., 2013), theophylline 0.06%, theobromine 0.03% (Sousa et al., 2010), total tannins 5.0%–14.1% (Fukumasu et al., 2006a; Marx, 1990; Ushirobira et al., 2004; Yamaguti-Sasaki et al., 2007), proteins 7.0%–8.0%, polysaccharides 30%–47% (Angelucci et al., 1978; Nazaré, 1998), sugars 6.0%–8.0%, fibers 3.0%, fatty acids 0.16% (Angelucci et al., 1978), total ash 1.06%–2.88%, moisture 4.3%–10.5% (Angelucci et al., 1978; Mattei et al., 1998; Nazaré, 1998).

Table 3.24.1 identifies some of the compounds present in guarana. Procyanidins or condensed tannins are formed by the association of several monomeric units (catechins and epicatechins). In guarana the dimers of procyanidins A2, B1, B2, B3, and B4 have already been identified as has the procyanidin trimer C1 (Yamaguti-Sasaki et al., 2007).

GUARANA AS A NUTRITIONAL AND NUTRACEUTICAL SUPPLEMENT

Guarana powder is easily available both in natural product stores and on the internet. It can be marketed alone or in combination with other herbal drugs, thereby raising the possibility of additive or synergistic effects (Spinella, 2001).

Dietary, food, or nutrient supplements (collectively known as supplements) are concentrated sources of nutrients or other substances with a nutritional or physiological effect which are administered to supplement the normal diet. In Brazil the term dietary supplement (DS) does not exist. However, such products are added in other food categories such as food for athletes, vitamins and/or mineral supplements, and foodstuffs with functional properties or health claims (Neves and Caldas, 2015). Thus, guarana has also gained popularity because it is regarded as a functional food.

The versatility of guarana lies in its pharmacological properties mainly due to their content of methylxanthines and polyphenols, as already described in the literature. They are considered cytoprotective (Leite et al., 2013; Oliveira et al., 2011), hepatoprotective, neuroprotective (Kober et al., 2015), chemopreventive (Fukumasu et al., 2006b), anticancer

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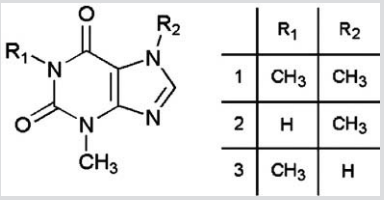
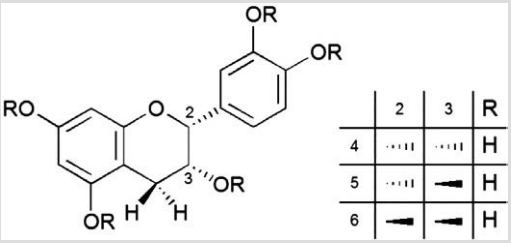
TABLE 3.24.1 Identification of the Main Chemical Constituents in Samples of Guarana

Name of substance	Chemical structure
Caffeine (1) (1,3,7-Trimethylxanthine) Theobromine (2) (3,7-dimethylxanthine) Theophylline (3) (1,3-dimethylxanthine)	
Epicatechin (4) Catechin (5) *ent*-epicatechin (6)	

(Fukumasu et al., 2011), anxiolytic (Roncon et al., 2011), panicolytic (Rangel et al., 2013), antidepressant (Otobone et al., 2007), antioxidant (Portella et al., 2013), and antimicrobial (Yamaguti-Sasaki et al., 2007). Despite such a variety of effects, supplements based on guarana are marketed primarily on the claim that guarana possesses stimulant properties (cognitive effect) (Pomportes et al., 2015a,b; Scholey et al., 2013) and is associated with weight loss (Andersen and Fogh, 2001; Bérubé-Parent et al., 2005; Boozer et al., 2001; Opala et al., 2006; Ruxton et al., 2007).

Herbal weight loss products generally attract users due to the health claims, assumed safety, easy availability, affordable price, extensive marketing, and the perceived lack of need for professional oversight (Bersani et al., 2015). The main stimulating effects of energy drinks have been attributed to caffeine and its combination with carbohydrates and sugar found in most beverages (Kaminer, 2010).

The efficacy of weight loss in overweight 25- to 55-year-old humans was evaluated by administering a supplement (Metabolife 356) containing guarana and ephedra (ma huang) as the main active ingredients (Boozer et al., 2001). Subjects were instructed to take 2 tablets, 30 min before each meal, 3 times a day. Each tablet contained 12 mg total ephedrine alkaloids and 40 mg caffeine. The supplement promoted short-term weight and fat loss as well as reduction in waist circumference, hip circumference, and triglyceride levels (Boozer et al., 2001).

Andersen and Fogh (2001) investigated weight loss for 10 days and 45 days, as well as weight maintenance over a year, in patients who ingested an herbal preparation containing yerba maté, guarana, and damiana. The dosage administered was 3 capsules per day which contained 112 mg yerba maté, 95 mg guarana, and 36 mg damiana. The results showed a significant delay in gastric emptying; it reduced the time of perceived gastric fullness and led to significant weight loss over 45 days in overweight patients. The exact amount of caffeine and tannins present in this formulation was not specified in this study.

Another study compared the effect of an herbal association containing green tea and guarana on body weight control (Bérubé-Parent et al., 2005). Subjects ingested 3 capsules per day, each capsule containing a fixed dose of caffeine (200 mg), and variable doses of epigallocatechin-3-*O*-gallate (90, 200, 300, and 400 mg). The authors suggested these mixtures should be considered a good complement to a weight loss programme and have potential for the treatment of obesity.

Another association containing extracts of asparagus, green tea, black tea, guarana, yerba maté, and kidney beans has been investigated for weight reduction and changes in body composition (Opala et al., 2006). The amount of each extract in the tablets and their major compounds were not reported. The results indicated reduction of fat and increase in lean body mass. However, there were no significant differences in weight and Body Mass Index measurements (Opala et al., 2006).

The administration of tablets (similar to the work of Andersen and Fogh, 2001) containing yerba maté (112 mg), guarana (95 mg), damiana (35 mg) (Zotrim), and 11.2 mg of caffeine were evaluated for weight reduction. A significant reduction in self-reported weight, waist circumference, and hip circumference was observed, and 22% of individuals showed clinically

significant weight loss (Ruxton et al., 2007). When the formulation was tested for appetite and food intake, alone or in combination with an inulin-based soluble fermentable fiber, the results demonstrated that it produced an acute effect on caloric intake and meal duration, suggesting it strengthens within-meal satiation and is enhanced by fiber (Harrold et al., 2013).

ADVERSE EFFECTS AND TOXICOLOGICAL PROPERTIES

Many people consume herbal drugs for the treatment of minor conditions or as supplements for the purposes of weight loss or exercise performance enhancement. Some of these have multiple natural caffeine sources (e.g., green tea, guarana, kola nut, and yerba maté). The health problems caused by their use include: intoxication due to their misidentification; contamination by pathogenic microorganisms, heavy metals, or pesticides; adverse effects by undeclared components; and drug interactions both between drugs and traditional pharmaceuticals. Given the consumption of herbal drugs by patients under pharmacological treatment, physicians and health administrators should report the health risks and the contraindications of these products. It has been proven that caffeine may not be as innocuous as many consumers have come to believe. Some caffeine-containing supplements, however, appear more risky than others (Gurley et al., 2015; Sanfélix Genovés et al., 2001).

Some supplements exert an effect on a body system in an effort to maintain good health, but might have physiological side effects. Guarana has been a cause of concern here. Approximately one in four supplement users reported health-related problems. The risk associated with the intake of DS is often underestimated by consumers (Kaminer, 2010; Kao et al., 2012; Restani et al., 2016).

The use of DSs is common, data suggesting they are used more than once a week with some consumers using three or more different DSs each week. Adverse outcomes associated with DSs include cardiac arrhythmias, exertional heat illness, rhabdomyolysis, syncope, seizures, stroke, and death. To identify health-related behaviors associated with potentially harmful DSs that include guarana in their composition, especially when related to bodybuilding, performance enhancement, and weight loss, it is necessary to explore common reasons and sources of information for DS use. Supplements are individually associated with specific risk-taking behaviors that may interfere with overall readiness. Improved reporting systems for adverse events associated with DS use and improved provider and patient education are two important public health steps that could be implemented to maintain health (Kao et al., 2012).

The health authorities of several countries have reported an increasing number of adulterations in supplements. Users frequently consume poorly controlled substances with little or no medical supervision. There is often a lack of scientific evidence or knowledge of the concentrations, doses, and/or effects of the substances they take. The presence of an undeclared substance in a supplement may be due to cross-contamination related to poor manufacturing practices or to the use of the same production line for several products, but it may be intentional and aimed at increasing the efficacy of the supplement. Adulterated supplements can cause serious adverse effects in humans including strokes, acute liver injury, kidney failure, pulmonary embolisms, heart palpitations, and death. Supplements that may contain toxic substances such as illegal anabolic steroids are a major concern. Educating the public regarding the potential risks they are taking when consuming adulterated or irregular products is essential to protecting human health (Neves and Caldas, 2015; Petroczi et al., 2011).

Adverse effects have been reported as palpitations, increased serum glucose, and transient increases in systolic blood pressure as a result of using supplements containing guarana and ephedra (12 mg of ephedrine alkaloids and 40 mg of caffeine) for weight loss (Boozer et al., 2001).

The epileptic population needs a lot of care in the administration of herbal medicines. Data suggest that products containing ephedrine (ephedra or ma huang) and caffeine (cocoa, coffee, tea, yerba maté, guarana, cola, or kola) can exacerbate seizures in people with epilepsy, especially when taken in combination. A dose that may be relatively safe in the general population may also interact adversely with the symptoms and treatments of epilepsy and other pharmaceutical medications. Patients and health professionals must educate themselves on the contraindications of herbal medicines, as well as pharmaceutical medications, to avoid improper use or adverse effects (Spinella, 2001). In patients with undetected idiopathic epilepsy or cryptogenic epilepsy provoked by the consumption of these drinks, there is a possibility of a dose-dependent effect and when a high volume is consumed, especially on an empty stomach, there may be more adverse effects in the central nervous system (Iyadurai and Chung, 2007). There is no doubt that overdosing on energy drinks can cause serious neurologic and cardiac toxicity which presents as hallucinations, seizure, coma, dysrhythmias, cardiac ischemia, cardiomyopathy, and rarely, cardiac arrest. Excessive consumption of caffeine and taurine followed by strenuous physical activity can initiate cardiovascular events and induce myocardial ischemia caused by coronary vasospasm with potentially fatal effects (Sanaei-Zadeh, 2013).

A growing number of reports also show caffeine intoxication to be clinically significant. Diagnostic criteria include five or more of the following signs: restlessness, nervousness, excitement, insomnia, flushed face, diuresis, gastrointestinal

disturbance, muscle twitching, rambling flow of thought and speech, tachycardia, cardiac arrhythmia, psychomotor agitation, dehydration, insomnia, and periods of inexhaustibility. Although individual responses to caffeine vary, it can increase heart rate and blood pressure, causing palpitations that may lead to going to emergency departments. The qualitative and quantitative effects resulting from excessive and chronic consumption of these supplements, as well as from potential interactions with medications and the dynamic innovation and availability of new street drugs, are not fully known (Kaminer, 2010).

Additives and contaminants including caffeine, indomethacin, and heavy metals (such as lead, mercury, and arsenic) have been found in herbal medicines. Misidentification of plants has led to serious adverse effects including renal failure. Considering the growing popularity of these products, physicians (particularly, cardiologists) must become familiar with the cardiovascular information available on herbal drugs (Valli and Giardina, 2002).

The presence of contaminatory metals is an important adverse effect and cause of toxicological symptoms. Investigating the presence of metals in 24 DSs from Mexico, some having guarana in their composition, demonstrated the presence of Cu (<0.19–137.85 μg/g), Zn (<2.83–4785.71 μg/g), Pb (<0.003–66.32 μg/g), Cd (<0.001–2.90 μg/g), and Hg (<0.24–0.85 μg/g). The toxicological implications of metals in DSs need to be studied and regulatory limits need to be established regarding the presence of contaminatory metals in DSs in order to safeguard human health (Garcia-Rico et al., 2007).

INTERACTIONS WITH PHARMACEUTICALS

Although some herbal drugs have health benefits, many may be intrinsically dangerous when ingested alone or in combination with other supplements or drugs. Because of the lack of quality control, consumers do not know the active ingredient is actually present in the product, the appropriate dosage, or what else is in the medicine besides the advertised ingredients. Adulteration, substitution, and contamination are possible. It is imperative for physicians to ask patients about supplement use. Physicians are ideally situated to help their patients in integrating herbal with conventional treatments and to assist them in treatment decisions (Sardesai, 2002).

Many pharmacokinetic interactions involve drug-metabolizing cytochrome P450 (CYP) enzymes (Goey et al., 2013). Exposure to caffeine induces the expression of the hepatic enzyme CYP1A2, but at a higher caffeine concentration the contribution of CYP1A2 to caffeine metabolism decreases in favor of CYP2C9, a situation that could affect medicine metabolism (Clapauch and Benchimol-Barbosa, 2012).

R-Warfarin is metabolized primarily by CYP1A2, CYP3A4, and carbonyl reductases, whereas *S*-warfarin is metabolized primarily by CYP2C9. It is recommended that coffee and other caffeine-containing beverages be assessed when considering the medical history of warfarin users and that caffeine restriction be tested as a way to overcome warfarin resistance (Clapauch and Benchimol-Barbosa, 2012). Concomitant use of herbal and anticancer drugs could lead to serious safety issues in patients. Herbal drugs have the potential to cause pharmacokinetic interactions with anticancer drugs, leading to either increased or decreased plasma levels of anticancer drugs primarily involving CYP3A4 (Goey et al., 2013; Hertz et al., 2015).

Ma huang is a natural source of ephedrine and brings about potent sympathomimetic activity. Several reports link the adverse response of ma huang to concurrent use of caffeine and guarana (a source of caffeine and theophylline; Valli and Giardina, 2002).

Guarana may be potentially useful in the prevention of diseases such as thrombosis and other vascular problems, since there are studies (Bydlowski et al., 1991, 1988; Nicoletti et al., 2007) that show it has an antiaggregant action on platelets. On the other hand, when given with anticoagulants it may increase the risk of bleeding (Nicoletti et al., 2007). Considering the role of enhanced platelet aggregation and reactivity in thrombosis, inflammation, and pathogenesis of atherosclerosis and stroke, especially after hormone replacement therapy, purified guarana preparations could offer considerable benefit as a DS through reduced platelet reactivity (Subbiah, 2007).

CONCLUSIONS

The various biological and pharmacological effects of guarana have been extensively studied and their use as a supplement investigated for more than a decade. Popularly, the main objectives of the use of supplements containing guarana, alone or in association with other plants, is weight loss as well as cognitive and stimulant effects. Numerous studies show that these supplements have positive effects on weight maintenance, decreased fat, increased lean body mass, cognitive performance, and functional brain activation, besides numerous other pharmacological actions.

However, numerous adverse effects have been reported associated with their use, mainly due to the high caffeine content of this plant and the particularities of consumers, as well as a higher propensity for heart disease or hypertension. In addition, interactions of guarana with pharmaceuticals have also been reported including their synergistic effects.

The ready availability of over-the-counter supplements in natural product stores and on the internet highlights the importance of professional monitoring of the use of this type of supplement.

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Chapter 3.25

Hawthorn: *Crataegus oxyacantha*, *Crataegus monogyna* and related species

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INTRODUCTION

Crataegus species are deciduous spring flowering shrubs, belonging to the Rosaceae family (Chang and Zuo, 2002). Etymologically, *Crataegus* is a derivative of the Greek word 'kratos' meaning strength, referring either to the hardness of the woody bark (Verma et al., 2007) or to its strong medicinal virtues. The nomenclature within the *Crataegus* genus is not clear. This is attributed to the high degree of hybridisation occurring among different species. The shrub has been extensively used for hedging hence the old English name 'hawe' referring to a space confined by a hedge (Kumar et al., 2012). Hawthorn is mainly distributed in North America, Europe and the Himalayas. Hawthorn species are found in several herbal supplements and are renowned for their chemopreventive properties (Kennedy 2005; Wu et al., 2011).

TRADITIONAL USES

Hawthorn has been used since the first century A.D. The virtues of the shrub as a heart and circulatory tonic have been recognized through many centuries in folk medicine. Early reports by Dioscorides and later Paracelsus, show that hawthorn is beneficial to treat heart conditions (Weihmayr and Ernst, 1996). According to the 'Doctrine of Signatures', the Greeks believed that the red juice of the fruit is beneficial in ailments associated with the blood or the cardiovascular system (Cox, 2005). William Coles, in 1657, stated that the powdered fruit mixed with wine was a cure for dropsy (Earwood, 1999). Various heart problems have been controlled by its extract since the nineteenth century. Problems of the nervous system have been treated in France by its blossoms. In 1894, following the death of the famous Irish physician, Dr. Green, who cured several cardiac ailments, his daughter revealed that the secret medicine was a tincture of the ripe berries of *Crataegus oxyacantha* (Kashyap et al., 2012). These berries were also used in Serbia for cardiovascular conditions (Jarić et al., 2007). Since the 19th century, Crataegus species were considered as wild food plants in Slovakia (Luczaj, 2012). In Germany and Kosovo, elixirs prepared from hawthorn flowers, fruit and bark were used as antispasmodics and for their effects on the sympathetic nervous system to control conditions related to the heart and circulatory system (Mustafa et al., 2012). *Crataegus monogyna* berries were consumed by children, hunters and shepherds in Portugal and Spain (Carvalho, 2005; Tardio et al., 2006), whilst consumed untreated as a snack in Albania (Pieroni et al., 2015) being considered as healthy and nutritious. Likewise, *Crataegus azarolus* fruit were consumed in Cyprus, for their health benefits (Della et al., 2006) and in India, *Crataegus songarica* fruit were consumed to maintain a healthy heart (Kumar et al., 2009). Within the Palestine area, *Crataegus aronia* was used to treat rheumatism, diabetes, digestive disorders, urinary tract problems and stones (Ali-Shtayeh et al., 2000). In Iran, *Crataegus pontica* fruit were used to control blood pressure (Baharvand-Ahmadi et al., 2016).

PHYTOCHEMISTRY

Hawthorn species store several constituents, with medicinal and/or nutritive values. Several studies have shown that the chemical constituents of *Crataegus* species are similar, and therefore their chemical properties and pharmacological activities are closely related. The main biological activity is attributed to a number of phytochemical classes mainly flavonoids, coumarins, amines and terpenoids (Nabavi et al., 2015).

The most quoted metabolites, in hawthorns, are the flavonoids. These are either found free or as glycosides (Nikolov et al., 1973) in the flowers, fruit and leaves. Flavonoids include apigenin, hyperoside, rutin, quercetin, luteolin-7-glucoside,

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vitexin, catechin, luteolin, epicatechin, anthocyanins (Shahat et al., 2002; Maharik et al., 2009; Wu et al., 2014), amongst others. Some studies quote the content of flavonoids, other phenylpropanoids and phenolic acids as the total phenols (Kostić et al., 2012; Jurikova et al., 2012; Edwards et al., 2012; Salmanian et al., 2014), determined by the Folin-Ciocalteu test (Attard, 2013). Other phenylpropanoids include lignans and coumarins (Wu et al., 2014). Esculine, a coumarin glucoside, is also present. Major organic acids include citric, succinic, oxalic and malic acids (Gundogdu et al., 2014)

In hawthorn species, terpenoids are also abundant. Mono- and sesquiterpenoids are mainly found in essential oils while triterpenoids and steroids are found in stem, fruit and leaf extracts (Wu et al., 2014). The two most abundant triterpenoids, are ursolic and oleanolic acids (Caligiani et al., 2013) (fig. 3.25.1).

Hawthorn fruits are abundant in several minerals mainly potassium (13531.96 mg/kg), calcium (3046.37 mg/kg), magnesium (1502.55 mg/kg) and phosphorus (1477.88 mg/kg) and traces of several other minerals such as sodium, boron, chromium, iron, cobalt, magnesium, manganese and zinc, amongst others (Özcan et al., 2005). Hawthorn fruit are found in several herbal multivitamin and mineral preparations.

USES OF HAWTHORN SPECIES

Several phytochemical studies conducted on hawthorn species include biological activities in terms of antioxidant properties using the DPPH method, with median inhibitory concentrations ranging between 0.003 to 1.570 mg/ml (Sözer et al., 2006; Ebrahimzadeh and Bahramian, 2009; Simirgiotis, 2013).

The main ethnobotanical uses for which hawthorn extracts were indicated include cardiac conditions, hypertension and diabetes. Diabetic and hypertensive subjects tend to develop cardiovascular diseases (CVDs). Hypertensive patients are more exposed to CVDs than a normotensive individual (Sowers et al., 2001). Several extracts have been tested for their pharmacological effects on the heart and cardiovascular system. Extracts, containing hawthorn constituents that have been marketed include Crataegutt®, Crataemon, Cardiplant®, Phyto H Complex, Crataegisan, WS® 1442, LI 132. These preparations and other experimental extracts are water, hydroalcoholic and alcohol-based. These are constituted of typical polar plant constituents such as flavonoids, triterpenic acids and oligomeric procyanidins. The main constituents implicated in the prevention of congestive heart failure (Zick et al., 2009; Zapfe, 2001) and CVDs, including vasodilation, antiarrhythmic and hypotensive effects are the flavonoids and condensed tannins (Wang et al., 2013). However, in *in vitro* and *in silico* studies, hawthorn extracts and triterpenic acids demonstrate that oleanolic acid is an angiotensin-converting enzyme inhibitor (Attard and Attard, 2006; Farrugia et al., 2013).

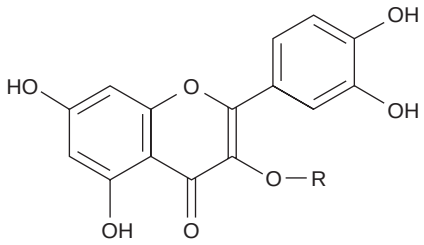
Several research groups report the effects of hawthorn fruit extracts as potential atheroscleroprotectants. *In vivo* studies with rats fed on a high-cholesterol diet, showed that hawthorn supplements suppress high total cholesterol and LDL-liproprotein and an increase in HDL-cholesterol levels (Xu et al., 2009; Kwok et al., 2010). In a rabbit model, it was suggested in part that hawthorn fruit extracts inhibited cholesterol absorption by the downregulation of intestinal AcylCoA cholesterol acyltransferase activity (Khalil et al., 2008; Zhang et al., 2002)

Though several ethnobotanical studies claim the use of hawthorn extracts for anxiety disorders, very few scientific studies were conducted to determine the rationale behind this. In a study on the hawthorn seed and pulp extracts, it was observed that both extracts caused CNS depression in a mouse animal model (Can et al., 2010)

Safety and Herb-Drug Interactions

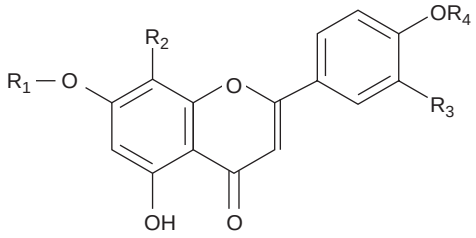
The safety of hawthorn extracts has only been assessed for proprietary products that reached the market (Schlegelmilch and Heywood, 1994). Safety is usually assessed through genotoxicity testing, using the AMES test as a first line assay and the mouse lymphoma assay, if a herbal product fails the AMES test (Attard, 2011). Registered hawthorn extracts did not show any mutagenic effects in these two assays. Though hawthorn extracts are generally safe, such products are contraindicated in consumers who are sensitive to *Crataegus* products. Due to the lack of information of the effects of hawthorn extracts in pregnancy, lactation and children under twelve years of age, these products are not recommended in these groups (Pöpping et al., 1995). Several studies showed that hawthorn extracts protect cells against damage induced by several drugs including cyclophosphamide (Hosseinimehr et al., 2008) and doxorubicin (Jalali and Hasanzadeh, 2013), and gamma radiation (Hosseinimehr et al., 2009). As a nutritional supplement, hawthorn extracts provide a good chemoprevention to several ailments. However, due to the nature of the phytochemicals present in different plant parts, special care should be considered when the consumer is managed on related cardioactive products due to the potential interaction between the two substances. A number of herbs that may potentially interact with hawthorn extracts include motherwort, squill, cola, peasant's eye herb, mate, shepherd'spurse, oleander, black hellebore and strophanthus (Robbers and Tyler, 1998), ginseng, turmeric and valerian (Ammon and Händel, 1981). Hawthorn extracts interact also with modern medicines. A particular study demonstrated

Flavonols



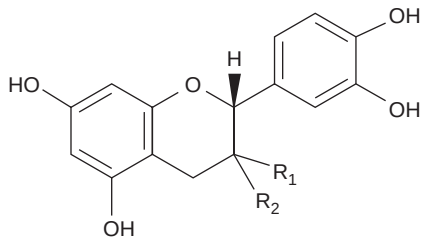
	R
Hyperoside	Gal
Quercetin	H
Quercetin 3-rhamnogalatoside	Rha-Gal
Quercetrin	Rha
Rutin	Rutinoside

Flavones



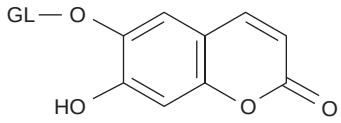
	R_1	R_2	R_3	R_4
Apigenin	H	H	H	H
Luteolin	H	H	OH	H
Luteolin-7-glucoside	Glu	H	OH	H
Luteolin-3′,7-diglucoside	Glu	H	OH	Glu
Vitexin	H	Glu	H	H
Vitexin-4′,7-di-D-glucoside	H	Glu	Glu	H
Vitexin-4′,-L-rhamno-D-glucoside	H	Rha-Glu	H	H
Vitexin-4′-rhamnoside	H	Rha	H	H

Flavan-3-ols



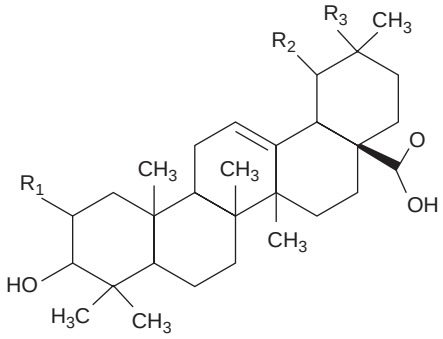
	R_1	R_2
(+)-Catechin	OH	H
(–)-Epicatechin	OH	H

Coumanrin



Esculin

Terpenic acids



	R_1	R_2	R_3
Ursolic acid	H	CH_3	H
Oleanolic acid	H	H	CH_3
Crataegolic acid	OH	H	CH_3

FIG. 3.25.1 Main constituents of *Crataegus* species.

the interaction of hawthorn with digoxin (Tankanow et al., 2003), though at the recommended dose for both, these products can be coadministered. Hawthorn may potentially interact with vasodilators, antihypertensive drugs and medicines used to control angina, heart failure and arrhythmias (Hahn et al., 1960).

CONCLUSION

Hawthorn extracts are widely used as nutritional supplements, a practice dating back since early ancient civilisations. These products should be considered more than supplements to food, as these have been tested for several biological effects, particularly cardiovascular effects, antioxidant, hypolipidaemic, anxiolytic amongst others. As a consequence, these products may interact with modern synthetics. Unfortunately, patients do not always mention the use of these supplements when advised to take a modern synthetic, and sometimes physicians do not take into account the effects of natural products when titrating a patient on a particular dose with a modern synthetic. The efficacy of hawthorn products is not yet fully understood notwithstanding the long term history of usage. It is advisable that patients and consumer consult with the physician or other health care professionals rather than self-medicate (Rigelsky and Sweet, 2002). This applies to all products containing hawthorn extracts whether marketed as food supplements or as herbal medicines.

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Chapter 3.26

Horse Chestnut

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INTRODUCTION

Aesculus hippocastanum (Hippocastanaceae), commonly known as horse chestnut or buckeye, is a large deciduous tree, showing a natural distribution in western Asia in the northern hemisphere. This species is native to the Balkans in southeast Europe, in small areas of northern Greece, Albania, Macedonia, Serbia, and Bulgaria, but it is widely cultivated in the parks, gardens, and roadsides of many European countries as well as the United States of America (Bombardelli et al., 1996; Zhang et al., 2010). A sturdy, domed tree, chestnut grows to a height of 25–30 m, its leaves comprise of 5–7 sessile leaflets, and its flowers are generally white or pink (Oszmiański et al., 2014).

Various parts of the plant (seeds, leaves, bark, and flowers) have been traditionally used in herbal medicine for centuries to treat many ailments. The brown seeds of horse chestnut are used as a cure for dysentery, bronchitis, hemorrhoids, and venous problems in folk medicine (Dudek-Makuch and Matławska, 2011; Sood et al., 2015).

BIOACTIVE COMPOSITION

The therapeutically effective compound escin has three types of alkylated triterpene glycosides (saponins): α-escin, β-escin, and crypto-escin (up to 10%). β-Escin comprises a mixture of over 30 different glycosides derived from the triterpene aglycones protoescigenin and barringtogenol C. Flavonoids (rutin, quercitrin, isoquercitrin, quercetin, kaempferol derivatives), hydroxycoumarins (esculin, fraxin, scopolin), sterols, essential oils, and starch have also been found. The toxic coumarin compound esculin found only in bark, root, and fruit bark does not exist in the seeds (Öztürk, 2011; WHO Monographs, 2002). Barringtogenol C and protoescigenin structures are presented in Fig. 3.26.1.

MEDICINAL PROPERTIES

Ever since the early 18th century there have been reports that the horse chestnut has therapeutic effects for fever and hemorrhoids (Sirtori, 2001). Leaves of the horse chestnut have been used to stop coughs. Moreover, *A. hippocastanum* has been employed to tone vessel walls and help prevent blood vessels from turning into varicose veins or hemorrhoids (Balch, 2002). The major active constituent β-escin, also known simply as escin, has demonstrated clinically significant activity in chronic venous insufficiency (CVI), hemorrhoids and postoperative edema (Sirtori, 2001). *A. hippocastanum* has been shown to have potent antiinflammatory effects due to escin. Antioxidant, antibacterial, and hypoglycemic activities of *A. hippocastanum* have also been reported in the literature (Roy et al., 2011; Matsuda et al., 1997a,b; Yoshikawa et al., 1994).

Usage in the form of compresses with elastic stockings to treat symptomatic CVI, sprain, and bruise is recorded (Öztürk, 2011). Topical gels containing 2% escin were tested in a randomized, placebo-controlled clinical trial to reduce sensitivity to pressure hematoma; it was found that escin gels significantly reduced sensitivity to pressure hematoma (WHO Monographs, 2002). In a different clinical trial, Diehm et al. (1996) mentioned that compression stocking treatment and horse chestnut therapy are effective alternative treatments for edema resulting from CVI. Decoctions of the bark are traditionally used as a remedy for skin disorders, such as sores, lupus, and ulcers (Wilkinson and Brown, 1999). Horse chestnut extracts have been reported to be included in a variety of commercial products such as shampoos, shower foams, foam baths, creams, lotions, and toothpastes, which are applied to the scalp, oral cavity, face, body, hands, legs, and feet (Wilkinson and Brown, 1999).

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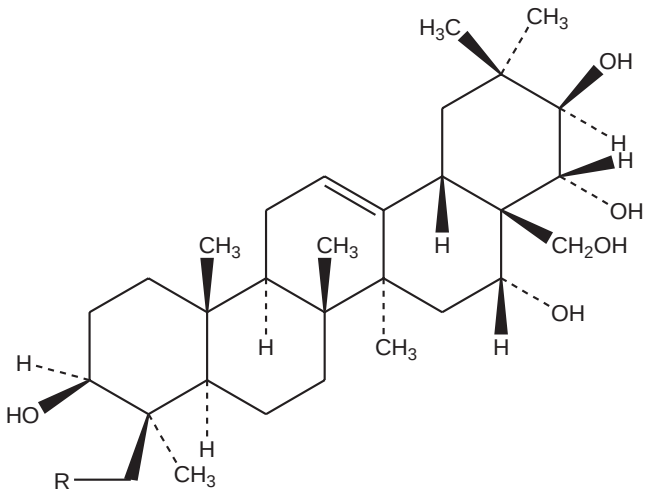

barringtogenol C R = H

protoaescigenin R = OH

FIG. 3.26.1 Structures of barringtogenol C and protoescigenin.

INDICATIONS

Although traditionally proposed for various medical conditions, CVI is the only indicator that has strong supporting scientific evidence (Ulbricht et al., 2002). Manufactured dry extract of horse chestnut seed is recommended by the German Commission E for the therapy of CVI, pain and sensation of heaviness in the legs, nocturnal systremma (leg cramps), pruritus, and swelling of the legs (Blumenthal et al., 2000). The European Scientific Cooperative on Phytotherapy (ESCOP) also offers horse chestnut for CVI, as well as for varicosis (ESCOP Monographs, 2003). It is registered for hemorrhoid treatment, varicosis, phlebitis characterized with pain, heaviness in the legs, swelling, itching, and night cramps. It has also been reported as being used as antiinflammatory agents to treat similar effects (WHO Monographs, 2002). Its usage in the treatment of coronary heart disease in traditional Chinese medicine has also been reported (WHO Monographs, 2002). It finds use in the treatment of bacillary dysentery and fever and is also used as a haemostat in extreme menstrual or other gynecological bleeding, but these treatments are not supported by experimental or clinical data (WHO Monographs, 2002).

DRUG AND OTHER INTERACTIONS

Because of the coumarin component of horse chestnut, it may interact with warfarin, salicylates, nonsteroidal antiinflammatory drugs, and other drugs with anticoagulant properties (PDR for Herbal Medicines, 2000). Horse chestnut may also interact with herbs or supplements such as garlic, ginger, *Ginkgo biloba*, red clover, turmeric, white willow, and others, which might affect platelet aggregation. However, literature reviews did not reveal any documented bleeding in clinically relevant cases (Grossberg and Fox, 2007; Ulbricht et al., 2002).

Two suspected cases of poisonous nephropathy have been reported, presumably due to very high escin doses. Therefore, *A. hippocastanum* semen should not be used with nephrotoxic drugs, such as gentamicin (WHO Monographs, 2002). Gentamicin and β-escin combined therapy led to a case of acute renal insufficiency (Aronson, 2009; Voigt and Junger, 1978).

Sodium escinate, the saponin content of horse chestnut seed extract, was approved by the Chinese Food and Drug Administration (CFDA) for postoperative edema treatment because of its significant antiinflammatory, antiedematous, and vasoprotective properties. In a case of acute renal failure in a patient who was treated with combination therapy utilizing sodium escinate and *G. biloba* extract injections, an interaction between sodium escinate and *G. biloba* was found. The mechanism underlying the interaction with sodium aescinate is not clear, but is believed to be associated with downregulation of CYP2C9 and CYP3A4 by the presence of amentoflavone in *G. biloba* extract. It has been reported that the protein-binding capacities of both drugs also contribute to their interaction (Ji et al., 2017).

A. hippocastanum semen extract has been reported to inhibit serum glucose levels in glucose-loaded rats (Sood et al., 2015; Yoshikawa et al., 1994). Taking horse chestnut with hypoglycemic drugs such as chlorpropamide, insulin, or metformin may increase the risk of hypoglycemia. Blood glucose–lowering effects and risk of hypoglycemia may increase when horse chestnut is used with herbs and supplements that lower glucose levels such as α-lipoic acid, chromium, devil's claw, *Panax ginseng*, and psyllium. However, literature reviews did not reveal any documented evidence in humans (Grossberg and Fox, 2007; Ulbricht et al., 2002).

The major saponin component escin binds to plasma protein and influences the binding of other drugs (Newall, 1996). The saponin-rich extract of *A. hippocastanum* is probably of little use for coeliac disease, fat malabsorption, and vitamins A, D, E, and K deficiency (Mills and Bone, 2005). There is no information about food that may interact with horse chestnut.

SAFETY

Symptoms such as diarrhea, vision and consciousness disorders, enlargement of the pupils, flushing of the face, severe thirst, and emesis have been recorded after consuming extreme amounts of horse chestnut seed (in one case a child ate five seeds) (PDR for Herbal Medicines, 2000).

The seeds are reportedly poisonous and must be specially prepared before medicinal use. Only properly processed products by responsible manufacturers should be used. Ingesting untreated seed has caused the death of children. The U.S. Food and Drug Administration (FDA) included horse chestnut on its former Unsafe Herbs list (Duke et al., 2002; Shenefelt, 2011).

Induced hepatic injury by Venoplant, an extract of *A. hippocastanum*, was first reported in 1986. A 37-year-old man treated with 65 mg Venoplant, who underwent a liver function test 17 days later, showed slight abnormality and 60 days after the injection complained of pruritus and jaundice (Takegoshi et al., 1986). Anaphylactic shock after intravenous injections of horse chestnut has been reported, along with renal toxicity or failure (Khan and Abourashed, 2010). Seeds of horse chestnut were tested for toxicity in 2-week-old Leghorn chicks and adult female Syrian hamsters; depression, muscular incoordination, paralysis, coma, and death were reported as toxic signs. The LD50 of horse chestnut seed extract (single dose) was found to be 10.6 mg/g of body weight for the chicks and 10.7 mg/g of body weight for the hamsters (Williams and Olsen, 1984).

SAFETY DURING PREGNANCY

The safety of horse chestnut during pregnancy and lactation has yet to be satisfactorily established. However, no side effects were reported in a clinical study involving pregnant women (WHO Monographs, 2002).

ADVERSE EFFECTS

Among the adverse effects reported of using horse chestnut are gastrointestinal complaints, dizziness, nausea, headache, and pruritus. Herbs rich in saponins may also cause reflux (Myers, 2002). One case report gave an account of a 15-year-old woman being hospitalized with symptoms including vomiting, dyspnea, burning in the nose and throat, and syncope, as a result of taking intranasally the snuff of horse chestnut seeds (Zając et al., 2014).

The consumption of three boxes of unpurified, traditionally acquired horse chestnut paste over a 6-week period brought about dyspnea in a 32-year-old male patient. Chest X-ray examination showed an enlarged cardiac shadow and bilateral pleural effusion which resulted in pericarditis as a result of using horse chestnut. The researchers suggested that development of the pericardial effusion could be due to the strong antiaggregant property of horse chestnut (Edem et al., 2016).

Bronchial asthma developed in a 57-year-old man employed in the pharmaceutical industry working with products such as *Plantago ovata* and escin. He complained of dry cough, dyspnea, sibilant wheezing, oppressive chest discomfort, and rhinitis. An escin-induced asthma diagnosis was established after several tests, but the mechanism by which escin can produce asthma is unknown. The results of the analyses suggest a non-IgE immunologic mechanism (Munoz et al., 2006).

A metaanalysis related to the efficacy of horse chestnut entailed a total of 10,725 patients and 3 observational studies. No serious adverse events were reported and, based on the metaanalyses, horse chestnut seed extract was found to be an effective and safe treatment for CVI (Siebert et al., 2002).

The incidence of pseudolupus was recorded in seven patients with varicose veins. Studies have shown that all the patients used the drug Venocuran, although it is not certain whether one or more components (horse chestnut seed extract, phenopyrazone, or cardiac glycoside-containing plant extracts) of this drug play an important role in the pathogenesis of this immunologically significant disease (Schuff-Werner and Berg, 1980).

Local application of Feparil gel (which contains escin) brought about generalized urticaria and dyspnea in a 51-year-old man. Prick tests were performed with the constituents of Feparil gel, but only escin and escin polysulfate were found positive. Although this case was diagnosed as contact urticaria from escin, the mechanism is uncertain (probably immunologic) (Escribano et al., 1997).

The gel or ointment form of *A. hippocastanum* should not be applied directly to broken or ulcerated skin (Braun and Cohen, 2007).

Renal failure has been reported in children receiving 0.5 mg/kg (at recommended dosages) of escin for 4 days. Escin may cause hemolysis after injection and released hemoglobin may accumulate in the kidneys and cause renal failure. These effects are not expected with the oral use of horse chestnut (McGuffin and Gardner, 2013; Mills and Bone, 2005).

POSOLOGY

Daily dosage: 250.0–312.5 mg twice daily of a standardized powdered extract of the crude drug (equivalent to 100 mg escin) containing 16%–20% triterpene glycosides, calculated as escin; topical gels containing 2% escin (WHO Monographs, 2002).

CONCLUSIONS

A. hippocastanum has been traditionally used in herbal medicine for centuries to treat many ailments. The major active compound escin is mainly connected with the activity of horse chestnut seed. Many *in vitro*, *in vivo*, and clinical trials have proven the antiedematous, antiinflammatory, vasculoprotective, and venotonic activity of escin. Despite caution expressed about interactions between preparations containing horse chestnut and other medicinal agents, herbs, and supplements, there is lack of evidence. Horse chestnut is associated with relatively few side effects and is generally considered to be safe when properly processed products produced by responsible manufacturers are given at recommended dosages.

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Chapter 3.27

Ispaghula (*Plantago ovata* Forssk.)

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INTRODUCTION

Ispaghula is a viscous water-soluble dietary fiber obtained from the seed husk of *Plantago ovata* Forssk. (Plantaginaceae). It is one of the most commonly used over-the-counter preparations commercialized worldwide by several companies using different brands and formulations, such as Metamucil, Fybogel, Isabgol, Ispagel, etc. Millions of people use ispaghula to regulate bowel habits, clean the intestine, and take as functional food and dietary supplemental products (Gilani et al., 1998). Ispaghula is also commonly known as *psyllium*, although some authors consider this term confusing since almost all the commercially available supplements are derived from *P. ovata* Forssk. and not from *Plantago psyllium* L. (Wärnberg et al., 2009; Fernandez et al., 2012). *P. ovata* is an herbaceous annual plant indigenous to Asia and the Mediterranean region of Europe and North Africa. It is widely cultivated in many countries with India being the leading producer (Talukder et al., 2016).

Ispaghula comprises 10%–30% of the seeds and is mainly composed of polysaccharides with high gel-forming properties. However, it also contains other active principals, such as 4-*O*-methylglucuronic acid, aucubin, campesterol, linoleic acid, oleic acid, palmitic acid, L-cystine, L-asparagine, mucilage, rhamnose, sterol, β-sitosterol, and tannins (Duke, 1992). According to the structural and chemical elucidation studies of Fischer et al. (2004) the gel-forming polysaccharides consist of a neutral arabinoxylan containing 22.6% arabinose, 74.6% xylose (molar basis), and some trace sugars. Many investigations have demonstrated the potential of ispaghula polysaccharides for hydrogel applications because of its easy availability, hydrophilicity, biocompatibility, biodegradability, biorenewability, and low toxicity (Singh, 2007; Thakur and Thakur, 2014). Scientific evidence of the efficacy of ispaghula for the treatment of several human diseases, such as constipation, diarrhea, inflammatory bowel diseases (IBDs), diabetes, hypercholesterolemia, has been demonstrated by many studies in recent years (Hussain et al., 2016). This chapter focuses on the main benefits of ispaghula for human health, its potential adverse effects and drug interactions, and the multifunctional applications of ispaghula-based hydrogels.

HEALTH BENEFITS

Among the numerous health benefits attributed to ispaghula (Table 3.27.1) its efficacy is particularly evident in the treatment of constipation, diarrhea, irritable bowel syndrome (IBS), IBDs, diabetes, and hypercholesterolemia. This was the reason these were selected for more detailed discussion in this chapter. However, the anticancer, antiseptic, antiamebic, and antinociceptive properties of ispaghula have also been reported (Hussain et al., 2016). A clinical trial showed that treatment with ispaghula after open hemorrhoidectomy reduces pain and the tenesmus rate. It also reduces the length of postoperative hospital stay (Kecmanovic et al., 2006). It might also be helpful for long-term weight management since it increases satiety. A recent randomized clinical trial by Brum et al. (2016) proved that its consumption before meals reduces hunger and the desire to eat between meals.

Constipation and Diarrhea

Ispaghula has been shown to have the paradoxical effect of both improving constipation and ameliorating chronic diarrhea (McRorie et al., 1998; Quitadamo et al., 2012; Rachlis et al., 2005; Washington et al., 1998). Stool softening is a step in the treatment of chronic constipation. Due to the absorption of water, ispaghula increases stool weight making its passage easier (Marlett et al., 2000). A study by McRorie et al. (1998) compared the stool-softening (stool water content) and laxative efficacy of ispaghula with docusate sodium in patients with chronic idiopathic constipation. Ispaghula was more

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TABLE 3.27.1 Most Important Uses of Ispaghula for Human Health

Disease	Important references
Constipation	McRorie et al. (1998), Mehmood et al. (2011), Quitadamo et al. (2012)
Diarrhea	Mehmood et al. (2011), Rachlis et al. (2005), Washington et al. (1998)
Irritable bowel syndrome	Bijkerk et al. (2004), Ford et al. (2008), Moayyedi et al. (2014), Vejdani et al. (2006)
Ulcerative colitis	Fernandez-Banares et al. (1999), Hallert et al. (1991)
Diabetes	Cicero et al. (2010), Clark et al. (2006), Sierra et al. (2002), Ziai et al. (2005)
Hypercholesterolemia	Anderson et al. (2000), Ribas et al. (2015), Uehleke et al. (2008), Van Rosendaal et al. (2004)
Obesity	Brum et al. (2016)
Hypertension	Cicero et al. (2007), Cicero et al. (2010)

effective than docusate sodium at increasing stool water content and consequently stool softening. In a randomized study the efficacy of a mixture of acacia fiber, ispaghula, and fructose was compared with polyethylene glycol (PEG) plus electrolytes in chronic functional constipation of children. PEG, ispaghula, and acacia fibers were similarly effective, although PEG was better accepted by children (Quitadamo et al., 2012). Pucciani et al. (2011) proved the usefulness of ispaghula in rehabilitation of obstructed defecation.

When investigating the effect of ispaghula in the treatment of lactulose-induced diarrhea, Washington et al. (1998) observed that ispaghula delays gastric emptying and reduces acceleration of colon transit, possibly by increasing meal viscosity and delaying the production of gaseous fermentation products, respectively. Protease inhibitors (PIs) are recognized as a common cause of diarrhea, and the treatment of PI-induced diarrhea is largely nonspecific. Ispaghula, loperamide, calcium carbonate, SP-303, and pancrelipase showed some efficacy for treatment of PI-induced diarrhea (Rachlis et al., 2005). Some explanations for the beneficial antidiarrheal properties of ispaghula are the gaseous products produced as a result of its delayed fermentation, which cause deceleration in colon transit, and its gel-forming capacity and soft nature, which are important in regulating the bowel (Hussain et al., 2016). Mehmood et al. (2011) studied the pharmacological basis for the use of ispaghula in gastrointestinal disorders, demonstrating that ispaghula has a gut-stimulatory effect (partially mediated by muscarinic and activation of 5-hydroxytryptamine receptor 4) and gut-inhibitory activity (possibly mediated by blockade of Ca^{2+} channels and activation of nitric oxide–cyclic guanosine monophosphate pathways).

Irritable Bowel Syndrome and Inflammatory Bowel Diseases

There is randomized controlled trial evidence that the use of fiber, particularly ispaghula-based products, reduces IBS symptoms (Bijkerk et al., 2004; Ford et al., 2008; Moayyedi et al., 2014). According to the results of a pilot study, Carmint (a mixture of extracts from different plants) plus ispaghula might be effective in relieving symptoms in patients with IBS (Vejdani et al., 2006). Additionally, some clinical trials demonstrated that ispaghula alleviates the more frequent symptoms associated with inactive ulcerative colitis, one of the two major forms of IBD (Fernandez-Banares et al., 1999; Hallert et al., 1991). Consumption of ispaghula increased fecal levels of n-butyrate as a result of colonic fermentation. It has been found to be as effective as mesalamine, an antiinflammatory drug usually used as frontline therapy in ulcerative colitis, at maintaining disease remission (Fernandez-Banares et al., 1999).

According to Bijkerk et al. (2004) the usefulness of ispaghula in the treatment of IBS lies mainly in its beneficial effects on constipation. The beneficial effects on IBD are probably related to anticonstipation activity and increased levels of short-chain fatty acids (Bijkerk et al., 2004). The anaerobic fermentation of ispaghula in the intestines results in the production of several fatty acids (Pylkas et al., 2005) with antiinflammatory and antioxidant properties.

Diabetes

Diabetes is an increasingly common condition that affects several million people worldwide. The reduction of blood glucose and cholesterol as well as weight reduction and maintenance are some of the beneficial effects of high-fiber diets, particularly those including soluble fiber, in diabetic patients. Several studies in humans showed reductions in blood sugar

levels after ispaghula consumption and proved its therapeutic effects in type 2 diabetic patients (Cicero et al., 2010; Clark et al., 2006; Sierra et al., 2002; Ziai et al., 2005). For example, Ziai et al. (2005) showed that 5.1 g of ispaghula per person with type II diabetes before breakfast and dinner is well tolerated and effective in improving glycemic control. Moreover, its consumption did not affect mineral or vitamin A and E concentrations (Sierra et al., 2002).

Hypercholesterolemia

High levels of low-density lipoprotein (LDL) cholesterol are associated with increased risk of cardiovascular disease. Dietary fibers are considered to be highly beneficial in lowering the concentration of LDL cholesterol in plasma, and ispaghula appears to be one of the most efficient and safe. A meta-analysis conducted by Anderson et al. (2000) showed that consumption of 10.2 g ispaghula/day reduced total cholesterol by 4% and LDL cholesterol by 7% relative to a placebo group. In a prospective observational study with 62 patients, Uehleke et al. (2008) observed that ispaghula supplements are effective in reducing cholesterol levels. More recently, Ribas et al. (2015) showed that ispaghula significantly reduces LDL cholesterol levels in dyslipidemic children and adolescents, and that it is generally well accepted and safe. Ispaghula reduces cholesterol as a result of the binding of bile acids in the intestinal lumen (Van Rosendaal et al., 2004). Although many studies prove the hypolipidemic activity of ispaghula, results must be carefully analyzed as the effects are time, dose, and gender dependent (Vega-López et al., 2001; Wei et al., 2009).

ADVERSE EFFECTS AND DRUG INTERACTIONS

Although natural products are generally considered nontoxic for humans, in many cases information on their real effects is nonexistent. The use of food products containing ispaghula was authorized by the US Food and Drug Administration (Anderson et al., 2000; FDA, 1998), and many studies showed that it is generally well tolerated and accepted (Oliver, 2000; Quitadamo et al., 2012; Ribas et al., 2015; Ziai et al., 2005). Nevertheless, some patients report some gastrointestinal effects including increased gas production, bloating, diarrhea, and constipation (Erdogan et al., 2016; Pittler et al., 2005), and other do not like its texture. Moreover, to avoid intestinal obstruction, it is important to take it with an adequate amount of water to fully wet the powder (Schiller, 2001). If consumed before meals, it can delay gastric emptying and reduce appetite (Brum et al., 2016). Some reports also indicate that ispaghula could be a potential allergen causing skin sensitivity problems, asthma, or anaphylaxis in nurses or patients handling the powder (Bernedo et al., 2008; Lantner et al., 1990).

The consumption of many natural supplements occurs outside medical control. It is possible that in some cases their consumption coincides with the administration of drugs, modifying their availability, kinetics, and pharmacological effects. Ispaghula is one of the most common forms of self-medication worldwide and sometimes is consumed in parallel with medications without medical advice (Fernandez et al., 2012). The few studies available analyzed the interactions of ispaghula with some drugs acting in the nervous, cardiovascular, or gastrointestinal systems such as hypoglycemic, hypocholesterolemic, or antiinflammatory drugs, and hormone therapy medications. A complete review of the few known drug interactions was published by Fernandez et al. (2012). Ispaghula can cause both desirable and undesirable effects when consumed simultaneously with some drugs (Table 3.27.2). For example, it can reduce the levels of lithium, digoxin, and levothyroxine in the blood, resulting in reduced bioavailability of these drugs. On the other hand, the administration of some other drugs concomitantly with ispaghula protects patients from their side effects. This is the case with orlistat, a pancreatic lipase inhibitor used to control obesity, which can cause troubling side effects such as oily spotting and fecal incontinence. Ispaghula has a protective effect against these events probably because it absorbs free fat and decreases flatulence (Camacho et al., 2011; Cavalieri et al., 2001).

DRUG DELIVERY APPLICATIONS

Nowadays, biorenewable polymer-based materials are being used to replace traditional synthetic materials (metals and ceramics) in a number of applications, particularly in the biomedical area. These materials are advantageous because they are biodegradable, ecofriendly, cheaper, and easily available. Furthermore, the interest in hydrogel-based natural polysaccharides has increased in recent years. Ispaghula is one of the most promising sources of natural polysaccharides because, among other advantages, it is easily available from an abundant biorenewable resource at very low cost (Singh, 2007; Thakur and Thakur, 2014). In recent years, several studies have reported on the synthesis of ispaghula-based hydrogels and their use in many applications such as drug delivery, toxic metal ion removal, and flocculants (Thakur and Thakur, 2014). The efficacy of ispaghula hydrogels for sustained delivery of different drug molecules (namely, anticancer, antiviral, antiinflammatory, antimicrobial, and antihypertensive drugs) has been reported (Table 3.27.3). Important studies

TABLE 3.27.2 Interaction of Ispaghula and Some Human Drugs

Drug	Application/disease	Result of the interaction	Important references
Glybenclamide	Diabetes	Contributes to the hypoglycemic effect	Ziai et al. (2005)
Levothyroxine	Hypothyroidism	Reduces bioavailability	Chiu and Shermab (1998), Liel et al. (1996)
Lithium	Psychiatric drug	Decreases blood level or absorption	Perlman (1990), Toutoungi et al. (1990)
Mebeverine	Irritable bowel syndrome	No interaction	Chapman et al. (1990)
Orlistat	Obesity	Reduces side effects	Camacho et al. (2011), Cavalieri et al. (2001)
Simvastatin	Hypercholesterolemia	Improves cholesterol reduction	Moreyra et al. (2005)

TABLE 3.27.3 Some Examples of the Use of Ispaghula-Based Hydrogels in Drug Delivery Studies

Drug	Activity	Important references
Acyclovir	Antiviral	Garg and Gupta (2009), Svirskis et al. (2014)
Aspirin	Antiinflammatory	Rosu and Bratu (2014)
Methotrexate	Anticancer	Singh and Bala (2014)
Metronidazole	Antimicrobial	Asnaashari et al. (2011)
Varsartan	Antihypertensive	Pawar and Varkhade (2014)

were carried out by Singh et al. (2008a,b), which analyzed the drug release mechanism from polymeric networks and the swelling kinetics of ispaghula-based hydrogels. There are some comprehensive reviews focusing on the drug delivery studies of ispaghula-based hydrogels that should be consulted for more detailed information on this subject (Hussain et al., 2016; Singh, 2007; Thakur and Thakur, 2014).

CONCLUDING REMARKS

Ispaghula has been used since ancient times to enhance functionality of the intestine. It is commercialized worldwide by several companies in diverse formulations and different brands. Due to its wide use in folk medicine the benefits of ispaghula for the treatment of several gastrointestinal and nongastrointestinal diseases have been clinically and scientifically evaluated in recent years. A review of the literature shows that ispaghula is particularly valuable for the treatment of constipation, diarrhea, IBS, and ulcerative colitis, and for regulating glucose and lipid levels. In addition, because of its low cost, hydrophilicity, biocompatibility, biodegradability, biorenewability, and low toxicity, it is one of the most promising materials for drug delivery, heavy metal ion scavenger, or waste water remediation applications. More clinical studies are needed to confirm some properties attributed to this supplement, to better understand its mode of action and guarantee its safety, and to assess the risks of the concomitant consumption of this supplement with other medications. Moreover, attention should be paid to the validation and standardization of available formulations.

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Chapter 3.28

Konjac (*Amorphophallus konjac*)

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INTRODUCTION

Amorphophallus konjac K. Koch is one of the most widely used species, belonging to the Araceae family. *Amorphophallus rivieri* Durand ex Carriere or konjac are its synonyms. It is commonly called "ju ruo" in Chinese, "konnyaku" (yam cake) in Japanese, and "gonyak" in Korean. It is also known as elephant-foot yam, devil's tongue, and snake palm (Hetterscheid and Ittenbach, 1996). *A. konjac* or simply konjac is an herbaceous perennial plant which has an underground stem in the form of a corm, a globose tuber having a diameter of 30 cm that produces rhizomatous offsets of 50 cm long and 3 cm thick. Its leaves are umbrella-shaped with dull pink type petioles of 1 m in length that are marbled dark green and dotted white, with highly dissected blades of 2 m, divided into many leaflets that are 3–10 cm long and 2–6 cm in diameter. Its peduncle is 1.1 m long, colored in the same ways as its petioles. The flowers are produced on spathe, with wavy margins, which is enclosed by a maroon spadix that is narrowly conic, having a length of 0.15–1.1 m. It emits a fetid odor for many days that easily attract flies (Brown, 2000; Chua et al., 2010; Gille et al., 2011). The World Checklist of Selected Plant Families suggests the following synonyms for konjac:

- *Amorphophallus mairei* H.Lév.
- *Amorphophallus nanus* H.Li & C.L.Long
- *Amorphophallus rivierei* Durand ex Carrière
- *Brachyspatha konjac* (K.Koch) K.Koch
- *Conophallus konjak* Schott
- *Hydrosme rivierei* (Durand ex Carrière) Engl.
- *Proteinophallus rivierei* (Durand ex Carrière) Hook.f.
- *Tapeinophallus rivierei* (Durand ex Carrière) Baill.
- *Amorphophallus palmiformis* Durieu ex Rivière
- *Conophallus konniaku* Schott ex Fesca

HABITAT AND TRADITIONAL MEDICINAL HISTORY OF GLUCOMANNAN

A. konjac was first reported in Southeast Asia (Hetterscheid and Ittenbach, 1996). The plant is found native to warm tropical and subtropical eastern Asian countries, from Japan extending into South China and Indonesia (Chua et al., 2010; Li et al., 2005). It is commonly distributed in tropical regions in Asia and Africa. In China, more than 26 different species can be found (Niu et al., 2005) which have extensive uses in traditional Chinese medicine (TCM) (Hetterscheid and Ittenbach, 1996). Besides its long use in China, currently in the West konjac is used as a food source and by nutraceutical industries. Commercially, konjac can be divided into two types, that is, starch and glucomannan. The glucomannan type is mainly produced in Asia. China and Japan are the top two konjac-cultivating countries in the world. Wild konjac is also cultivated and harvested in Southeast Asia on a small scale (Zhou et al., 2004a).

Initially in China (206 BC-08 AD) konjac was studied and utilized as a therapeutic plant in *Shen Nong Materia Media*. The Ben Cao Gang Mu, an old Chinese pharmacopoeia, declared konjac as toxic, pungent, and a cool natured herb (Xu et al., 2001). Due to such phytochemicals, TCM approved it for detoxification, as a tumor suppressant, for alleviating blood stasis, and for its phlegm liquefaction properties (Niwa et al., 2000; Xu et al., 2001). Besides this, konjac flour is also used as a food source being used to produce noodles, tofu, and snacks. Konjac flour is prepared from the corm of konjac. The underground stem of konjac (its corm) is first washed, and then peeled, sliced, dried, and finally ground

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to form konjac flour. Konjac flour is also used in the form of a cake/gel after boiling it with plant ash. It is used for the treatment of various disorders like asthma, hernias, burns, coughs, breast pain, and blood and skin disorders. Konjac flour is also used as a food source being used to produce noodles, tofu, and snacks (Brown, 2000; Douglas et al., 2004; Long, 1998; Wootton et al., 1993). In southeast China, flour derived from the leaves and corms of konjac, is also used as an insect repellent and as animal fodder (Long, 1998). Furthermore, konjac is also grown as an ornamental plant outside of Asia, due to its beautiful appearance and marbled petioles (Follett et al., 2002). During the 6th century AD, konjac was introduced as a medicinal food in Japan by the King of North Korea, and from this point, new processing technologies for the commercial production of kanjoc were developed (Liu, 2004).

PHYTOCHEMISTRY OF GLUCOMANNAN

Konjac glucomannan (KGM) is the plants main active constituent. *Amorphophallus* is the only plant species which is rich in glucomannan concentration. It is, therefore, cultivated in large planting areas (Liu, 2004). More than 60% of the dry weight of glucomannan is found in the bulbs of various species of *Amorphophallus* (Chen, 2008; Li et al., 2005). Besides this, *Amorphophallus* also consists of unsaturated fatty acids, starches, proteins, amino acids, and alkaloids. Therefore, it can be preferably used as a food for patients with diabetes, obesity, high blood pressure, colon cancer, and other digestive diseases (Long-quan, 2008).

KGM is a soluble fiber extracted from the corm that is commonly used as a food additive. Besides this, it is also used in the development of dietary supplements and nutraceuticals. Recently konjac stems have been used for dietary fiber, that is, the exogenous plant material in the diet which is resistant to digestive enzymes. Dietary fiber may be classified as soluble or insoluble (McCleary, 2003). KGM is considered as one of the most viscous and water soluble dietary fibers (Sheng and Teng, 2008). It is a high molecular weight polysaccharide (Fang and Wu, 2004), mainly composed of β-1,4-linked D-mannosyl and D-glucosyl residues at a molar ratio of 1.6:1 (Katsuraya et al., 2003; Maeda et al., 1980). Due to its β-1,4-linkages, KGM is resistant to salivary or pancreatic amylase enzymes, therefore, it directly passes in an unchanged form into the colon and is then fermented by colonic bacteria (Keithley and Swanson, 2005). The backbone of glucomannan possesses between 5% and 10% acetyl-substituted residues (Williams et al., 2000).

THERAPEUTIC USES

KGM also exhibits a distinctive chemical structure and composition, giving it several physiological actions (Alonso-Sande et al., 2009). KGM is very effective at enhancing the immune function, helping with weight loss, and reduction in blood cholesterol (Chua et al., 2010; Sim et al., 2011). Besides this, KGM also modifies intestinal microbial metabolism and serves as a valuable "functional food additive" or prebiotic carbohydrate (Chen et al., 2005; Venter et al., 1990). KGM is mostly used as a useful food component, that is, a stabilizer and thickener, in many fields, such as the food industry, biopharmaceutical industry, chemical industry, and fields associated with human health as well as agriculture among others (Gläser et al., 2004; Tye, 1991). Since 1994, KGM has been authorized as a food additive and dietary supplement in England and has been classified as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (Phillips and Williams, 2009; Zhang et al., 2005). In 1996, the US Department of Agriculture (USDA) passed KGM to be used as a binder in meat and poultry products (Jiménez-Colmenero et al., 2012). In Europe, agreement number E425 was allotted to KGM by the European Food Safety Authority (FSA, 2007). Moreover, KGM has also shown its worth in pharmaceutical dosage preparations such as controlled drug delivery systems (Alvarez-Manceñido et al., 2008; Alonso-Sande et al., 2009) and in the production of various absorbent materials like sanitary towels and disposable nappies (Kök et al., 2009). KGM is also used as a pharmaceutics excipient for oral, colon-targeting, drug-delivery systems (Zhang and Yang, 2014).

Chitosan, that is, deacetylated chitin, is water insoluble and brittle in its dry state and therefore has very limited biological and medical applications. For this purpose a research group modified chitosan by blending it with KGM. Blended films were prepared by blending an excess aqueous solution of KGM with chitosan (in a ratio 7:3) in acetate solution. Transparent films were obtained after drying at 40°C on glass plates for 4 h. The study found that an increased concentration of KGM decreased the crystallinity of blended films. Whereas, the mechanical properties and thermal stability of the blended film improved because of the strong interaction of hydrogen bonding between the amino group (chitosan) and OH group (KGM). Thus, as a result the potential exists to coat pills with soluble antiseptic (Xiao et al., 2000). Besides this, palmitoylated KGM (PKGM) has the ability to act both as a surfactant and as a polymer. PKGM has the ability to form both stable types of emulsion, that is, w/o and o/w. PKGM acts as an excellent w/o emulsifier, by producing strong interactions with the interface of oil droplets, while it acts as a good o/w emulsifier because of its strong hydrophilic characteristics, by creating strong interactions with the interfaces of water droplets (Tian et al., 1998).

KGM interacts synergistically when combined with starch, thus, it enhances its viscosity. Additionally, KGM also inhibits starch granule association, thus, it is suggested that KGM may slow down rice starch gel retrogradation (Charoenrein et al., 2011). Different studies reported that the addition of sodium carbonate (Na_2CO_3) to KGM–starch mixtures showed normal phase separation. Because Na_2CO_3 prevents both the mixtures from concentrating into two phases it was found that Na_2CO_3 encouraged strong interchain association between KGM and starch gel, thus, modifying and controlling the pasting and rheological properties of starch (Zhoua et al., 2014).

In Chinese traditional medicine, KGM is available in capsule form, for the treatment of coughs, asthma, hernias, hematological disorders, burns, breast pain, and skin disorders (Chua et al., 2010). Recently, KGM was marketed in the form of capsules to be used as a drink mixture as well as in food products (Talbott, 2003). It has been reported that KGM has the ability to treat obesity (Kraemer et al., 2007), obesity-related dyslipidemia (Gallaher et al., 2000; Vasques et al., 2008), and diabetes (Vuksan et al., 1999, 2000, 2001). Besides this, it has also been suggested that KGM has the ability to be used as a prebiotic (Chen et al., 2005; Chen, 2008) and as an immunomodulator (Onishi et al., 2007a,b).

LIMITATIONS OF GLUCOMANNAN

The corm of the diploid species *A. konjac* (Chauhan and Brandham, 1985) possesses a high concentration of glucomannan and has acquired wide adaptability. These plants are susceptible to serious diseases, mainly soft rot disease (Zhou et al., 2004b). These plants have poor disease resistance and a low coefficient of propagation (Cun et al., 2009). In contrast, the wild *Amorphophallus bulbifer* has a high coefficient of propagation and has high disease resistance. Besides this, it contains relatively high concentrations of good quality glucomannan (Zhang et al., 2009).

As compared to other commercially available gums, it formulates an extremely high-viscosity solution with a distinct shear-thinning property (Vuksan et al., 2001; Wang et al., 2012). The apparent viscosity of 1% (w/w) KGM is about 30,000 cps (Tatirat and Charoenrein, 2011). Due to their high viscosity and low mobility, KGM solutions are inconvenient for people to consume as a food matrix (Pan et al., 2012). Many countries banned KGM for confectionary use, because of its choking effect in the throat—it was found to cause excess swelling (EFSA, 2005). Various techniques have been used in order to reduce its viscosity (Alonso-Sande et al., 2009; Zhang et al., 2005). Among these techniques, alkali plays a vital role in elucidating the gelation behavior of KGM by increasing salvation, along with facilitating the deacetylation of its chain (Williams et al., 2000). Alkaline concentration strongly influences the gelation rate of KGM and thus determines the elastic modulus of the gel (Huang et al., 2002). Furthermore, utilization of glucomannan dietary supplements is also associated with various other side effects, like abdominal pain, esophageal obstruction, flatulence, lowering gastrointestinal (GI) obstruction, and the possible alteration of the bioavailability of other drugs (González et al., 2003).

CLINICAL STUDIES ON GLUCOMANNAN

A randomized clinical trial was conducted for the antiobesity activity of KGM. A total of 53 participants were assigned to take 1.33 g of glucomannan with 236.6 ml of water at least 1 h before each meal for a period of 8 weeks. The study supported the idea that glucomannan, at a dose of 3.99 g/day, is well tolerated and did not promote weight loss (Keithley et al., 2013). In a double-blind, crossover, and placebo-controlled study, the effects of glucomannan at a dose of 3.9 g/day for 4 weeks, was investigated in 63 healthy candidates. The study's findings revealed that glucomannan fibers reduced total cholesterol (TC) concentration by 10%, with no undesired effects, and thus considered glucomannan an effective cholesterol-lowering dietary supplement (Arvill and Bodin, 1995). Several randomized controlled trials in children with plasma hypercholesterolemia revealed that the group treated with glucomannan significantly reduced TC and low-density lipoprotein (LDL)-cholesterol compared to a control group. Thus, glucomannan may represent a potential supplement to assist dietary therapy (Martino et al., 2005; Sood et al., 2008; Vido et al., 1992). Similarly, a randomized trial was conducted on 200 obese patients, in order to check the effects of two doses of 3 g of *Plantigo ovate* husk and 1 g of glucomannanon (two and three times) on body weight and metabolic variables. Significant reduction in weight was observed along with an increase in postprandial satiety for both doses of fiber compared to a placebo. Both types of fiber also showed a significant reduction in the concentration of plasma LDL-cholesterol (Salas-Salvadó et al., 2008).

A successful single-blind study was carried out in 48 newly diagnosed hyperthyroid patients and concluded that combination therapy of glucomannan (with methimazole and propranolol) had the ability to significantly reduce the serum level of all four thyroid hormones (T3, T4, FT3, and FT4) compared to a placebo group (Azezli et al., 2007).

A randomized, controlled clinical trial study was conducted in 11 hyperlipidemic and hypertensive type 2 diabetic patients. It proved that glucomannan improved glycemia along with other related risk factors for coronary heart disease (CHD) like

hypertension and blood lipid profile in high risk, type 2 diabetic patients (Vuksan et al., 1999). Similarly in another controlled metabolic study, 278 subjects, with insulin resistance syndrome, were treated with glucomannan and wheat bran fiber. The results supported that a high-viscosity diet (rich in glucomannan) significantly controlled glycemia and the lipid profile compared to wheat bran fiber (Vuksan et al., 2000). The use of glucomannan dietary supplements before loading glucose in type 2 diabetes patients significantly improved the decline in prandial ghrelin. Glucomannan delayed the increase in fasting ghrelin, thus, attenuating ghrelin-induced feeding (Chearskul et al., 2009).

A double-blind, randomized, crossover study was conducted on children complaining of chronic constipation with or without encopresis. The children received either glucomannan fiber or a placebo at a dose of 100 mg/kg body weight, taken with 500 ml of fluid for 4 weeks. The results revealed that use of glucomannan as a fiber supplement was beneficial in the treatment of functional constipation. Besides this, no potentially serious side effects were noted (Loening-Baucke et al., 2004). Several other studies, that is, placebo-controlled and diet-controlled trials, proved that glucomannan acts as a natural laxative, and taken in a modest dose was found to improve the colonic ecology of constipated adults, thus, enhancing weekly defecation frequency of patients by possibly increasing stool bulk, thus, encouraging colonic fermentation and growth of lactic acid bacteria (Chen et al., 2006; Chen et al., 2008).

INTERACTION OF GLUCOMANNAN WITH OTHER SUPPLEMENTS/DRUGS/FOODS

Glucomannan can alter overall therapeutic response. For instance, it has a hypoglycemic effect, and when given in combination with oral hypoglycemic agents, can lead to hypogylcemia. Similarly, it decreases the bioavailability of some oral medications. In order to avoid such interactions, it is recommended to take other medications at least 1 h before, or 4 h after, administration of glucomannan (Keithley and Swanson, 2005).

CONCLUSIONS

In short, konjac has diverse therapeutic and nutritious effects. The available scientific data strongly recommend its safe and effective use as a nonvitamin, nonmineral food supplement. In this regard, further strategy is required for its formulation on a commercial scale.

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Chapter 3.29

Mangosteen (*Garcinia mangostana* L.)

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MANGOSTEEN FRUIT AND ITS SOURCES

Mangosteen's scientific name is *Garcinia mangostana* L. (GML). Nearly 400 species of the genus *Garcinia*, which comprises evergreen trees and shrubs from the Clusiaceae (Guttiferae) family, have been found. The fruit is naturally found in South East Asia and Indonesia and is endemic to the Malay Peninsula, Myanmar, Thailand, Cambodia, Vietnam, and the Moluccas. Mangosteen has been cultivated in tropical regions (e.g., India, Honduras, Brazil, and Australia) for the last two centuries. The species thrives in warm, humid, or tropical climates and has a narrow range of adaptability (Obolskiy et al., 2009). To date, Thailand is the main producer of mangosteen in the world, producing approximately 240,000 trees annually (Maliar et al., 2011). Mangosteen is a slow-growing plant which ranges from 6 to 25 m in height and produces flowers that are red or green and roughly 4–5 cm in size. The exocarp of the fruit is dark purple or reddish (Fig. 3.29.1) and is recognized as a rich source of red pigment. It also contains a soft and juicy edible white pulp enclosed typically within 6–8 arils. The flavor is slightly acidic yet sweet with a delightful smell (Cen et al., 2013; Parthasarathy and Nandakishore, 2014).

BIOACTIVE COMPOSITES OF THE MANGOSTEEN FRUIT

Mangosteen contains secondary metabolites such as prenylated compounds and polyphenols. It has recently been noted that mangosteen contains a plentiful source of a class of polyphenols known as xanthones (Wang et al., 2012). The three-ring arrangement—which holds diverse functional groups comprising isoprene, methoxy, phenyl groups, aromatic protons, phenolic hydroxyl groups, hydroxyl protons, and dihydrofuran rings—is the main chemical structure of a xanthone (Shan et al., 2011; Watanapokasin et al., 2010). More than 60 types of xanthones have been isolated from its roots, while its pericarp and bark contain α-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, and 9-hydroxycalabaxanthone. The important compounds in mangosteen are phenolic. In fact, 10 phenolic acids have been found in mangosteen fruit and the major one is protocatechuic acid, which is found in mangosteen peel. Xanthonoids and other phytochemicals are also found in mangosteen peel (Chaivisuthangkura et al., 2009; Zadernowski et al., 2009). Significant quantities of additional bioactive compounds, such as terpenes, tannins, calcium, phosphorus, iron, thiamine, riboflavin, niacin, and ascorbic acid are also found (Patil et al., 2014). The more bioactive components and their content are found in Tables 3.29.1 and 3.29.2.

VARIOUS BIOLOGICAL EFFECTS OF MANGOSTEEN

The rind, leaves, fruit, and bark of mangosteen have been used in folk medicine for thousands of years (Hiranrangsee et al., 2016; Reverentia and Sargowo, 2014). The rind of the fruit, which contains resin, is used to stop diarrhea, dysentery, and wound infection in South East Asia. The bark and young leaves are effective against ailments of the genitourinary tract and are used in the formulation of astringent medicines for use in dysentery and enteritis (Ibrahim et al., 2016; Wang et al., 2012). More interestingly, some reports have recently indicated that mangosteen extract can help reduce the build-up of fat that leads to steatohepatitis (Hafeez et al., 2014; Tsai et al., 2016). There are a number of therapeutic benefits associated with mangosteen: cardioprotective, antiinflammatory, anticarcinogenic, antioxidant, antiallergic, antibacterial, antifungal, antiviral (Alsultan et al., 2016; Johnson et al., 2012), cytotoxic, antidepression, anti-Alzheimer, antiglaucoma, and antideterioration (the latter applies to oxidizable products such as cosmetics; Gutierrez-Orozco and Failla, 2013; Pal et al., 2013). Sakagami et al. (2005) found that α-mangostin was very effective against five strains of vancomycin-resistant

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FIG. 3.29.1 The various parts of mangosteen fruit including leaves, exocarp, and white pulp.

TABLE 3.29.1 Total Phenolic and Anthocyanin Content and EC_{50} of Each Part of Mangosteen

Mangosteen parts	Total phenolic content (mg/GAE/100 g)	Anthocyanin content (mg Cyn-3 Glu/100 g)	EC_{50} (μg/mL)
Outer pericarp	2930.49 ± 318.10	179.49 ± 10.80	4.73 ± 0.55
Inner pericarp	3404.09 ± 321.92	19.71 ± 22.98	1.35 ± 0.13
Flesh	133.29 ± 20.44	Not detected	133.33 ± 25.17

EC_{50}, The concentration of an agonist that produces 50% of the maximal possible effect of that agonist.
Chaovanalikit, A., Mingmuang, A., Kitbunluewit, T., Choldumrongkool, N., Sondee, J., Chupratum, S., 2012. Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products. Int. Food Res. J. 19, 1047–1053.

TABLE 3.29.2 The Nutritional Composition of *Garcinia mangostana*

Nutrient	Content/100 g
Water	80.94 g
Energy	73 kcal
Carbohydrates	17.91 g
Protein	0.41 g
Total fat	0.58 g
Cholesterol	0 mg
Dietary fiber	1.8 g
Vitamins	
Folates	31 μg
Niacin	0.286 mg
Pantothenic acid	0.032 mg
Pyridoxine	0.041 mg
Riboflavin	0.054 mg
Thiamin	0.054 mg

TABLE 3.29.2 The Nutritional Composition of *Garcinia mangostana*—cont'd

Nutrient	Content/100 g
Vitamin A	35 IU
Vitamin C	2.9 mg
Minerals	
Sodium	7 mg
Potassium	48 mg
Calcium	12 mg
Copper	0.069 mg
Iron	0.30 mg
Magnesium	13 mg
Manganese	0.10 mg
Phosphorus	9.21 mg
Zinc	0.21 mg

Note: Values for canned mangosteen (syrup pack).

Enterococci (VRE) and nine strains of methicillin-resistant *Staphylococcus aureus* (MRSA) with minimal inhibitory concentration (MIC) values of 6.25 gm/mL and from 6.25 to 12.5 gm/mL, respectively. This result established α-mangostin as important in reducing VRE and MRSA infections (Sakagami et al., 2005). In another study it was found that both the antimicrobial and antitumor activity of mangosteen ethanol extract highlighted the possibility of its therapeutic use in infectious diseases and in cancers such as melanoma. Mangosteen ethanol extract obtained in the resin, leaf, and fruit showed antimicrobial activity against *S. aureus* and *Escherichia coli* with an MIC of 0.1 mg/mL. Moreover, mangosteen leaf extract induced genotoxicity and apoptosis in B16 to F10 cells (Cunha et al., 2014).

ANTIOXIDANT ACTION OF BIOACTIVE COMPOSITES OF MANGOSTEEN

Sánchez-Pérez et al. (2010) demonstrated α-mangostin's protective role in cisplatin (Platinol) which brought about programmed cell death by inhibiting p53 (tumor suppressor gene) expression and free radical generation. Buelna-Chontal et al. (2011) pointed out α-mangostin's effectiveness against cardiac reperfusion damage by reducing the infarcted area. In addition, it inhibited cardiac adenosine triphosphate (ATP) and phosphocreatine levels decreasing in the reperfused myocardium. Mangosteen peel extract could be supplied as food additives due to its antioxidant properties, which increase the shelf life of food by means of avoiding lipid peroxidation. It could also be used as a protective oxidative burst in living systems (Suttirak and Manurakchinakorn, 2014).

ANALGESIC EFFECT AND POTENTIAL ACTION OF MANGOSTEEN

The basic ethanol extract of mangosteen peel, α-mangostin, and γ-mangostin has been found to possess potent peripheral and central antinociceptive effects in mice, signifying that xanthones from mangosteen peel could be developed as new analgesic drugs (Sukma et al., 2011). Moreover, γ-mangostin displayed analgesic effects in hot-plate and formalin tests, which may involve the histamine (H1), bradykinin, and serotonin (5-HT2) receptors (Cui et al., 2010). Mangosteen xanthones, α-mangostin, and γ-mangostin repressed allergic mediators in bone marrow-derived mast cells induced by phorbol 12-myristate 13-acetate (PMA) plus A23187 by inhibiting synthesis of interleukin (IL)-6, prostaglandin D2 (PGD2) with leukotriene C4 (LTC4), degranulation of histamine, and cyclooxygenase-2 (COX-2) expression (Chae et al., 2012).

ANTIINFLAMMATORY ACTION OF MANGOSTEEN

Mangosteen extracts can decrease the levels of nitric oxide (NO) in RAW 264.7 cells exposed to lipopolysaccharides (LPS) (Tewtrakul et al., 2009). Mangosteen ethanol extract inhibits inflammation in atherosclerotic rats by restricting the scattering

of nuclear factor-κB (NF-κB) p65/p50 into the nucleus; it also decreases tumor necrosis factor-α (TNF-α) and NO levels (Adiputro et al., 2013). α-Mangostin also decreased activation of several signaling pathways including interleukin-1 (IL-1), mitogen-activated protein kinase (MEK), c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription 1 (STAT-1), and activator protein 1 (AP-1) in human U937 macrophage-like cells (Liu et al., 2012). Gutierrez-Orozco et al. (2013) investigated the inhibitory effects of α-mangostin on the secretion of proinflammatory mediators by transformed and primary human cells and proved that α-mangostin inhibited the secretion of proinflammatory mediators such as IL-8 or TNF-α in human cell lines from various tissue origins.

ANTICANCER ACTION OF MANGOSTEEN COMPOUNDS

The distinctive physical and chemical properties of xanthones show promise for dietary chemoprevention due to their putative health benefits and the protection they clearly provide (Shan et al., 2011). It has long been advocated that many xanthones from this fruit including α-mangostin possess anticancer properties that could be used in prostate, breast, lung, colorectal, and cutaneous cancer by initiating and regulating cell death pathways, suppressing cancer cell propagation and spread, and arresting the cell cycle (Arcangeli et al., 2012; Li et al., 2013). α-Mangostin in the human breast cancer cell line MDA–MB231 induced programmed cell death through the mitochondrial pathway and significantly elevated the ssDNA, caspase 3, 8, and 9 levels (Kurose et al., 2012). α-Mangostin has a significant effect against head and neck squamous cell carcinoma (HNSCC) cell lines (Kaomongkolgit et al., 2011). α-Mangostin reduces cancer growth and spread to the lymph nodes via an immune-competent xenograft model of metastatic mammary cancer carrying a p53 mutation (Shibata et al., 2011). Mangosteen extract containing α-mangostin and γ-mangostin completely suppresses the growth of HCT 116 colorectal carcinoma in vitro and in vivo by caspase-mediated apoptosis (Buelna-Chontal et al., 2011). The antiapoptotic properties of xanthones in α-mangostin have been examined and documented as antimelanoma agents via caspase stimulation, which involves increasing 25-fold the activity of caspase-3 and caspase-9 and disrupting the mitochondrial cell cycle membrane when matched to untreated cells (Wang et al., 2011).

ANTIBACTERIAL ACTION OF BIOACTIVE COMPOSITES OF MANGOSTEEN

Mangosteen extracts present inhibitory effects against two Gram-positive bacteria (*S. aureus* ATCC11632 and *Bacillus cereus* ATCC10876) and one Gram-negative bacterium (*E. coli* ATCC10536) in a dose-dependent way. When the amount of extract increases from 0 to 80 μL a comparable increase in inhibition zone diameter is detected. The inhibitory activity of seed extract (80 μL) is the strongest (twice as strong against *S. aureus* ATCC11632), followed by pericarp and pulp extracts (Lim et al., 2013). The lowermost MIC of xanthones against five *Leptospira* serovars has been estimated at 100μg/mL, which is essentially greater than traditional antibiotics for the treatment of leptospirosis (Seesom et al., 2013). Mangosteen pericarp is more effective in lowering the MIC and maximum bacterial concentration (MBC) values of *Lactobacillus acidophilus* (MIC 25 mg/mL, MBC 50 mg/mL) and oral *Streptococcus* (MIC 50 mg/mL, MBC 100 mg/mL) than other tested organisms, namely *Streptococcus mutans*, *Streptococcus salivarius*, and *Streptococcus sanguis* (MIC 100 mg/mL, MBC 200 mg/mL), showing that mangosteen pericarp acts as an actual antibacterial agent at low concentrations (Janardhan et al., 2017). All the derivatives of α-mangostin showed maximum antibacterial activity (up to 12 mm) at 100 μg/mL concentration against *E. coli*, *Bacillus subtilis*, *S. aureus*, and *Pseudomonas aeruginosa* bacterial strains that are associated with other synthesized compounds (Narasimhan et al., 2017).

ANTIPARASITIC AND ANTHELMINTIC PROPERTY EFFECTS OF BIOACTIVE COMPOSITES OF MANGOSTEEN

α-Mangostin has a larvicidal influence on botanic third-instar mosquito larvae (Larson et al., 2010). Amazing toxicity effects were demonstrated by *G. mangostana* against the third and fourth larval stage of *Aedes aegypti* (Torres et al., 2015). α-Mangostin shows promise against trematodes including *Schistosoma mansoni*, *Echinostoma caproni*, and *Fasciola hepatica* in vitro (IC_{50} of 2.9–15.6 mg/mL) (Keiser et al., 2012).

ANTIFUNGAL ACTION OF BIOACTIVE COMPOSITES OF MANGOSTEEN

α-Mangostin shows promise as a good antifungal candidate against *Colletotrichum gloeosporioides* (EYL131) and *Neosartorya spathulata* (EYR042) (Arunrattiyakorn et al., 2011). α-Mangostin had the most significant effect against fungal strains, inhibiting *Candida albicans* by 12 mm and *Aspergillus niger* by 13 mm (Narasimhan et al., 2017). A dichloromethane

extract from mangosteen showed a pronounced inhibitory effect (IC_{50}) against *Plasmodium falciparum* (IC_{50} of 2.7 μg/mL) and *Trypanosoma brucei* (IC_{50} of 0.5 μg/mL), while ethyl acetate extract showed no antiprotozoal activity at all (Al-Massarani et al., 2013).

ANTIOBESITY ACTION OF α-MANGOSTIN

α-Mangostin demonstrated its capacity to treat or prevent obesity by suppressing the build-up of intracellular lipids in differentiating adipocytes and stimulating lipolysis in mature adipocytes (Quan et al., 2012). Udani et al. (2009) conducted an 8-week randomized, double-blind, placebo-controlled trial with 40 subjects to evaluate the potential antiinflammatory effect of a commercialized mangosteen juice blend (XanGo, 18 oz/day). Interestingly, they found that XanGo significantly reduced C-reactive protein (CRP) levels in humans compared with those taking a placebo (Udani et al., 2009).

MANGOSTEEN INTERACTION WITH DRUGS

Based on laboratory studies, mangosteen may possibly increase the risk of bleeding when administrated with acetyl salicylic acid (Aspirin), anticoagulants such as warfarin (Coumadin) or heparin, antiplatelet drugs such as clopidogrel (Plavix), and nonsteroidal antiinflammatory drugs such as ibuprofen (Motrin, Advil) or naproxen (Naprosyn, Aleve).

Mangosteen may have an antihistamine effect; therefore, caution is advised when taking antihistamine medication. Due to its antioxidant effects, mangosteen may cooperate with chemotherapeutic agents (i.e., anthracyclines, platinum compounds, and alkylating agents) whose mechanism of action involves oxidation. Moreover, mangosteen may possibly inhibit phosphodiesterase; therefore, close attention should be given to patients taking phosphodiesterase inhibitors (www.naturalstandard.com).

INTERACTIONS WITH HERBS AND DIETARY SUPPLEMENTS

Mangosteen may possibly increase the risk of bleeding when taken with herbs and supplements that are supposed to increase the risk of bleeding. There have been numerous cases of bleeding with the use of *Ginkgo biloba*, and some with the use of garlic and saw palmetto (Standard, 2016).

CONCLUSIONS

Mangosteen is a most valuable fruit as a result of its high antioxidant content and other beneficial nutrient components. This makes it a good natural agent in the fight against all manner of diseases including cancer. However, more human and experimental research is needed to prove its therapeutic effects.

LIST OF ABBREVIATIONS

ERK Extracellular signal-regulated kinase
IL- Interleukin-1
JNK c-Jun N-terminal kinase
MEK Mitogen-activated protein kinase
LPS Lipopolysaccharide
NF-Kb Nuclear factor-κB
NIK NF-κB-inducing kinase
TNF-α Tumor necrosis factor-α
P53 Tumor-suppressor gene
NO Nitric oxide

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FURTHER READING

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Chapter 3.30

Milk Thistle (*Silybum marianum*)

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INTRODUCTION

Silymarin is a flavonoid obtained from the medicinal plant *Silybum marianum* (L.) Gaertn., belonging to the family Asteraceae/Compositae. The plant is otherwise known as milk thistle, since the leaves of the plant have "milky veins," and sometimes as *Cardus marianus*, Marian thistle, and Mary thistle. Silymarin has mainly been used for the treatment of liver and gall bladder–related disorders like jaundice, hepatitis, and cirrhosis for over 2000 years (Gazak et al., 2007). Apart from its liver-protecting properties, it is also used to treat various types of disorders such as cancer, inflammation, and neurodegeneration; its wide pharmacological activity is mainly attributed to the excellent antioxidant property of silymarin (Maryana et al., 2016). Ever since ancient times, *S. marianum*, which yields silymarin, has been cultivated for its wide pharmacological properties, which can be seen in ancient Egyptian literature and in the representation of this plant in the Bible under its older Latin name *Lebanon cardus* (Federico et al., 2017). Pliny the Elder (AD 23–79) reported that the plant had the ability to remove bile. Even Nicolas Culpepper (a herbalist; 1616–54) and Albrecht von Haller (a Swiss physician; 1708–77) mentioned the efficacy of the plant in preventing liver disorders (Mayer et al., 2005). After advances in research and development in recent years, such historic evidence was confirmed through the documentation of scientific reports on the pharmacological properties of silymarin. A brief report on *S. marianum* with special emphasis on silymarin is outlined in this chapter.

COMPOSITION

Silybin is the main component of silymarin (constituting more than 50%, sometimes as much as 70%). Other flavonolignans such as silychristin, silydianin, isosilybin, and unidentified organic polymers are also present (Fig. 3.30.1). The compounds present in minor quantities include flavonols such as quercetin, taxifolin, and kaemferol. However, the composition of silymarin varies greatly between the variety of *S. marianum* and the conditions under which it is grown. Hence, the composition variation of silymarin among suppliers greatly influences its biological activity (Zholobenko and Modriansky, 2014). Since the standardized extract generally contains only 80% silymarin, the plant tissue culture technique is considered the better alternative to enhance the content of silymarin. Culture conditions can be manipulated with different growth regulators such as chitosan, methyl jasmonate and salicylic acid, which enhance the activity of chalcone synthase, the key enzyme responsible for the synthesis of silymarin (Gabr et al., 2016).

BIOAVAILABILITY AND SAFETY

The bioavailablity of silymarin is very low, since only 20–50% of silymarin administered orally is absorbed by the gastrointestinal tract. The factors responsible for its low availability include its low solubility, rapid metabolism and excretion through urine and bile, and reduced penetration through the epithelial cells of the intestine (Maryana et al., 2016). Toxicity studies have demonstrated that silymarin is well tolerated and has very low general toxicity. The maximum tolerated dose and oral 50% lethal dose are 300 mg/kg in dogs and 10,000 mg/kg in rats (Fraschini et al., 2002). However, when administered at 50, 100, and 200 mg/kg per day to pregnant female BALB/c mice, it affected organogenesis and caused embryotoxicity including intrauterine growth retardation, abnormalities in the craniofacial region and vertebral column and fetal resorption, which cautions the administration of silymarin to pregnant women (Gholami et al., 2016).

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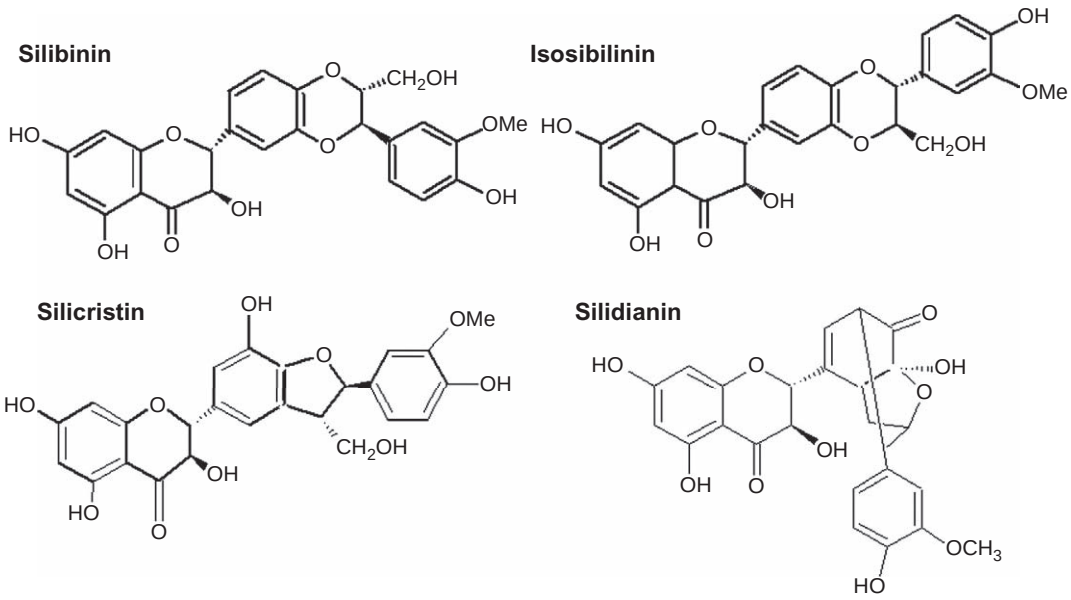

FIG. 3.30.1 Chemical structure of silibinin, isosilibinin, silidianin, and silicristin. *(Adapted from Kiruthiga, P.V., Pandian, S.K., Devi, K.P., 2010. Silymarin protects PBMC against B(a)P induced toxicity by replenishing redox status and modulating glutathione metabolizing enzymes—an in vitro study. Toxicol. Appl. Pharmacol. 247(2), 116–128.)*

PHARMACOLOGICAL PROPERTIES OF SILYMARIN

Silymarin exhibits varied pharmacological properties and protects all the major organs against toxicity brought on by different factors, mainly due to its antioxidant properties. Many reviews emphasizing the pharmacological significance of silymarin have been written. Silymarin has been used for the treatment of chronic liver-related disorders for many years. Silymarin treatment significantly reduces nonalcoholic fatty liver disease (NAFLD), which is a clinical condition resulting from excess fat deposition in the liver—not from alcohol. Steatosis results when deposition of fat in the liver exceeds more than 5–10%. Silymarin when administered as a food supplement along with L-cysteine, L-methionine, L-glutathione, and vitamin E reduced the biochemical changes induced by NAFLD. Ultrasonographical changes also revealed that silymarin supplementation reduced the accumulation of fat in the liver (Cacciapuoti et al., 2013). Silymarin protects the liver mainly by triggering antioxidant defense in the liver. For example, silymarin enhances the level of the endogenous antioxidant glutathione by increasing the availability of the amino acid cysteine, which is important for the synthesis of glutathione (Kwon et al., 2013). Since the liver plays a vital role in the detoxification of foreign compounds, it is one of the most viable targets for toxicity brought on by these compounds. Silymarin has been found to be beneficial in preventing toxicity induced by chemicals such as acetaminophen, carbon tetrachloride and thioacetamide. Oxidative stress triggered by these chemicals activates proteins like c-Jun N-terminal kinase (JNK), extracellular signal–regulated kinase (ERK), mitogen-activated protein kinases (like p38 MAP kinase) and leads to apoptosis of liver cells. Treatment of mice with silymarin at different doses (50, 100, 150, 200 and 250 mg/kg body weight) prevented apoptosis triggered by thioacetamide by triggering the PI3K-Akt cell survival pathway (Ghosh et al., 2016). Pretreatment of albino rats with silymarin at 200 mg/kg triggered antioxidant defense in liver cells and protected against lead-induced hepatotoxicity (Jalali et al., 2016). Even in combination with other drugs like L-methionine, silymarin (at 25 mg/kg) was able to prevent acetaminophen overdose from bringing about damage to the liver, kidneys, and cerebral cortex (Onaolapo et al., 2017).

Silymarin has a protective effect against liver damage caused by viruses like hepatitis C virus (HCV) infection. It suppresses the virulence of HCV by inhibiting the activity of viral NS5B RNA-dependent RNA polymerase and nuclear factor-κB (NF-κB) expression, thus neutralizing the inflammatory cytokines produced during viral infection (Ahmed-Belkacem et al., 2010). Cholestatic liver disease is one of the liver disorders that occur when the formation of bile or bile flow to the small intestine is impaired. If the disease is untreated, obstructive jaundice will lead to cirrhosis followed by liver failure. Administration of 300 mg/kg per day of silymarin to experimental obstructive jaundice model rats revealed that silymarin prevented obstructive jaundice–induced changes in the liver of the animals mainly by reducing oxidative stress (Onalan et al., 2016).

Research carried out in many in vitro and in vivo model systems has supported the fact that silymarin interferes with the cell cycle, induces apoptosis, and exhibits antiinflammatory and antimetastatic effects in a variety of cancer cells (Ramasamy and Agarwal, 2008). Silymarin is considered as a better alternative for the treatment of multidrug-resistant cancer cells, such resistance is an escaping mechanism developed by the cancer cells against many chemotherapeutic drugs. Treatment of the chemoresistant breast cancer cell lines MCF-7 and MDA-MB-435 with 200 μM silbinin inhibited the growth of these cancer cells and augmented the sensitivity of the cells to the standard chemotherapeutic drugs doxorubicin and paclitaxel by inhibiting expression of the oncogenic proteins STAT3 (signal transducer and activator of transcription 3) and Bcl2 (Molavi et al., 2017). Silymarin administration to mice bearing Lewis lung cancer cells exhibited activity against lung cancer by reducing the number of myeloid-derived suppressor cells (MDSCs), the accumulation of which results in loss of T cells and tumor development (Wu et al., 2016).

Chronic obstructive pulmonary disease is a progressive lung disease caused by different factors including cigarette smoke, which can inflame the airway. Treating the bronchial epithelial cell line (Beas-2B) with silymarin (10, 20, 30, or 40 μM) suppressed the ERK/p38 MAP kinase pathway and autophagy in cells and prevented cigarette smoke–induced airway inflammation, which suggests that silymarin can be effectively used for treatment of inflammatory pulmonary diseases (Li et al., 2016). Oxidative stress has been implicated in different disorders including diabetes mellitus, which is one of the endocrine disorders that occur due to insufficient levels of insulin synthesized by pancreatic cells. Administration of silymarin at 50 mg/kg orally to diabetic experimental rats prevented diabetes-induced initiation of oxidative stress, elevated insulin levels, and subsequently reduced glucose levels, by restoring the number of pancreatic cells (Amniattalab et al., 2016). In type 2 diabetes, excessive reactive oxygen species (ROS) are generated within islet β-cells, which affects the secretion of insulin by these cells. Although silymarin is reported to reduce glucose levels in diabetic patients the effect of silymarin on islet β-cells is not known, hence the studies were carried out on the β-cell line HIT-T15. It was observed that silymarin treatment enhanced the exocytosis process in islet β-cells and caused an increase in glucose-stimulated insulin secretion, mediated through its ability to reduce oxidative stress (Meng et al., 2016).

The glomerulus, which is a meshwork of capillaries, plays a vital role in filtration of urine in the kidneys. During inflammatory reactions, glomerular mesanglial cells secrete proinflammatory mediators and cause injury to cells. In vitro experiments carried out with mouse mesanglial cells showed that silymarin treatment at 50 U/mL inhibited cytokine mixture–mediated induction of iNOS gene expression by inhibing the ERK1/2 kinase pathway, which is primarily involved in inflammatory response (Youn et al., 2017). Oxidative stress has also been implicated in cardiac diseases, one of the commonest causes of death worldwide. The antioxidant silymarin has been observed to offer a protective role against cardiac problems induced by metals, anticancer drugs, and environmental pollutants, which induce oxidative stress (Razavi and Karimi, 2016). Though platelet activation is considered as a protective mechanism for preventing blood loss, uncontrolled platelet activation leads to disturbance of the cardiovascular system. Hence, to maintain proper hemostatic stability, there is a search for effective antiplatelet agents. Evaluation of the antiplatelet activity of flavonolignans (silybin, silychristin, and silydianin) against adenosine diphosphate–induced blood platelets revealed that these flavonolignans bind with the P2Y12 receptor and inhibit platelet aggregation by decreasing the expression of P-selection and by activation of integrin $\alpha_{IIb}\beta_3$ (Bijak et al., 2017).

The antioxidant properties of silymarin support its pharmacological action against a variety of oxidative stress disorders including neurological disorders such as Alzheimer's and Parkinson's disease (Devi et al., 2016). The flavonoid silibinin, which is isolated from silymarin, exhibited therapeutic activity against Alzheimer's disease (AD) by inducing an inhibitory mechanism on cholinesterase enzymes and on aggregation of amyloid beta (Aβ) peptide (Duan et al., 2015). The antiaging effect of silymarin (at 25 and 50 μM) in the *Caenorhabditis elegans* model system revealed that silymarin increases the lifespan of animals by 10.1% and 24.8%, respectively. It was also able to reduce the progression of AD in the transgenic CL4176 *C. elegans* model for AD by reducing the expression of Aβ protein 1–42 in muscles (Kumar et al., 2015). Silymarin not only prevents the neurotoxicity caused by different disease pathologies, it also prevents the neurotoxicity induced by many chemicals like acrylamide. Treating PC12 cells with different concentrations of silymarin (12, 24, 48, 96, or 192 μg/mL) revealed that silymarin protects the cells by triggering Nrf2 signaling and Nrf2 expression (Li et al., 2017). Silymarin also prevents depression, which is a common neuropsychiatric disorder affecting the normal well-being of a person. When experimental mice were injected with 100 and 200 mg/kg of silymarin extract, it induced an antidepressant effect that mediated modulation of the corticosterone response (Thakare et al., 2016).

Silymarin and its active constituent silibinin are considered a better alternative to antibiotics, since they exert an antimicrobial effect against a variety of microbes such as *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Candida albicans*. Silymarin and silibinin have been observed to act as a better antimicrobial agent when used in combination with antibacterial drugs. The results suggest that silymarin could be used as an adjuvant in antibiotic therapy for treating multidrug-resistant bacteria, so that bacterial resistance problems

can be overcome (de Oliveira et al., 2015). The antifungal activity of silymarin against *C. albicans* indicated that silymarin damages the membrane of the fungi by altering its permeability, inducing membrane lipid peroxidation, favoring potassium release, and promoting membrane depolarization (Yun and Lee, 2017).

CONCLUSIONS

Many scientific reports have proved silymarin to be a promising pharmacological agent that could be used to treat various disorders such as jaundice, hepatitis, and cirrhosis. However, since only 20–50% of silymarin is absorbed due to low water solubility and poor bioavailability the effectiveness of silymarin is extremely limited. Hence, further studies are required to overcome this problem by developing encapsulation techniques to enhance the bioavailability of silymarin.

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Chapter 3.31

Orthosiphon stamineus (Java Tea)

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HISTORICAL NOTE

Orthosiphon stamineus (vernacular name: barbiflore, Java tea, misai kucing, kabling-gubat, kumis kucing, cat's whiskers, kidney tea, rau meo, remujung, balbas pusa, moustaches de chat, and yaa nuat maeo; synonym: *Clerodendranthus spicatus*, *Ocimum aristatum*, *Orthosiphon aristatus*, *Orthosiphon grandiflorus*, *Orthosiphon spicatus*, *Orthosiphonis folium*) has long been used in traditional medicine in East India, Indo China, South East Asia, and tropical regions of Australia where the plant is usually found. *O. stamineus* belongs to the Lamiaceae family and is a perennial herb. The stem is four-angled reaching a height ranging from 0.3 to 1 m, and the flowers are white or pale lilac. The flower has stamens (>2 cm) extending from the corolla tube. The leaves are about 2–4 cm wide and 4–7 cm long and have a lanceolate-like, elliptical, or rhomboid shape. The aerial parts (dried stem and leaves) are commonly brewed as a tea for a variety of purposes, from treating inflammatory disorders to ailments of the urogenital tract. This plant is commonly used in South East Asian folk medicine for diabetes, hypertension, gallstone, tonsillitis, epilepsy, rheumatoid diseases, menstrual disorder, gonorrhoea, syphilis, renal calculus, lithiasis, edema, eruptive fever, influenza, hepatitis, and jaundice. However, scientific studies on the medicinal benefits of *O. stamineus* do not confirm all its traditional uses. Nevertheless, *O. stamineus* is well known for its potent diuretic effect, which is stronger than other natural diuretics (Burkill, 1966).

CHEMICAL COMPONENTS

Chemical compounds extracted from *O. stamineus* leaves vary greatly with the type of solvent used and the auxiliary energy the process is subjected to. Major compounds often found from *O. stamineus* extracts are rosmarinic acid, eupatorin, and sinensetin. In fact, these compounds are often used to standardize products derived from this plant. Extraction using water often produced a lower yield of flavonoids (i.e., sinensetin, eupatorin, eupatorin-5-methyl ether) due to the lower solubility of flavonoids in water (Pang et al., 2017). Extraction using pure organic solvents such as ethanol and isopropanol yielded the highest concentration of flavonoids (methoxylated compounds), but a very low concentration of rosmarinic acid (hydroxylated compounds). The presence of excessive energy during the extraction process may induce thermal degradation of polyphenolic compounds (Pang et al., 2014) and may produce danshenshu (Nuengchamnong et al., 2011) and caffeic acid. Extracts from *O. stamineus* were found to contain essential oil fragments, diterpenes, phenolic acid, and flavonoids. In addition, *O. stamineus* extract was found to be rich in potassium (Basheer and Majid, 2010). The full list of compounds found in *O. stamineus* is listed in Table 3.31.1.

TRADITIONAL USE

The diuretic effect of *O. stamineus* leaf extract has long been well known in the South East Asian community. Owing to such a benefit, decoctions of *O. stamineus* have been used in folk medicine for treatment of various kidney diseases from infection to renal calculi. Normally, urinary tract infection is treated with a decoction of fresh leaves taken twice a day, despite there being no systematic knowledge on the dosage required for effective treatment. A decoction of dried leaves is often used for treatment of strangury and dysuria. The whole plant either dried or fresh is used to treat kidney stones by traditional medical practitioners (Muhlisah, 2007). The Filipinos also take a decoction of the leaves to relieve gout (De Padua et al., 1987). The Kenyah people of Sarawak, Malaysia, use the young twigs and leaves of *O. stamineus* for treatment of backache (Chai, 2006).

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TABLE 3.31.1 Active Compounds From *Orthosiphon stamineus*

Type	Compounds	References
Phenolic acid	Danshensu Salvionolic acid B Caftaric acid Sagerinic acid	Nuengchamnong et al. (2011)
	Rosmarinic acid Caffeic acid Lithospermic acid Chicoric acid	Sumaryono et al. (1991)
Flavonoids	Ladanein Vomifoliol 7′,3′,4′-Tri-O-methylluteolin 6-Hydroxy-5,7,4′-trimethoxyflavone	Tezuka et al. (2000)
	Eupatorin Tetramethylscutellarein 5-Hydroxy-6,7,3′,4′-tetramethoxyflavone 3′-Hydroxy-5,6,7,4′-tetramethoxyflavone Sinensetin Pillion Salvegenin Cirsimaritin Rhamnazin Apigenin trimethyl ether Luteolin tetramethyl ether	Malterud et al. (1989)
Essential oil fragments	β-Caryophyllene α-Humulene β-Elemene 1-Octen-3-ol β-Bourbonene β-Pinene Caryophyllene oxide Camphene Limonene α-Pinene 1,8-Cineol Borneol Linalool Camphor Eugenol p-Cymene Carvone Bornyl acetate δ-Cadinene	Hossain et al. (2008)
Diterpenes	Orthosiphols A–Z	Awale et al. (2001, 2002, 2003), Masuda et al. (1992), Nguyen et al. (2004), Stampoulis et al. (1999), Takeda et al. (1993) and Tezuka et al. (2000)
	Staminols A–D	Awale et al. (2003), Nguyen et al. (2004), Stampoulis et al. (1999) and Tezuka et al. (2000)
	Staminolactones A–B	Stampoulis et al. (1999) and Tezuka et al. (2000)
	Secoorthosiphols A–C	Awale et al. (2002) and Nguyen et al. (2004)
	Nororthosiphonolide A	Awale et al. (2002)
	Norstaminolactone A	Awale et al. (2002)

TABLE 3.31.1 Active Compounds From *Orthosiphon stamineus*—cont'd

Type	Compounds	References
	Norstaminols A–C	Awale et al. (2002, 2003), Stampoulis et al. (1999) and Tezuka et al. (2000)
	Norstaminone A	Awale et al. (2001)
	Neoorthosiphonone A	Awale et al. (2004)
	Neoorthosiphols A–B	Awale et al. (2001, 2003), Nguyen et al. (2004), Ohashi et al. (2000) and Shibuya et al. (1999)
	Siphonols A–E	Awale et al. (2003)
	Orthochromene A	Ohashi et al. (2000)
Triterpene	Ursolic acid Oleanolic acid Betulinic acid	Tezuka et al. (2000)
	Hydroxybetulinic acid Maslinic acid α-Amyrin β-Amyrin	Hossain and Ismail (2013)
Benzochromene	Methylripariochromene A	Guerin et al. (1989)

MAIN ACTIONS

Antidiabetic Activity

Extracts from *O. stamineus* have antidiabetic properties which are believed to be due to their interaction with glucose metabolism (i.e., both inhibiting and increasing glucose uptake by diaphragm muscles). This plant is also reported as a popular antidiabetic alternative medicine for type II diabetes.

A chloroform subfraction of *O. stamineus* leaves containing eupatorin (1.48%) and sinensetin (2.26%) was found to inhibit glucose uptake in streptozotocin-induced diabetic rats at an intestinal absorption rate of 500–2000 μg/mL, although the effect is no better than the reference drug metformin (Mohamed et al., 2013). Mohamed et al. (2012) showed that a 50% ethanolic extract of *O. stamineus* leaves inhibits α-glucosidase (IC_{50} of 4.63 mg/mL) and α-amylase (IC_{50} of 36.70 mg/mL), which they attributed to the presence of sinensetin. Sinensetin had an IC_{50} of 0.66 g/mL and maximum inhibition of 89% (2.5 mg/mL) for α-glucosidase and an IC_{50} of 1.13 mg/mL and maximum inhibition of 85.8% (2.5 mg/mL) for α-amylase. Thus, the plant extract inhibits the process of converting carbohydrates to glucose and hence helps regulate glucose levels in diabetic patients. They stated that sinensetin outperformed the reference drug acarbose. Earlier, Sriplang et al. (2007) reported up to 35% reduction of blood glucose in streptozotocin-induced diabetic rats using 1000 mg/kg of *O. stamineus* water extract. They noted an effect comparable with that of the reference drug glibenclamide. Blood glucose reduction is not caused by sinensetin alone because the water extract usually has an almost undetectable amount of sinensetin (Pang et al., 2017). The fact that water extract is responsible for blood glucose reduction according to Sriplang et al. (2007) means water-soluble compounds other than sinensetin must be responsible for the same actions. Elucidation of these compounds still awaits further investigation.

Water extract does not stimulate insulin secretion when tested in vitro (Sriplang et al., 2007). Similarly, a chloroform subfraction of plant leaves containing eupatorin at 1.48% and sinensetin at 2.26% also fails to stimulate insulin secretion in diabetic rats at doses up to 1000 mg/kg for 2 weeks (Mohamed et al., 2013), although it was found to suppress insulin release in response to subcutaneous glucose load (Mohamed et al., 2011a) and oral glucose test (Sriplang et al., 2007).

O. stamineus extract has also been found to stimulate glycogen synthesis. Mohamed et al. (2013) reported that chloroform extract containing 1.48% eupatorin and 2.26% sinensetin increased glucose uptake by diaphragm muscles at a rate of 2 mg/mL in a different way from that of insulin, despite not being effective at a lower concentration (i.e., 0.5–1 mg/mL). It is possible that *O. stamineus* stimulates glucose uptake into muscle cells, even though it is not known whether this applies to the largest body of muscle (i.e., skeletal muscle).

Mohamed et al. (2013) reported that a chloroform subfraction of plant leaves could reduce blood glucose level in streptozotocin-induced diabetic rats. The dose ranged from 500 to 1000 mg/kg taken orally twice daily for 14 days. They noted potency was significantly less than the reference drug metformin at 500 mg/kg. *O. stamineus* water extract also yielded a 13% reduction in fasting blood glucose for a similar dose and duration (Sriplang et al., 2007). In terms of percentage reduction in fasting blood glucose a dose of 1000 mg/kg had a similar effect to that of the reference drug glibenclamide, but the glucose concentration of the latter was slightly lower. According to Mohamed et al. (2011b) the extract could reduce blood glucose in a subcutaneous glucose tolerance test without inducing hypoglycemia, which suggests that the mechanism of action is related to 5′-AMP-activated protein kinase (AMPK). *O. stamineus* plant extract has the potential to reduce blood glucose levels in diabetic subjects.

Diuretic Activity

O. stamineus water extract increased urine output in Sprague-Dawley rats and the diuretic activity was dose dependent (Adam et al., 2009). The diuretic effect was lower than those of furosemide and hydrochlorothiazide, the medicine often used for diuresis. They observed higher than normal urinary potassium excretion and elevated blood urea nitrogen, creatinine, and glucose levels, but all remained within the normal range. Methanol–water extract of *O. stamineus* leaves showed diuretic effects comparable with those of hydrochlorothiazide for both acute and chronic administration (Arafat et al., 2008). They reported a reduction in uric acid level after 6 h in much the same way as occurs with allopurinol for hyperuricemic rats.

The diuretic effect of *O. stamineus* extract is attributed to the presence of methoxy flavonoids (i.e., sinensetin and tetramethylscutellarein) which have an antagonistic effect on adenosine receptors (Yuliana et al., 2009). Thus, the diuretic properties of *O. stamineus* are due to the affinity of its active compounds to adenosine receptor ligands.

Nephroprotective Activity: Kidney Stone Treatment

Zhong et al. (2012) reported that *O. stamineus* extracts can reduce the synthesis of calcium-based stones in the kidneys. In their work calcium oxalate induced nephrolithiasis in rats fed with 80–160 mg/kg of *O. stamineus* extract. They used the phenolic, flavonoid, and polysaccharide content derived from the main *O. stamineus* extracts for the test. They observed that urinary calcium and oxalate excretion increased and that the effect was dose dependent. They concluded the polysaccharide extracts of *O. stamineus* provided the most significant reduction of CaOx crystal nucleation and aggregation. Rodgers et al. (2014) reported that *O. stamineus* water extract reduced the growth rate of calcium oxalate in much the same way as Cystone, although Cystone was more effective (30.9%) than *O. stamineus* water extract (20%) in reducing overall CaOx crystal size.

Premgamone et al. (2001) evaluated the effect of drinking *O. stamineus* tea made from 2.5 g of sun-dried plant material on 48 renal calculi subjects split into 2 groups for 18 months. They compared the effect with that of the reference drug (i.e., 5–10 g granular sodium potassium citrate). They found a significant reduction in kidney stone size for both study groups at an annual rate of $28.6 \pm 16.0\%$ and $33.8 \pm 23.6\%$ for *O. stamineus* and sodium potassium citrate, respectively. At the end of treatment, 90% of initial clinical symptoms (i.e., back pain, headaches, and joint pain) were relieved. No side effects were reported from subjects with *O. stamineus*; however, fatigue and loss of appetite were observed in 26.3% of subjects treated with sodium potassium citrate. Currently, there is no evidence that the plant extract is more potent than the currently used drug. However, the plant has the potential to reduce calcium-based stones production in the kidneys with no side effects.

Antihypertensive Activity

A chloroform subfraction of *O. stamineus* extract containing methylripariochromene A was shown to reduce blood pressure in spontaneously hypertensive rats (Matsubara et al., 1999). Manshor et al. (2013) reported that 50% methanolic extract and water extract of *O. stamineus* at a dose of 1000 mg/kg can also reduce blood pressure in spontaneously hypertensive rats. It is not clear whether methylripariochromene A was present in the extract, since quantification of the compound was not reported. The greatest effect was found with the methanolic extract, but it was still less than that of the reference drug losartan.

In a random clinical study of 80 subjects, Cicero et al. (2012) reported a mild but notable reduction in systolic, diastolic, and pulse pressure in hypertensive dyslipidemic subjects treated with a nutraceutical containing *O. stamineus* for 8 weeks. They reported no significant difference in cardiovascular disease risk at the end of the trial. The antihypertensive effect of *O. stamineus* is notable, although less potent than that of the reference drug hydrochlorothiazide. Elsewhere, Trimarco et al. (2012) showed a significant reduction in mean 24-hour systolic and diastolic blood pressure levels compared with baseline

values for subjects treated with supplements containing *O. stamineus* from their clinical trial involving 27 subjects. In contrast, the subjects treated with a supplement containing a combination of policosanol, red yeast rice extract, berberine, folic acid, and coenzyme Q10 (without *O. stamineus*) showed no significant reduction in blood pressure. Earlier, Supari (2002) reported systolic and diastolic blood pressure equivalent to amlodipine in mild and moderate hypertensive subjects taking a mixture supplement of *Apium graveolens* and *O. stamineus*.

Hepatoprotective Activity

Yam et al. (2007) observed the hepatoprotective effect of *O. stamineus* 50% aqueous methanolic extracts on CCl_4-induced rats. They monitored normalization of the liver rate function using alanine transaminase and aspartate transaminase as indicators. They found the effect was dose dependent and that it was only effective at higher doses (i.e., >250 mg/kg). Alshawsh et al. (2011) studied the hepatoprotective effect that a 95% ethanolic extract of *O. stamineus* given at rates of 100 and 200 mg/kg daily for 2 months had on thioacetamide-induced liver cirrhosis in rats. They found the hepatoprotective effect was dose dependent and that it was only significant at the higher dose (i.e., 200 mg/kg). They suggested the hepatoprotective effect was due to neutralization of the toxic compounds through the cytochrome p450 pathway. Nevertheless, the hepatoprotective effect was less potent than the reference drug silymarin (50 mg/kg) which comes from milk thistle. All these studies show that *O. stamineus* extract possesses hepatoprotective properties, although clinical trials on humans are not available.

Gastroprotective Activity

Yam et al. (2009) studied the antiulcerogenic activity of *O. stamineus* methanolic extract using an ethanol-induced gastric ulcer rat model. They reported a marked histological improvement in the healing of mucosal damage in groups receiving *O. stamineus* methanolic extracts. They concluded the gastroprotective effects of *O. stamineus* extract were due to its ability to inhibit lipid peroxidation and stimulate gastric mucus secretion.

Antiproliferative Properties and Cancer Treatment

O. stamineus contains diterpenes and flavonoids that display mild antiproliferative properties against liver metastatic colon 26-L5 carcinoma and human HT1080 fibrosarcoma cell lines (Awale et al., 2001, 2002). *O. stamineus* ethanolic extract was found to suppress HCT116 colorectal tumors in mice (Ahamed et al., 2012). The latter authors' in vitro test shows that *O. stamineus* is noncytotoxic to colon cancer and endothelial cells, but it appears to suppress vascular endothelial growth factor. The anticancer properties of *O. stamineus* lack clinical evidence, and more studies are needed to further understand its efficacy.

TOXICITY

The LD_{50} of *O. stamineus* is estimated to be greater than 5000 mg/kg with no apparent toxicity reported in Sprague-Dawley rats after 14 days. Despite a benign increase in liver weight and a reduction in serum enzymes (Chin et al., 2008) and despite the absence of toxicity at this dose being reproduced acutely (Abdullah et al., 2009) and subchronically over a period of 28 days with 50% ethanolic extract (Mohamed et al., 2011a), *O. stamineus* water extract showed no genotoxic effects in *Salmonella* and doses up to 4000 mg/kg showed no genotoxic effects in a mouse bone marrow test (Muhammad et al., 2011). *O. stamineus* extract is neither toxic nor genotoxic with tested doses five to ten times higher than effective supplemented doses.

ADVERSE EFFECTS AND INTERACTIONS WITH DRUGS

In most clinical studies, pregnant or lactating women, persons with liver or heart failure, and persons who have had a stroke are often excluded for safety reasons due to limited information available on interaction with *O. stamineus*. Caution should be observed in patients with hypertensive therapy when taking *O. stamineus* because there is a possibility of bringing about an orthostatic hypotensive attack (Globinmed, 2017). Garcia-Moran et al. (2004) wrote a letter to *Gastroenterology and Hepatology* complaining about potential development of acute hepatitis as a result of consuming *O. stamineus* tea; however, this claim has yet to be substantiated elsewhere. Moreover, *O. stamineus* is consumed widely as herbal tea in South East Asia without any reported complaint related to hepatitis.

O. stamineus–based supplements should not be taken with other diuretics because such a combination may cause hypertension and congestive cardiac failure. In the presence of cardiac and renal insufficiency, caution is advised when taking this supplement. Adam et al. (2009) noted that there is a risk of hypoglycemia when *O. stamineus* is taken together with antidiabetic drugs.

CONCLUSIONS

It is clear from reviewing preclinical and clinical trials on *O. stamineus* that the plant shows significant promise as diuretic, antidiabetic, antihypertensive, nephroprotective, hepatoprotective, gastroprotective, antiproliferative and anticancer drug. The effective dose of the plant extract ranging from 200 to 1000 mg/kg is not toxic with the LD_{50} estimated to be greater than 5000 mg/kg. At present, how it interacta with other drugs is not yet fully understood. However, as a precaution it is recommended that *O. stamineus*–based supplements should not be taken in conjunction with other diuresis or hypertensive therapy.

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Chapter 3.32

Papaya (*Carica papaya* L., Pawpaw)

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INTRODUCTION

Botany

Carica papaya L. is an herbaceous laticiferous tree-like plant that grows to about 10 m in height. It is native to tropical and eastern Central America where seeds that remain viable for a long time in a dried state are transported to different parts of the world. *C. papaya* belongs to the family Caricaceae and is grown in most tropical countries of the world as a food crop (OECD, 2005). It is known by different names in different parts of the world: pawpaw in the UK and Sri Lanka, tepaya in East Malaysia, lechosa in Venezuela, papali in India, and ibepe in the southwest of Nigeria (West Africa). The ripe fruit is the part most commonly consumed. However, the unripe fruit is popular because it produces large amounts of latex, which is rich in enzymes known to be used in a range of applications: industrial, nutritional, and therapeutical (Azarkan et al., 2003). An increasing number of varieties of *C. papaya* are being cultivated throughout the world. Reproductively, the plants are naturally dioecious (having male and female organs on separate plants); however, monoecious (true hermaphrodite) plants are thought to have been cultivated as a result of adaptation after being introduced to different regions of the world. Therefore, some species of *C. papaya* have evolved to become gynodioecious (female organ on some plants and hermaphrodite organs on others) and andromonoecious (male organ and hermaphrodite organs on the same plant). Different cultivars exhibit diverse reproductive morphology (Niklas and Marler, 2007). Many *C. papaya* cultivars grown from several varieties abound in different regions of the world (e.g., the Solo type is the oldest variety and has given rise to "Kapoho Solo," "Dwarf Solo," and "Waimanalo"). *C. papaya* cultivars are innumerable and scientists are continuously exploring conventional and modern/advanced plant-breeding techniques—selective, cytogenetical, and biotechnological—to develop new cultivars with desirable fruit characteristics, disease resistance, and increased yield of such other products as *C. papaya* cysteine proteinases and some secondary metabolites, which can be classified as nonvitamin nonmineral (NVNM) nutritional supplements of *C. papaya*. Cultivars like "Kapoho Solo," which was cultivated in Hawaii, is known for its pear shape, greenish-yellow skin, and sweet taste, while "Sunrise Solo," cultivated in both Brazil and Hawaii, is known for its orange-red attractive flesh color, precocious low-fruiting habit, high sugar content, and desirable flavor. "Pusa Majesty," which was developed from Pusa-type varieties, is gynodioecious with a medium-sized (1–1.5 kg) fruit. It is known for its high tolerance to viral diseases and root knot nematodes. The CO5 cultivar, which was cultivated in India and developed from Coimbatore-type varieties, has been selected/bred for its high papain production. It consistently produces 14–15 g dry papain per fruit. Studies have developed/documented *C. papaya* varieties and cultivars in different countries to preserve selected characteristics and more still are being developed in different parts of the world as part of modern agricultural programs seeking improved yield of food and cash crops (Singh and Sudhakar Rao, 2011) (Table 3.32.1).

Origin and Global Distribution of *Carica papaya*

The exact place of origin of *C. papaya* is not known, but the literature reports the presence of *C. papaya* seeds in Panama and the Dominican Republic before 1525. It is believed that cultivation of *C. papaya* spread from here to southern Mexico, Central America, the West Indies, the Bahamas, and Bermuda in 1616 (Morton, 1987). The Spanish are said to have brought seeds of *C. papaya* around 1550 to the Philippines and Malacca, from where the plant reached India and the Kingdom of Naples in 1626. The plant was introduced later in all tropical and subtropical regions of the world (Australia, Hawaii, Sri Lanka, and South Africa) as a plantation crop. In the 1950s, *C. papaya* was brought to Miami and New York from Santa Marta (Colombia),

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TABLE 3.32.1 *Carica papaya* **Cultivars, Varieties and Country of Origin**

Varieties	Cultivars	Countries	Characteristics	References
Solo	Sunrise solo	Brazil, Hawaii	Pear-shaped fruit with a slight neck. Averages 22–26 ounces depending on location. Skin smooth, flesh firm, reddish-orange, sweet, sugar content high. Quality similar to Solo. Seed cavity not as deeply indented as other Solo strains, making seed removal easier. Plant precocious, maturing fruit about 9 months after transplanting, at a height of about 3 feet attractive flavor, taste, sweet, soft, and red pulp	Fabi et al. (2007, 2014), De los Santos de la et al. (2000) and FAO (2003)
	Kapoho solo	Hawaii	Pear-shape, greenish-yellow skin, sweet taste yellow-orange flesh	Wall (2006)
	Frangi or Paiola	Malaysia	Small size, bright yellow skin, sweet taste, pleaseant aroma	Oga et al. (2013)
	Dwarf Solo/ Sunset	Hawaii	Solo type. Small to medium-sized, pear-shaped fruit. Orange-red skin and flesh. Very sweet. Dwarf, high yielding plant	De los Santos de la et al. (2000), FAO (2003) and Morton (1987)
	Waimanalo	Hawaii	Fruit round with a short neck, average weight 16–39 ounces. Skin smooth, and glossy, cavity star-shaped. Flesh thick, firm, orange-yellow in colour, flavor and quality high, keeps well. Recommended for fresh market and processing. Fruits of female plants rough in appearance. Average height to the first flower is 32 in.	De los Santos de la et al. (2000), FAO (2003) and Samson (1986)
	Vista Solo	USA	Medium to large fruit depending on climate, 5 in. wide, up to 18 in. long. Skin yellow, flesh orange to yellow-orange. Hardy, compact Solo type producing high quality fruit. Needs fairly hot weather to develop sweetness	De los Santos de la et al. (2000), FAO (2003), Popenoe (1974) and Morton (1987)
Deshaies Plant	NA	Guadeloupe	NA	Ocampo Pérez et al. (2005)
MTQ2	NA	NA	NA	NA
Pusa	Pusa Delicious	India	This is a gynodioecious. The fruits are medium in size with deep orange flesh colour having excellent flavour	Chan and Paull (2008) and Chan (2009)
	Pusa Giant	India	This is a dioecious and bears fruit at one month. height. The plant can withstand strong wind and storm. Fruits have attractive big size and weight ranging from 2.5 to 3.5 kg per fruit used for vegetable and canning industry	Chan and Paull (2008) and Chan (2009)
	Pusa Dwarf	India	This is a dioecious cultivar having dwarf stature and more precocious in bearing. The plants start bearing from 25 to 30 cm. above ground level. Fruit size is medium (1 to 2 kg) and oval in shape. Most suitable for high density orcharding, nutrition garden and kitchen garden	Chan and Paull (2008) and Chan (2009)
	Pusa Majesty	India	This is a gynodioecious line. The fruits are medium in size and round in shape. Fruit flesh solid in texture and yellowish in colour having good keeping quality tolerant to viral diseases	Chan and Paull (2008) and Chan (2009)

TABLE 3.32.1 *Carica papaya* Cultivars, Varieties and Country of Origin—cont'd

Varieties	Cultivars	Countries	Characteristics	References
	Pusa Nanha	India	It is a dioecious line, dwarf and precocious. Fruiting starts at a height of 40 cm within 239 days of planting with the total height of the plant as 130 cm. Fruit medium to small, oval, seed cavity 12 ×8 cm. and flesh 3.5 cm thick of blood red to orange colour with TSS (6.5%–8.0%) resistant to lodging (Pusa series release from IARI, New Delhi)	Chan and Paull (2008) and Chan (2009)
Coimbatore	CO1	India	It is a selection from progenies of cv. Ranchi. Plants are dwarf and fruits are borne within 1.2–1.5 m. from the ground level. Fruits are medium sized and sweet with golden yellow skin and orange coloured flesh	Chan and Paull (2008) and Chan (2009)
	CO2	India	It is a pure line selection from a local type. The plant is medium-tall in height. Fruits are obvate and large in size; skin yellowish green, flesh orange coloured, soft and moderately juicy. It is a good table fruit and high papain yielder	Chan and Paull (2008) and Chan (2009)
	CO3	India	This is a hybrid between Co-2 × Sunrise Solo. It is a tall vigorous plant. The fruit is medium-sized, sweet with good keeping quality	Chan and Paull (2008) and Chan (2009)
	CO4	India	This is a cross between Co-1 × Washington. Plant medium-tall. Fruit large, flesh thick, yellow with purple tinge, taste sweet and good keeping quality. (Co-1 to Co-4 are released from TNAU, Coimbatore)	Chan and Paull (2008) and Chan (2009)
	CO5	India	It is a selection from Washington and isolated for its high papain production. It produces consistently 14–15 g dry papain/fruit. It gives 75–80 fruits/tree in 2 years with an average yield of 1500–1600 kg dried papain/ha	Chan and Paull (2008) and Chan (2009)
Faircild	NA	NA	It was introduced by the US Department of Agriculture in 1938 and named after Dr. David Faircild. The fruit weighs about 4 pounds, 8–9 in. long by 5–6 in. and is rounded-oblong in shape. The fleshy portion is thick with a large edible portion. The flavor is medium cressy combined sweetness. The pulp is an attractive orange yellow, smooth texture and cold resistant than most other varieties tested	Annegowda and Bhat (2016)
Kissimmee	NA	NA	NA	
Coorg honey	Sekaki	NA		Chan (2001)
	Eksotika	NA	NA	Chan (2001) and Farouk-Idnan et al. (2013)
Madagascar	NA	NA	NA	Caro et al. (2000)

Puerto Rico, and Cuba by Albert Santo, an Italian entrepreneur. *C. papaya* began being grown as a food crop at the commercial scale in southern and central Florida around 1959. Today, farmers in Hawaii, tropical Africa, the Philippines, India, Ceylon, Malaya, and Australia are the largest commercial producers of *C. papaya* in the world (Nakasone and Paull, 1998).

Global *C. papaya* production in 2014 was about 12.7 million tonnes (FAOSTAT, 2016), which is about a 32% increase over world total *C. papaya* production in 2004. The quantity of *C. papaya* produced in different geographical regions in

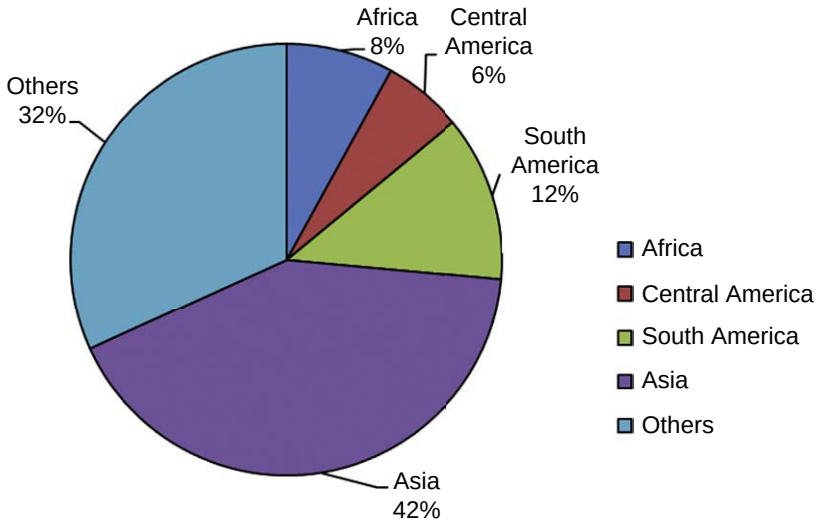

FIG. 3.32.1 Quantity of *Carica papaya* fruit produced in different geographical regions in 2014. Food and Agriculture Organization of the United Nations, Statistics (FAOSTAT, 2016).

2014 is illustrated in Fig. 3.32.1. Asia remains the world's largest producer of *C. papaya*, mainly India (Brazil runs it a close second). Countries like Nigeria, Indonesia, and Mexico also contributed significantly to world total production and along with other countries figures in the list of the top-10 producing countries of the world in 2014 (Table 3.32.2). Importantly, the volume of *C. papaya* production is dependent on factors relating to national agricultural policies, as well as land, pest, and disease control in different countries.

Traditional Uses of *Carica papaya*

C. papaya is a popular plant used traditionally by people of several nations. It has widely reported nutritional and health benefits (Anuar et al., 2008; Nayak et al., 2007; Starley et al., 1999). The ripe fruit is the part of the plant most commonly eaten and is a rich source of nutrients. Decoctions of unripe fruit and of leaves are also widely used in folkloric medicinal preparations. In most ethnomedicinal reports, *C. papaya* is used as a component of herbal mixtures containing other herbs.

TABLE 3.32.2 Total Production (tonnes) and Area (hectares) of Papaya in the Top-Ten Producing Countries of the World in 2014

Country	Production (thousand tonnes)	Area (thousand hectares)
India	5,639,300 million	133,360
Brazil	1,603,351 million	32,031
Nigeria	850,000	94,200
Indonesia	840,121	9,384
Mexico	836,370	14,533
Dominican Republic	704,786	2,676
Congo	220,483	13,043
Philippines	172,628	7,918
Venezuela	165,102	8,664
Thailand	157,571	4,320

Culled from *Food and Agriculture Organization of the United Nations, Statistics (FAOSTAT) Database, 2016*.

In Nigeria the plant is used by traditional medicine practitioners in the management of diabetes (Gbolade, 2009) and malaria, and in fungal and helminthic infections (Okpe et al., 2016). In Cameroon, it is a component of Nefang, a popular antimalarial herbal mixture (Tarkang et al., 2015). Leaf decoctions are used as anticancer remedies by aboriginal Australians (Nguyen et al., 2013) and as an abortifacient in India. Thus, the traditional use of *C. papaya* seems to be well established. One of the proximate implications of this is that there is increasing scientific interest in evaluating the pharmacologic and therapeutic potential of the plant and its components.

Carica papaya: An Important Source of Nonvitamin Nutritional Supplements

C. papaya has been classified as an herb because of its hollow nonwoody stem. The current definition of a dietary supplement according to the Dietary Supplement and Health Education Act (DSHEA) of 1994 enacted by the US Congress includes vitamins, minerals, herbs, botanicals, amino acids, concentrates, metabolites, constituents, extracts, or a combination of any of these ingredients. In addition, the ingredients must carry a label showing it is a dietary supplement intended for ingestion and not to be traded as conventional food or the main ingredient in a food. The Act separately mentions vitamins, minerals, complex botanics (herbs and extracts), and simpler ones (amino acids, concentrates, metabolites, constituents, or mixtures of these) to show that all such plant-derived bioactive natural products fall under the class of NVNM nutritional supplements (Scarbrough, 2004). Hence, extracts, juice, parts of whole plants processed in certain ways, and natural products isolated from different parts of *C. papaya* added to regular food to improve its nutritional value or taken to prevent the development of diseases and improve body structures/functions are NVNM dietary supplements of *C. papaya*. The comprehensive list of NVNM dietary supplements found in various parts of papaya, their potential nutritional benefit, specific sources, and references are contained in Table 3.32.3. The NVNM components found in papaya include different classes of proteins, secondary metabolites, and parts of the whole plant from both ripe and unripe fruits, latex, roots, and leaves.

Therapeutic Potential of *Carica papaya* Nonvitamin Nonmineral Components

There is considerable interest in the use of functional foods, botanicals, and herbals to maintain human well-being. *C. papaya* is a popular component of many traditional medicines and has been used to treat bacterial, fungal, and plasmodial infections. It is also used as a component of diabetic preparations in Nigeria. Scientific reports and clinical studies have evaluated and documented the therapeutic potential of *C. papaya* under various experimental conditions (Table 3.32.4). Generally, only a few clinical studies have evaluated the therapeutic efficacy of *C. papaya* in humans. These clinical studies have used different parts of the plant making generalizations on some of the actions difficult. More importantly, all these studies are small, randomized, usually open-labeled clinical trials. While no large randomized, double-blind clinical trials have yet been conducted, these studies including systematic reviews generally provide robust evidence of safety and efficacy warranting further clinical studies to ascertain clinical efficacy.

Clinical Efficacy of Carica papaya *in Dengue Fever*

Dengue fever is a neglected tropical disease with no current drug approved for its management. Thrombocytopenia is a common pathological feature and may predict disease severity. *C. papaya* is a promising candidate for dengue therapy primarily because of its ability to improve platelet count. A recent systematic review of clinical studies evaluating the safety and efficacy of *C. papaya* in dengue fever provides evidence in support of the possible therapeutic efficacy of the plant in improving platelet counts in dengue fever patients (Charan et al., 2016). This review included four studies that were mainly small, randomized clinical trials evaluating the efficacy of formulations of *C. papaya* leaf extract (Charan et al., 2016). In a double-blind randomized clinical trial (Kasture et al., 2016), leaf extract significantly improved platelet count in dengue fever patients receiving the extract compared with controls. Thus, dengue fever is a clinical condition where *C. papaya* has interesting therapeutic potential that requires evaluation in larger randomized clinical trials.

Several possible mechanisms by which *C. papaya* improves platelet count have been suggested. Chinnappan et al. (2016) evaluated the ability of *C. papaya* leaf extract to inhibit platelet aggregation after exposure to dengue-infected plasma. They concluded that the action of *C. papaya* leaf extract on platelet count is dengue specific. However, the specific effect of *C. papaya* leaf extract on dengue requires further evaluation.

Therapeutic Potential of Carica papaya *in Microbial Infections*

Though there is a wide armamentarium of antibacterial agents available for the treatment of bacterial infections, the specter of the emergence of bacterial strains resistant to current antibiotic chemotherapy looms large. Thus, continuous

TABLE 3.32.3 NVNM Component of *Carica papaya* Plants, Processing and Therapeutic Potentials

Categories of NVNM	Plant parts	Processing/Usage	Application	References
Extract	Root	Blended/ pulverized/ soxhelation	Antibacterial	Doughari et al. (2007) and Tiwari et al. (2011)
	Stem	Grinded/pulverized	Antibacterial, antimicrobial	Nirosha and Mangalanayaki (2013), Sumathi and Gowthami (2014)
	Leave	Blended, pulverised, crushed, lypholization, soxhlet extraction, liquid extraction	Antimalarial, antimicrobial, antiproliferative, antidiabetes, antioxidant, wound healing, antitumor, antithrombocytopenic, anticancer, antihyperglycaemic, antiinflammatory	Kovendan et al. (2012a,b), Baskaran et al. (2012), Pandey et al. (2017), Indran et al. (2008), Juárez-Rojop et al. (2014), Imaga et al. (2010), Anuar et al. (2008), Vuong et al. (2013, 2015), Alabi et al. (2012), Otsuki et al. (2010), Zunjar et al. (2015), Sasidharan et al. (2011) and Owoyele et al. (2008)
	Fruit	Grinded, macerated, homogenizing, fermentation, liquid extraction	Antihepatotoxic, antibacterial, antisickling, antioxidant, antiiflammatory	Rajkapoor et al. (2002), Ocloo et al. (2012), Mojisola et al. (2008), Nafiu and Rahman (2015a,b)
	Seed	Pulverized, grinded, liquid extraction	Hepatoprotection, antihypoglycemic, antioxidant, antiinflammatory	Adeneye et al. (2009), Adeneye and Olagunju (2009), Kothari and Seshadri (2010) and Amazu et al. (2010)
	Pulp/Juice/Latex	Blended	Anxiolytic	Kebebew and Shibeshi (2013)
Whole plant parts	Root	Decoction, boiled	Abortifacient, diuretic, checking irregular bleeding from the uterus, piles, antifungal activity	Krishna et al. (2008) and Aravind et al. (2013)
	Stem	Decoction	Jaundice, antihemolytic activity, STD, store teeth (inner bark), antifungal activity	Krishna et al. (2008) and Aravind et al. (2013)
	Leave	Cut into small pieces, squeeze, and filter	Tumour-destroying agent, Young leaves as vegetable, Jaundice (fine paste), urinary complaints & gonorrhea (infusion) dressing wound fresh leave, antibacterial	Nwofia and Okwu, 2015, Krishna et al. 2008 and Aravind et al. (2013)
	Fruit	Eaten raw	Round worm infection, dyspepsia, constipation, amenorrhoea, stomachic, digestive, carminative diuretic, dysentery and chronic diarrhea, expectorant, sedative and tonic, relieves obesity, bleeding piles, wound of urinary tract, ringworm and skin disease psoriasis	Burkill (1985), Krishna et al. (2008) and Aravind et al. (2013)

TABLE 3.32.3 NVNM Component of *Carica papaya* Plants, Processing and Therapeutic Potentials—cont'd

Categories of NVNM	Plant parts	Processing/Usage	Application	References
	Seed	Chew, pounded and crushed	Intestinal worms, clear nasal congestion, carminative, emmenagogue, vermifuge, abortifaciant, counter irritant	Elizabeth (1994), Krishna et al. (2008) and Aravind et al. (2013)
	Pulp/Juice/Latex	Incision, pressing, milled	Antioxidant, antifungal, anthelmintic, warts, sinusitis, eczema, cutaneous tubercles, bleeding piles and enlarged liver and pectoral properties, anathematic, relieves dyspepsia, cures diarrhea, pain of burn and topical use, bleeding hemorrhoids, stomachic, whooping cough	Mehdipour et al. (2006), Giordani et al. (1996), Adu et al. (2009), Calvache et al. (2016), Satrija et al. (1995), Aravind et al. (2013), Krishna et al. (2008) and Nafiu et al. (2016)
Isolated fraction	Root (Nicotine, Carposide, Myrosine)	Isolation and characterization	Antibacterial	Krishna et al. (2008)
	Stem (Papain, Myricetin, Kaemferol, Quercetin)	Isolation and characterization	Antioxidant analgesic antipyretic hypoglycemic	Anibijuwon and Udeze (2009), Baskaran et al. (2012) and Krishna et al. (2008)
	Leave (Quercetin, Kaempferol, Quercetin-3-rutinoside, Myricetin-3-rhamnoside, Kaempferol-3-rutinoside, Papain, Chymopapain, Caricain, Glycylendopeptidase, Carpaine, Carpinine, Pseudocarpaine, Dehydrocarpaine I and II, Nicotine, Choline, Bispiperidine, Caffeic acid, P-coumaric acid, Chlorogenic acid, Tryptophan, Methionine, Lysine, Cystatin, Saponins, Tannins, Anthraquinolones, reducing sugars, steroids)	Isolation and characterization	Peroxynitrite scavenging activity, antitumor anticancer analgesic antiinflammatory diuretic amoebicide antibacterial antisplasmodic, antimalarial heart depressant antihelmintic, antioxidant, antioxidant analgesic antipyretic hypoglycemic	Nugroho et al. (2017), Aravind et al. (2013), Baskaran et al. (2012), Burdick (1971), Krishna et al. (2008), Tuffley and Williams (1951), and Baskaran et al. (2012)
	Fruit (Danielone, isopropyl 5-β-Dglucopyranosyloxy-2-hydroxybenzoate, methyl β-D-glucopyranoside, α-linolenic acid, Chitinase, Carpaine)	Isolation and characterization	Antimicrobial, antioxidant	Echeverri et al. (1997), Galang et al. (2016), Krishna et al. (2008), Aravind et al. (2013)
	Seed (Myrosin, Carpaine, Caricin, β-sitosterol, Carpasemine)	Isolation and characterization	Cytotoxic	Krishna et al. (2008) and Aravind et al. (2013)
	Pulp/Juice/latex proteinases (papain, chymopapain, and papain proteinases A and B), N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic, palmitic, stearic, Linoleic, Linolenic and C is -vaccenic and oleic acids	Isolation and characterization	Proteolytic, antiinflammatory, antioxidant, antioxidant Analgesic antiinflammatory antibacterial antiviral antifungal anticancer antiallergic aid in digestion laxative assist wound healing boost platelets	Milivoevich (1978), Mezhlumyan et al. (2003), Gazetov and Kalinin (1990), Aravind et al. (2013), Broke (2013)

TABLE 3.32.4 Therapeutic Potential of *Carica papaya* in Clinical and Experimental Studies

Papaya preparation used in study	Route of administration – dose	Findings	References
Crude ethanolic extract of unripe *C. papaya* fruit	Intravenous, 20 mg/kg	Etracted significantly lowered mean arterial pressure in animal models of hypertension	Eno et al. (2000)
Methanolic extract for *C. papaya*	Oral, 100 mg/kg twice daily for 30 days	Extract lowered blood pressure in spontaneously hypertensive rats	Brasil et al. (2014)
Standard fermented *C. papaya*	Oral – 0.2 g/kg	8 weeks administration to db/db mice improved macrophage function ant wound site	Collard and Roy (2010)
C. papaya leaf aqueous extract	Oral – 0.75, 1.5, 3 g/100 mL	All doses exhibited glucose lowering effect and raised insulin levels in streptozotocin induced diabetic rats	Juárez-rojop et al. (2012)
C. papaya fruit aqueous extract	Oral 100 mg/kg/day for 10 days	Wound area was significantly reduced in STZ-induced diabetic rats compared to control. Wound epithelialized faster in extract treated rats	Nayak et al. (2007)
C. papaya leaf aqueous extract	Oral 100, 200 and 400 mg/kg for 21 days	In alloxan-induced diabetic rats, only 400 mg/kg dose resulted in significant hypoglycemic effect	Maniyar and Bhixavatimath (2012)
Fermented *C. papaya*	Oral, 6 g/day for 14 weeks	No significant changes in fasting blood glucose and glycated hemoglobin during supplementation period	Somanah et al. (2012)
Standardized fermented *C. papaya* gel	Topical 7 g/day for 10 days	In this human study, clinical symptoms of gingivitis were improved as well as inflammatory marker reduction was achieved	Kharaeva et al. (2016)
Ethanolic extract of *C. papaya* leaves	Oral, 100 mg/kg	Significant hypoglycemic effect in STZ treated mice	Sasidharan et al. (2011)
C. papaya leaf juices	Oral, juice extracted from 50 g of leaves, administered once daily for 3 days	Ability to induce platelet production in dengue patients	
C. papaya leaf extract tablets	Oral, 1100 mg 3 times daily for 5 days	Rapid induction in rise in platelet number in 30 patients who received standard therapy plus c papaya treatment compared to control that received standard therapy alone	
C. papaya ethanol leaf extract capsules	Oral, 550 mg	Capsules shortened hospitalization stay and induce rise in platelet count	
C. papaya leaf extract capsule	Oral 500 mg once daily for 5 days	Leaf extract raises platelet count in dengue fever patients without side effect	
Fermented *C. papaya*	Oral, 4.5 g twice a day for 90 days	Significant improvement in skin features such as elasticity, evenness and moisturization. Effect is associated with changes in cyclophilin A and CD147 genes	Bertuccelli et al. (2016)
C. papaya leaf extract	Oral, three times daily for 5 days	*C. papaya* leaf extract accelerates rise in platelet count	Kasture et al. (2016)
Fermented *C. papaya*	Oral, 3- or 9 g daily for 30 days	Dose-dependent increase in natural killer cytotoxicity	
C. papaya seed	Oral	Effective stool parasite clearance	Okeniyi et al. (2007)
Fermented *C. papaya*	Oral, 500 mg/kg for 92 days	Improvement in key antioxidant enzyme activity and reduced liver peroxidation	Somanah et al. (2016)
Fermented *C. papaya*	Oral (150, 300, 450 mg/kg) or intraperitoneal (1600/4000 mg/kg)	Suppressed tumorigenesis on balb/mice	Murakami et al. (2016a,b)

efforts in search of novel antibacterial agents subsist. Phytochemicals, especially essential oils, have been shown to exhibit antimicrobial properties on many strains including multidrug-resistant strains when used alone or in combination as synergists/potentiators of less effective products (Ohene-Agyei et al., 2014). The main classes of phytochemicals with recognized antibacterial activity (phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, terpenoids and essential oils, alkaloids, polyamines, isothiocyanates, and glycosides) are detected in varying amounts in different parts of *C. papaya*. Several lines of evidence are in support of the potential use of *C. papaya* parts in bacterial infections. Although an early study reported lack of microbiocidal activity against bacterial strains, recent studies have continued to show *C. papaya* antibacterial activity (Asghar et al., 2016; Mbosso Teinkela et al., 2016; Yismaw et al., 2008). Asghar et al. (2016) reported the in vitro antibacterial activity of several extracts of the pulp, leaf, stem, and roots of *C. papaya* against multidrug-resistant strains of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Ethanolic extract of *C. papaya* seem to be the most active. Ethanolic extract of the sap was also found effective against *S. aureus* (Mbosso Teinkela et al., 2016). There seems to be a dearth of quality peer-reviewed studies that have examined the antibacterial activity of *C. papaya* in experimental animal models. This notwithstanding, human clinical studies have evaluated the plant's antimicrobial effects.

In a phase 2 randomized clinical trial, children with stool-confirmed microbial infections were treated with *C. papaya* extract (Okeniyi et al., 2007). This regimen was found to be safe in these children. A recent study conducted by Fujita et al. (2017) further corroborates this finding showing that *C. papaya* reduces the number of firmicutes, *Clostridium scindens* and *Eggerthella lenta*, and boosts immune activity by modulating bacterial clearance from the gut.

It has been suggested that papaya exerts a proteolytic effect on bacteria by producing a coagulum that immobilizes microorganisms and improves the efficiency of phagocytic cells thereby protecting the host against bacterial infections (Gurung and Škalko-Basnet, 2009; Wimalawansa, 1981). The antimicrobial properties of papaya are also connected with its alkaloid and carpaine content (Hewitt et al., 2002).

Therapeutic Potential of Carica papaya *in Aging*

There is evidence suggesting the efficacy of *C. papaya* as an antiaging agent in humans. The study of Bertuccelli et al. (2016), a double-blind, randomized clinical trial, evaluated the antiaging effects of oral supplementation with a fermented papaya preparation (FPP). The FPP caused marked improvement in skin quality and integrity in treated patients compared with those without FPP. This antiaging effect was further corroborated by significant change in the gene expression of proteins involved in skin integrity.

Therapeutic Potential of Carica papaya *in Wound Healing*

Wound healing is a complex process that involves several cells and mediators (Velnar et al., 2009). Traditionally, it is thought to occur in nonlinear phases including inflammation, proliferation, and remodeling (Broughton et al., 2006). Wound impairment is a major pathological condition in the clinic. Wound healing may generally be impaired as aging progresses and by pathological conditions such as diabetes mellitus (Brem and Tomic-Canic, 2007; Greenhalgh, 2003).

Several plants have been evaluated for their ability to promote wound healing including several parts and components of *C. papaya*. *C. papaya* dried latex was shown to promote wound healing in a mouse burn model (Gurung and Škalko-Basnet, 2009). Water extract of the fruit also possesses wound-healing potential as it shortens the period of reepithelialization in diabetic wounds in mice (Nayak et al., 2007). The seeds (Nayak et al., 2012) and unripe fruit (Nafiu and Rahman, 2015a) were reported to promote wound healing in an excision wound model. These animal studies provide a scientific basis for further evaluation of this effect in human studies. The pulp of *C. papaya* fruit is a major component of burn dressings in the Pediatric Burn Unit of the Royal Victoria Hospital in the Gambia. Experience of this preparation shows it prevents wound infection and appears to aid shedding of necrotizing tissue in the wound. The preparation is well tolerated by children (Starley et al., 1999). The study by Murthy et al. (2012) shows that in cesarean patients receiving partially ripe *C. papaya* wound dressings, incision wounds healed faster. This randomized, open-labeled study shows the superiority of *C. papaya* over hydrogen peroxide in rapid induction of development of healthy granulation tissue and comparable safety of the *C. papaya* preparation. Several factors may play a role in the wound-healing effect of *C. papaya*. Wound healing requires recruiting fibroblasts to the wound site which play important roles in tissue repair. *C. papaya* has been reported to induce the expression of vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β1) (Nafiu and Rahman, 2015b), factors that promote recruitment of fibroblasts to the wound, thereby promoting the healing process. The wound-healing properties of *C. papaya* may also be related to its antibacterial activity (Dawkins et al., 2003; Yismaw et al., 2008). Bacterial infections are known to alter the course of wound healing by prolonging the inflammatory state.

Therapeutic Potential of Carica papaya *in Periodontitis*

A fermented papaya preparation was evaluated for its clinical efficacy in periodontitis patients. In this randomized open-labeled clinical trial, *C. papaya* effectively lowered inflammatory cytokines improving clinical indices including disease severity, bleeding, and depth of cavity (Kharaeva et al., 2016). This study is a proof-of-concept study supporting further evaluation of *C. papaya* in larger randomized double-blind clinical studies. Tadikonda et al. (2017) also reported the antiplaque and antigingivitis efficacy of a dentifrice containing papain and other related phytochemicals in a randomized controlled clinical trial. When compared with a standard dentifrice among patients undergoing fixed orthodontic treatment, papain-containing dentifrice reduced plaque and gingival indices. The study concluded that the test dentifrice can be used as a home-based adjunct to clinical therapy in orthodontic patients.

Therapeutic Potential of Carica papaya *in Diabetes Mellitus*

C. papaya has been used as a traditional agent in the management of diabetes (Gbolade, 2009). Scientific evidence further suggests the antidiabetic potential of *C. papaya*. In animal models of diabetes, *C. papaya* was reported to lower blood glucose (Juárez-Rojop et al., 2012; Maniyar and Bhixavatimath, 2012; Sasidharan et al., 2011) and increase beta cell area suggesting regenerative capacity. A recent evaluation of *C. papaya* in diabetes shows that it restores basal insulin levels in diabetic animals. While these studies suggest the plant effects may be attributable to beta cell regeneration, in vitro studies have reported other potential antihyperglycemic effects including antiglucosidase activity (Loh and Hadira, 2011; Oboh et al., 2014). Furthermore, the usefulness of *C. papaya* may extend beyond its glucose-lowering effect. Two studies in animals report the wound-healing properties of the extract in diabetic wounds (Collard and Roy, 2010; Nayak et al., 2007). The antidiabetic effect of *C. papaya* has subsequently been evaluated in human patients. In this human study, *C. papaya* shows antidiabetic potential due to its hypoglycemic effect (Somanah et al., 2012). These studies showed that *C. papaya* may be beneficial in diabetes therapy and warrants further studies in larger populations.

Therapeutic Potential of Carica papaya *in Hypertension*

A few scientific reports have evaluated the antihypertensive potential of this plant. Eno et al. (2000) found that intravenous administration of *C. papaya* reduced mean arterial pressure in renal and deoxycorticosterone acetate–induced hypertension in male Wistar rats. In another study, Brasil et al. (2014) reported *C. papaya* had an antihypertensive effect in spontaneously hypertensive rats. While Eno et al. (2000) suggest the effect may be via adrenoceptor antagonism, the studies by Brasil et al. (2014) suggest angiotensin-converting enzyme inhibitory activity corroborating earlier in vitro studies reporting angiotensin-converting enzyme inhibition by the plant (Loh and Hadira, 2011). These studies point to the potential of *C. papaya* in hypertension. There is need to evaluate this pharmacologic effect in human patients.

Therapeutic Potential of Carica papaya *in Cancer*

C. papaya has been demonstrated to possess excellent in vitro antiproliferative activity in various cancer cell lines including breast, lymphocyte, and human peripheral mononuclear cells. It is a potential source of anticancer agents (Nguyen et al., 2013, 2015; Pandey et al., 2017). Animal chemopreventive studies further demonstrate the chemopreventive potential of *C. papaya* (Pathak et al., 2014). Somanah et al. (2016) also reported the ability of fermented *C. papaya* to modulate hepatocellular carcinoma in mice. Murakami et al. (2016a) also showed *C. papaya* tumor-suppressive activity in vivo.

Antisickling Potential of Carica papaya

A very early report suggested the antisickling potential of unripe *C. papaya* (Thomas and Ajani, 1987). Thereafter, the use of *C. papaya* in thalassemias was evaluated. Fermented papaya preparations improved red blood cell sensitivity to hemolysis and oxidative stress markers in a mouse model of beta thalassemia and in patients (Amer et al., 2008; Fibach and Rachmilewitz, 2010). In another in vitro study, which evaluated the antisickling effect of plants, *C. papaya* showed promising antisickling effects as it significantly prevented the sickling effect of 2% sodium metabisulfite (Nurain et al., 2016).

Pharmacological Properties of *Carica papaya*

The use of *C. papaya* in humans is based on some pharmacological actions that are associated with disease prevention and therapeutic targets. Pathological factors such as tissue oxidative damage, inflammation, immune disorder, and microbial infections form the bases and underlying changes leading to the development of most diseases. Therefore, this section focuses

on the antioxidant, antiinflammatory, and immunomodulatory properties of *C. papaya* and its products. The antimicrobial property of *C. papaya* was discussed in the "Therapeutic potential of *Carica papaya* in microbial infections" section.

Antioxidant Properties of Carica papaya

Complex metabolic activities involving many enzymes, nutrients, and molecular oxygen to produce the energy needed for normal body functions yield oxidants that at the basal level could impact positively on the body. However, altered tissue metabolism under disease conditions (diabetes mellitus, chronic wound healing, microbial infections, and chronic inflammation-associated diseases) increases oxidant production such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and peroxides. Altered tissue metabolism also suppresses the endogenous antioxidant defense system, a prelude to tissue/cellular oxidative damage. Exogenous antioxidants find relevance in this condition. Studies have shown that phytochemicals such as carotenoids, phenolic acids, flavonols, and flavonols, which are found abundantly in *C. papaya*, play important roles as antioxidant nutritional supplements.

Recently, Somanah et al. (2017) showed that ripe and unripe Mauritian *C. papaya* (Solo-type) seed, peel, and pulp extracts (2 mg/mL) significantly reduced oxidative stress levels in human preadipocytes (SW872) and hepatocellular carcinoma cells (HepG2) exposed to hydrogen peroxide (H_2O_2) by mediating proinflammatory cytokine—such as tumor necrosis factor-alpha (TNF-α), interleukin (IL-6), and monocyte chemoattractant protein-1 (MCP-1)—secretory levels, reducing intracellular ROS and maintaining mitochondrial viability. *C. papaya* peel water extracts were found to contain the highest total phenolic content and reducing activity, while total flavonoid content and radical scavenging activity was found in 80% ethanolic extract (Siddique et al., 2017). FPP also induced increased nuclear translocation of nuclear factor erythroid 2–related factor (Nrf2) and release of Nrf2-regulated neuroprotective antioxidants and detoxifying molecules in striatal astrocyte culture. It also suppressed 6-hydroxydopamine–induced dopaminergic neuronal loss in neuron–astrocyte mixed cultures and neuron-rich cultures pretreated with glial-conditioned medium. The protective role of FPP against neurooxidative damage was confirmed in vivo. FPP induced striatal Nrf2 activation and increased the glutathione level in mice treated with FPP for 2 weeks (Murakami et al., 2016b). The antiaging property of FPP, mentioned above, was also linked to its antioxidant effect on skin tissue (Bertuccelli et al., 2016).

Antiinflammatory Properties of Carica papaya

Inflammation is a component of several diseases including rheumatoid arthritis, asthma, and type 2 diabetes mellitus as well as of wound healing. Papain from papaya latex or unripe pulp produced therapeutic effects in patients with inflammatory disorders and was effective in controlling edema and inflammation associated with surgical or accidental trauma (Rakhimov, 2000). *C. papaya* leaf ethanol extract significantly reduced carrageenan-induced paw edema and cotton granuloma tissue formation. Similarly, the extract reduced edema in a formaldehyde-induced arthritis model (Owoyele et al., 2008). Many studies (human and animal) on the wound-healing effect of papaya consistently implicated the antiinflammatory properties of *C. papaya* as the main mechanism for wound healing (Mikhalchik et al., 2004; Nafiu et al., 2015a,b; Rakhimov, 2000). Ironically, Gupta et al. have used *C. papaya* latex as an inflammagen in a new model of inflammation for testing antiinflammatory drugs (Sindhu et al., 2017). A latex suspension injected in the hind paw of a rat produced concentration-dependent inflammation similar to carrageenan-induced inflammation, but more responsive to slowly acting antiarthritic drugs. A recent study on a mouse model of allergic airway inflammation showed that *C. papaya* leaf extract reduced inflammatory cell infiltration of the lungs as well as total and differential leukocyte counts in both blood and bronchoalveolar lavage fluid samples. These were associated with *C. papaya*–induced downregulation of inflammatory genes: IL-4, IL-5, eotaxin, TNF-α, nuclear factor-κB (NF-κB), and inducible nitric oxide synthase (iNOS) (Inam et al., 2017). An ethanol acetate fraction of *C. papaya* leaf water extract (500 μg/mL) was shown to inhibit the release of TNF-α (60.2% inhibition) by human monocytic cells derived from an acute monocytic leukemia (THP-1) following *Porphyromonas gingivalis* lipopolysaccharide activation. The extract was active when compared with dexamethasone and is a major bioactive component in Gencix. Gencix is a toothpaste recommended for the protection of gums and prevention of gingivitis. The antiinflammatory property was linked to the flavonol (quercetin and kaempferol) content of the extract (Dejoie et al., 2016). A complex fruit/vegetable concentrate (Juice Plus + Orchard, Garden, and Berry Blends), prepared from *C. papaya* and 24 other fruits and vegetables, reduced systemic inflammation in a double-blind, randomized placebo-controlled trial. The subjects were given 6 capsules of the concentrate daily for 8 weeks. The concentrate markedly reduced plasma TNF-α without change in plasma C-reactive protein, especially in subjects with higher baseline systemic inflammation (serum CRP ≥3.0 mg/mL). The concentrate also altered the expression pattern of several genes: NF-κB, 5′-adenosine monophosphate–activated protein kinase (AMPK) signaling, phosphomevalonate kinase (PMVK), zinc finger AN1-type containing 5 (ZFAND5), and calcium-binding protein-39 (CAB39). The concentrate improved blood lipid and metabolic profiles through reduction of systemic inflammation to alleviate the risk of obesity-induced chronic disease (Williams et al., 2017).

Immunomodulatory Properties of Carica papaya

Enhancing the immune response is an important approach to prevent infection under conditions that can compromise immune cell functions. Studies have shown that *C. papaya*–based products exhibit remarkable immunomodulatory properties, and this has been reviewed by Pandey et al. (2016). Recently, Jayasinghe et al. (2017) used in vitro, ex vivo, and in vivo techniques to establish the immunomodulatory properties of mature leaf concentrate of *C. papaya* (MLCC). They found that oral MLCC considerably increased platelet, total leukocyte, lymphocyte, and monocyte subpopulations and bone marrow cells in rats. The highest MLCC dose tested in the study markedly reduced proinflammatory cytokine (IL-6 and TNF-α) concentrations. The phagocytic index of rat peritoneal macrophages, in vitro and in vivo, increased at all doses tested (0.18, 0.36, 0.72 mL/100 g body weight of rats) with T helper 1 (Th_1)-biased cytokine response elicited in vitro by the MLCC. MLCC (31.25 and 62.5 μg/mL) also stimulated proliferation of bone marrow cells and splenocytes ex vivo, but induced cytotoxicity of both bone marrow cells and splenocytes at 500 and 1000 μg/mL via modulation of cytokines. An oral subacute toxicity test, targeting hepatorenal function biomarkers, revealed that MLCC was safe.

C. papaya leaf and seed extracts are most commonly investigated for their immunomodulatory properties. *C. papaya* seed extract enhances mitogen-activated proliferation of lymphocytes. Fractions separated from the seed extract significantly inhibited the classical complement-mediated hemolytic pathway in a study that used lymphocyte proliferation assay and complement-mediated hemolytic assay (Mojica-Henshaw et al., 2003). In another study, it was shown that *C. papaya* water extract upregulates interleulin-12 subunit p40 (IL-12p40), interleukin-12 subunit p70 (IL-12p70), interferon-gamma (IFN-γ), and TNF-α, but inhibits IL-2 and IL-4 to enhance survival of peripheral blood mononuclear cells (PBMC) in the culture supernatant. Using microarray technology and real time polymerase chain reaction, 23 genes classified by gene ontology analysis as immunomodulatory were upregulated following the addition of *C. papaya* extract to the cells. The addition of papaya extract to activated PBMC significantly enhanced cytotoxic activity against cancer cells. The study concluded that *C. papaya* extract may serve as an immunoadjuvant for vaccine therapy (Otsuki et al., 2010). A fermented papaya preparation, when administered on raw 264.7 macrophages in a culture, significantly increased production and expression of nitric oxide (NO) and iNOS, respectively. It also enhanced TNF-α secretion independent of bacterial lipopolysccharide (Rimbach et al., 2000). *C. papaya* leaf water extract was shown to increase neutrophil adhesion to nylon fibers, which corresponds to the in vivo process of margination (Ghaisas et al., 2012). Unripe *C. papaya* fruit were also reported to markedly enhance immunoglobulin IgG and IgM levels in a rat model of acrylamide-induced oxidative stress (Sadek, 2012). In a comparative study on the immunomodulatory potentials of ripe and unripe, transgenic and native papaya fruits using an ovalbumin-sensitized mouse model, native green and ripe papaya fruit supplementation considerably increased ovalbumin-specific IgG_{2a} titer while native green papaya fruit only decreased ovalbumin-specific IgE (Chen et al., 2011). Human subjects (male and female) fed 100 g fresh papaya fruit for 2 days exhibited decreased Th_1 T-cell phenotypes, but increased Th_2 and T_{regs} T-cell phenotypes in their PBMCs. The increased T_{regs} phenotype of the male sample was markedly associated with IL-1β level in the in vitro culture supernatant (Abdullah et al., 2011).

Dietary Supplements and Drug Interactions

In the last decade, plant-derived dietary supplements have become a common alternative to medical remedies throughout the world for a number of reasons: increased cost of medical care; belief that phytochemicals are natural and safe; lack of strict guidelines for development; approval, use, and reports of enhanced efficacy with concomitant use of plant-derived dietary supplements and drugs (Ernst, 2005; Iwu et al., 1999; Sahoo et al., 2010). This means patients are now using plant-derived dietary supplements at the same time as allopathic drugs for improved therapeutic efficacy. However, in recent years there has been an increasing number of reports on complications emanating from interactions between drugs and dietary supplements secondary to excessive pharmacological actions (adverse events) or constrained bioavailability of drugs (treatment failure). Most plant phytochemicals when ingested either as food or dietary supplements mimic drugs and are capable of interfering with the bioavailability and bioactivity of other drugs and food (nutrients) taken together or separately as they share the same metabolic pathways. Dietary supplements from *C. papaya* are no exception to such interactions as they are capable of modifying the absorption, distribution, metabolism, and excretion (ADME) properties of drugs through their effects on drug-metabolizing and transporting proteins in the body (Rodríguez-Fragoso et al., 2011).

Interactions Between Carica papaya *and Drugs*

Studies have reported potential interactions between *C. papaya* and different drugs. Most reported cases are at the preclinical level and their clinical significance cannot be confirmed; however, some cases show that interactions between *C. papaya* and drugs have raised serious concern about clinical implications (Rodríguez-Fragoso et al., 2011). The mechanisms under-

lying the interactions between *C. papaya* and drugs depend on two principal groups of factors: drug factors (pharmacokinetic or ADME properties of the drug, pharmacodynamics or drug/body interaction, and physical/biochemical interactions between papaya and drugs) and factors relating to characteristics of the constituent parts of *C. papaya* ingested (plant part, species, geographical origin, maturity, manufacturing processes, storage conditions, and seasonal variability). Additive interactions between *C. papaya* phytochemicals and drug actions can be beneficial (enhanced pharmacological actions) or detrimental (adverse reactions and treatment failure). Drugs, *C. papaya* dietary supplements, phytochemicals, their mode of interactions, and implications for body/structure functions are presented in Tables 3.32.5 and 3.32.6.

TABLE 3.32.5 Plants and Bioactive Principles That Form the Starting Point for Modern Drugs

Plant of origin	Bioactive principle	Pharmacological benefit	Remark and references
Artemisia annua	Artemisinin	Antimalaria	A sesquiterpene lactone found to be effective against all the malaria-causing protozoan organisms in the genus *Plasmodium*. The drug is used in the treatment of malaria involving chloroquine-resistant parasites and multidrug-resistant *P. falciparum* (Guo, 2016)
Atropa belladonna	Atropine	Spasmolytic	An anticholinergic drug used to treat slow heart rate, organophosphate insecticides and nerve gases poisoning and inhibit salivary and mucus glands (Máthé, 2014)
Cinchona succirubra	Quinine	Antimalaria	An alkaloid bioactive phytochemical in the extracts of cinchona which have been used as antimalarial drug since before 1633 (Achan et al., 2011)
Carica papaya	*Chymopapain*	Antiinflammation (slipped disk)	A cysteine proteinase enzyme used for intradiscal injection in patients suffering prolapsed intervertebral discs (Anibijuwon and Udeze, 2009)
Claviceps purperea	Ergot alkaloids (ergotamine)	Antimigraine	An ergot alkaloid vasoconstrictor. It is an alpha-1 selective adrenergic agonist and is commonly used in the treatment of migraine disorders (Schardl et al., 2006)
Coffee Arabica	Caffeine	Vasotherapeutics	A central nervous system (CNS) stimulant of the methylxanthine class used to treat bronchopulmonary dysplasia and apnea of prematurity and can enhance cognitive and physical performance (Nehlig et al., 1992)
Erythroxylon coca	Cocaine	Local anesthetics	A strong stimulant of tropane alkaloid class used as local anesthetic and vasoconstrictor (Luft and Mendes, 2007)
Ephedra sinica	Ephedrine	Sympathomimetics and sympatholytics (β-blockers)	alpha- and beta-adrenergic agonist of phenethylamine class. It has central nervous system stimulatory effects and has been used in the treatment of several disorders including asthma, heart failure, rhinitis, urinary incontinence, narcolepsy and depression (Kobayashi and Zhang, 2003)
Theobroma cacao	Theobromine	Vasodilator	A xanthine alkaloid, formerly used in the treatment of angina pectoris, hypertension and as a diuretic, commonly used as a bronchodilator and as a vasodilator (Usmani et al., 2005)
Camellia sinensis	Theophylline		Is a xanthine derivative used as a drug to treat muscle pain in people with peripheral artery disease. It is indicated mainly for asthma, bronchospasm, and COPD (Hansel et al., 2004)

(Continued)

TABLE 3.32.5 Plants and Bioactive Principles That Form the Starting Point for Modern Drugs—cont'd

Plant of origin	Bioactive principle	Pharmacological benefit	Remark and references
Rauwolfia serpentine	Reserpine	Antihypertensives	Reserpine is an adrenergic blocker of indole alkaloid class. It has been used for the control of high blood pressure and psychotic symptoms, though seldomly used nowadays because of its numerous side-effects (Shamon and Perez, 2009)
	Ajmaline	Antiarrythymics	Ajmaline is an alkaloid belonging to class Ia antiarrhythmic agent. It is used diagnostically to reveal typical findings of ST elevations in patients suspected of having Brugada syndrome (Wolpert et al., 2005)
Physostigma venenosum	Physostigmine	Antidementia	A parasympathomimetic alkaloid, used as an antidote against anticholinergic drug overdoses as it reversibly inhibit cholinesterase activity (Stilson et al., 2001)
Papaver somniferum	Morphine	Analgesics	Morphine alkaloid is a narcotic pain reliever that acts directly on the central nervous system to decrease the feeling of acute and chronic pain
	Papaverine	Spasmolytic	Is an opium alkaloid vasodilator drug, used primarily to improve blood flow in patients with visceral spasm (especially those involving the intestines, heart, or brain), and can be used in the treatment of erectile dysfunction (Kim et al., 2008)

TABLE 3.32.6 Herb-Drug Interactions: *Carica papaya* Phytochemicals and Various Classes of Drugs

C. papaya phytochemicals/ dietary supplements	Molecular target	Interacting drug	Remark and reference
Freeze dried hot aqueous infusion extract	Papaya metal chelation of ciprofloxacin. May also involved inhibition of a member of the influx transporter protein family (organicanion transporter polypeptide; OATP.)	Ciprofloxacin	In vivo. Metal cations chelate ciprofloxacin in aqueous solution and limit their antibacterial activity and associated adverse events. *C. papaya* reduced the bioavailabilitysince it is rich in cation mineral elements.
Fermented papaya preparation	ND. Inhibitionof P-gp is proposed in line with an in vivo finding	Glybenclamide	*Clinical,* fasting and postprandial plasma glucose were significantly reduced both in healthy and diabetic patients. The patients were induced to reduce or suspend their oral antidiabetic drug. FPP could inhibit P-gp to enhance bioavailability of glybenclamide. Dose adjustment of glybenclamide may be required
Ethanol leaves extract	the extract may bind part of glimepiride in GIT thereby delays it absorption	Metformin, glimepiride	In vivo. The extract delayed the hypoglycaemic activity of glimepiride but hastened that of metformin. The extract is thought to bound part of glimepiride or stimulates production and activity of enzymes that degrade it. (Fakeye et al., 2007)

TABLE 3.32.6 Herb-Drug Interactions: *Carica papaya* Phytochemicals and Various Classes of Drugs—cont'd

C. papaya phytochemicals/ dietary supplements	Molecular target	Interacting drug	Remark and reference
GMP certified Standardized *C. papaya* extract (proteolytic activity > 6000 NFPU/ mg papayafruit; Bio Serae LaboratoriesBram, France)	*C. papaya* reduced intestinal motility to inhibit the intestinal propulsion movements? It could also inhibit drug metabolizing (CYP isoenzymes; CYP3A and CYP2E1) and transporting proteins (P-gp) efflux activity	Amiodarone	In vivo and in vitro. Co-administration of single doses of Amiodarone and *C. papaya* in rats caused delay in t_{max} but could not alter the amiodarone C_{max} significantly. Thus, *C. papaya* extract or some of its phytochemical constituents increases the bioavailability of amiodarone through reduced intestinal absorption and inhibition of drug metabolizing and transporting proteins. Repeated administration of *C. papaya* extract also alters tissue distribution and may contribute to tissue accumulation and the target organ toxicity associated to amiodarone and MDEA
C. papaya fruit juice	Activation or induction of CYP3A4-mediated pre-systemic felodipine metabolism	Felodipine	In vivo. *C. papaya* reduced bioavailability of felodipine
Papain (complex proteases enzyme mixture)	Potentiate the effect of warfarin	Warfarin	Clinical. Patient developed skin, urinary and gastrointestinal bleeding after ingestion of the complex proteases enzyme mixture (as nonsteroidal antiinflammatory drug) and exhibited prolonged prothrombin time, activated thromboplastin time, and low functional and antigenic levels of prothrombin. Fresh frozen plasma and parenteral vitamin K ameliorated the condition (Perez-Jauregui et al., 1995)
C. papaya extract containing papain	Potentiate the effect of warfarin	Warfarin	The patient was admitted for cardiac surgery with an INR of 7.4 which decreased to 2.0 after withdrawal of both papaya extract (used as weight-loss aid) and warfarin (Shaw et al., 1997)
Ethanolextract of unripe fruit of *C. papaya*	Potentiation of digoxin effect by inhibition of P-gpefflux activityin Caco-2 cells	Digoxin	In vitro. *C. papaya* inhibited p-gp mediated transport of 3H-Digoxinon Caco-2 cellmonolayers using digoxinasa modelp-gpsubstrate. Therefore, interactions with conventional p-gp substrate drugs are likely to occuron co-administration which may result inaltered therapeutic outcomes
Aqueous extract of *C. papaya* leaves	Inhibition of P-gpefflux activityin the intestinal segment mounted in Ussing chamber	Digoxin	In vivo and ex vivo. *C. papaya* leaves extract increased the permeability of the P-gp substrate digoxin in the mucosal-to-serosal direction in intestinal segments mounted in Ussing chambers. However, co-administration of the leaf extract of *C. papaya* and digoxin in rats did not determine a significant increase in the extent of systemic drug exposure
C. papaya aqueous crude leaf extract	ND, pharmacodynamic interaction was suggested. *C. papaya* antioxidants activity could counteract artesunic acid mediated oxidants production during malaria parasite infection thus, isobolar equivalence reveal antagonism	Artesunic acid	In vivo, co-administration of *C. papaya* and artesunic acid enhanced antimalarial activity, prolonged the survival time and increased the cure rate compared to artesunic acid alone. Nevertheless, quantitative assessment of synergistic potential of the drug and *C. papaya* using isobolar equivalence showed that the combinations of artesunic acid and *C. papaya* are antagonistic (Onaku et al., 2011)

Interactions Between Carica papaya *and Anticoagulants/Antiplatelets*

There is a dearth of information on the clinical significance of interactions between *C. papaya* and anticoagulants in the body (Rodríguez-Fragoso et al., 2011). However, what little data are available on the potential interaction with anticoagulant drugs have been widely reported (Perez-Jauregui et al., 1995; Shaw et al., 1997). There is little or no information available in the literature characterizing the interaction between *C. papaya* and various classes of anticoagulants, but the interaction between *C. papaya* and warfarin has been described in a few reports. Warfarin, a cheap anticoagulant that has long been used in clinical settings, receives more attention regarding its potential interaction with herbs and foods. Therefore, patients and users of *C. papaya* or *C. papaya*-derived nutraceuticals and dietary supplements for whatever purpose should inform their doctors when taking other classes of anticoagulants.

We found two case reports of the interaction between warfarin and papain in the literature. One was the 1991–95 toxicology review conducted by the National Poisons Information Service in the United Kingdom. Although full details of the case have not been published, the patient reportedly maintained a therapeutic international normalized ratio (INR) while receiving warfarin, but began to take papaya extract containing papain as a weight loss supplement. The patient was admitted later for cardiac surgery with an INR of 7.4 which reduced to 2.0 after withdrawal of both papaya extract and warfarin (Shaw et al., 1997). In another case, a 47-year-old woman ingested an overdose of a nonsteroidal antiinflammatory complex mixture containing pancreatin, bromelin, papain, lipase, amylase, trypsin, alpha chymotrypsin, and rutin. She developed skin, urinary, and gastrointestinal bleeding which was thought to be the effect of a coumadin overdose. The patient exhibited a prolonged prothrombin time during an activated partial thromboplastin time test as well as low functional and antigenic levels of prothrombin. Administration of fresh frozen plasma and parenteral vitamin K to the patient reverted all laboratory and clinical abnormalities back to normal (Perez-Jauregui et al., 1995). The adverse reaction may be connected to components of the drug mixture, although experimental testing and high-performance liquid chromatography analysis revealed that the drug might have been contaminated by coumadin. Until further information about this potential interaction becomes available, patients receiving warfarin should be advised to avoid papain supplementation. The exact mechanisms behind the interaction between *C.papaya* and anticoagulants (warfarin) is not yet known; however, possible mechanisms include potentiation of inhibition of vitamin K-dependent coagulation factor by warfarin as well as inhibition of cyclooxygenase and prostaglandins through its antiinflamatory effect.

There are no clinical or preclinical reports on the interactions between *C. papaya* and any class of antiplatelet drug in the literature. Despite the lack of studies, there is theoretical concern here. *C. papaya* and its bioactive components are widely reported as having antiinflammatory properties and have been shown in many studies to increase platelet counts, reduce platelet aggregation in dengue-infected platelet-rich plasma in vitro, and significantly induce upregulation of platelet-specific genesin patients with dengue fever and dengue haemorrhagic fever. Platelet-type lipoxygenase (ALOX 12) and platelet-activating factor receptor (PTAFR) genes have been shown to influence platelet production and platelet aggregation. There is a need for research here to reveal possible interactions between *C. papaya* and antiplatelet drugs. This will have significant implications for patients suffering thrombotic events, especially those associated with dengue fever. A patient suffering a dengue-associated large-vein thrombotic event was recently reported in Brazil (da Costa et al., 2012).

Interactions Between Carica papaya *and Antidiabetic Drugs*

The available reports on the interaction between *C. papaya* and antidiabetic drugs are mainly preclinical. Therefore, the clinical significance cannot be ascertained. Coadministration of *C. papaya* ethanol leaf extract with metformin or glimepiride in an alloxan animal model of diabetes led to significant interactions with glimepiride or metformin which affected the hypoglycemic activities of the drugs. The extract delayed the onset of hypoglycemic activity of glimepiride and increased the hypoglycemic activity of metformin. The investigator proposed that the extract might delay absorption of the drug by binding up part of the drug or stimulating production and activity of enzymes that degrade some of the drug, especially in the gut (Fakeye et al., 2007). In a separate study, a fermented papaya preparation was shown to reduce both the postprandial and fasting plasma glucose level to a level that influenced patients who were already on an oral antidiabetic drug (glybenclamide) to reduce the dosage of their oral antidiabetic therapy and even suspend the drug in one patient (Danese et al., 2006). This is a serious clinical concern in patient management. Further studies are needed to elucidate the mechanism behind the interaction.

Interactions Between Carica papaya *and Antiarrhythmic Drugs*

Antiarrhythmic drugs (cardiac dysrhythmia medications) are a diverse group of drugs that are used in the treatment of abnormal heart rhythms (cardiac arrhythmias) such as atrial fibrillation, ventricular fibrillation, ventricular tachycardia, and atrial flutter. Preliminary studies in animals and in vitro have shown the potential interaction between *C. papaya*

and antiarrhythmic drugs, such as amiodarone and digoxin. Amiodarone is one of the most widely prescribed class III antiarrhythmic agents with a narrow therapeutic index. Coadministration of single doses of amiodarone and *C. papaya* in rats caused delay in the time taken to reach the maximum plasma amiodarone concentration, but did not alter the extent of systemic exposure to amiodarone. However, in rats pretreated with *C. papaya* extract for 14 days, before single-dose amiodarone, the extent of systemic amiodarone exposure was markedly increased while the peak of systemic exposure to amiodarone showed no significant change (Rodrigues et al., 2014). Thus, *C. papaya* extract or some of its phytochemical constituents could have antimotility properties that inhibit intestinal propulsion movements (Ezike et al., 2009), prolong the transit time of drugs, and increase the extent of absorption of amiodarone (Tarirai et al., 2010). In addition, *C. papaya* could have an inhibitory effect on drug-metabolizing (CYP isoenzymes) and transporting protein (P-gp) efflux activity that could be responsible for the increased bioavailability of amiodarone, since amiodarone is known to be a cytochrome p450 (CYP) isoenzyme and a P-gp substrate.

Tissue distribution and resultant change in the tissue-pharmacodynamic effect of a drug is another area of concern in herb–drug interaction. With regard to this, repeated administration of *C. papaya* extract before amiodarone treatment increases the concentration of amiodarone in the lungs and results in greater exposure of heart and lung tissues to mono-N-desethylamiodarone (MDEA), the main metabolite of amiodarone. Therefore, repeated administration of *C. papaya* extract could contribute to tissue accumulation and target organ toxicity associated with amiodarone and MDEA (Rodrigues et al., 2014). Digoxin, a class V antiarrhythmic agent, is commonly used to treat heart failure. *C. papaya* leaf water extract significantly increased (54.50%) the apparent permeability of digoxin in the mucosal-to-serosal direction in intestinal segments mounted in Ussing chambers. However, coadministration of *C. papaya* leaf extract and digoxin in rats did not bring about significant change in the extent of systemic drug exposure (Oga et al., 2013). Note that these reports are taken from preclinical studies which may not represent the clinical experience; however, they do indicate the need for a clinical study to ascertain the clinical significance of the reports.

Interactions Between Carica papaya *and Antimalaria Drugs*

There is an increasing tendency in patients to take herbs (*C. papaya*) at the same time as antimalaria drugs. This is understandable because malaria remains one of the world's foremost infectious diseases causing the death of children and pregnant women in poor tropical regions including West Africa, India, and Central and South America where traditional medicines (herbs) are as popular as allopathic medicines. Again, as a result of the increasingly resistant malaria parasite concomitant use of alternative (herbs) and allopathic medicines in most developing malaria-endemic countries is on the increase. To date, there has been no clinical case reporting on the interaction between *C. papaya* and any of the antimalaria drugs, despite concerns that the citizens of poor developing countries are most likely taking herbs (including papaya products) at the same time as commonly prescribed antimalaria drugs. The reasons are obvious and relate to the high cost of new and safe antimalaria drug and the belief that herbs (*C. papaya* products) enhance the efficacy of antimalaria drugs. This may be connected to the attitude of patients toward disclosure of previous or concomitant herbal therapy to their doctors. However, preclinical data do show the potential interaction between *C. papaya* and artesunic acid (antimalaria drug). Onaku et al. (2011) showed that coadministration of *C. papaya* and artesunic acid enhanced antimalarial activity, prolonged survival time, and increased the cure rate compared with artesunic acid alone. Nevertheless, quantitative assessment of the synergistic potential of the drug and *C. papaya* using isobologram analysis showed that combinations of artesunic acid and *C. papaya* are antagonistic, despite the fact that artesunic acid enhances its activity.

Interactions Between Carica papaya *and Antimicrobial Drugs*

C. papaya has been found to interact with antimicrobial drugs. Ciprofloxacin, a quinolone antibacterial drug, is used in the treatment of a wide range of bacterial infections. It is known to be associated with many side effects ranging from altered biochemical markers of hepatorenal function, of the endogenous antioxidant system, of dyslipidemia, to changes in immune system functions. When *C. papaya* and ciprofloxacin are coadministered in rats, elevated oxidative stress and other markers in ciprofloxacin-treated rats are markedly reduced without any adverse event in body organs and erythrocytes. This shows that combined use of *C. papaya* and ciprofloxacin can potentially prevent the side effects commonly experienced by patients on ciprofloxacin treatment alone. This can be linked to the interaction between *C. papaya* and ciprofloxacin. The mechanism behind the interaction is not understood; however, decreased absorption of ciprofloxacin or the direct effect of *C. papaya* leaf extract in the body is suspected (Imaga et al., 2012). In a recent in vivo pharmacokinetic analysis of ciprofloxacin in combination with *C. papaya* leaf water extract, *C. papaya* was shown to reduce the bioavailability of ciprofloxacin by decreasing the rate of absorption and volume of distribution as well as increasing the elimination rate, which confirm an earlier report on the possible interaction between *C. papaya* and ciprofloxacin (Ukpo et al., 2017). In another study, concomitant

use of *C. papaya* seed methanol extract and amoxicillin revealed a potential additive interaction against *E. coli* ATCC 25922 with a fractional inhibitory concentration index of 0.99, but no interaction was observed when used against *S. aureus* ATCC 25923 with a fractional inhibitory concentration of 2.51 (Bridge et al., 2015). Giordani et al. (1997) reported that *C. papaya* latex and fluconazole brought about a synergistic bactericidal effect on *Candida albicans* by enhanced cell wall degradation. Likewise, Adejuwon et al. (2011) and Rakholiya and Chanda (2012) showed a possible synergistic antimicrobial interaction between *C. papaya* root and leaf methanol extract and certain known antimicrobial drugs such as penicillin G, ampicillin, amoxyclav, cephalothin, polymyxin B, rifampicin, amikacin, nilidixic acid, gentamicin, chloramphenicol, and ofloxacin against a wide spectrum of Gram-positive and Gram-negative bacteria, but not with tetracycline.

Interactions Between Carica papaya *and Other Herbs/Dietary Supplements*

There is increasing awareness of the drug–herb interaction, but little is known about the interaction between herbs and other dietary supplements in the mainstream medical literature. However, it is common practice among traditional medical experts (herbalists) to combine two or more herbs/dietary supplements in an effort to enhance therapeutic efficacy. Many studies have reported the use of combinations of *C. papaya* and other herbs or dietary supplements to achieve enhanced therapeutic efficacy or to multitarget in the healing process.

Interaction Between Carica papaya *and* Vernonia amygdalina

C. papaya combined with *Vernonia amydalina* has long been used in folkloric medicine among Nigerian (West Africa) tribes to treat malaria. In an attempt to provide scientific evidence of the therapeutic and synergistic potential (interaction) of this herb–herb combination the leaf water extracts of *C. papaya* and *V. amygdalina* were coadministered in mice infected with *Plasmodium berghei*. The combination markedly reduced the parasite load and at the same time enhanced recovery of hepatic cell damage and hematological parameters (Okpe et al., 2016).

Interaction Between Carica papaya *and Flamboyant Bark (*Delonix regia*)*

Delonix regia flamboyant bark extract and *C. papaya* extract have both long been used in folkloric medicine as antimalaria agents, but separately. Recently, Fatmawaty et al. (2017) investigated the potential synergistic interaction between *C. papaya* leaf and flamboyant bark ethanol extracts in *P. berghei*–infected mice. A ratio of 1:1 was found the most efficacious and exhibited synergistic or additive effects in vivo. It had much better antiplasmodial properties than every single-dose plant extract tested in the study.

Interaction Between Carica papaya *and* Carissa spinarum

A potential synergy has been demonstrated for combined use of methanol extracts of *C. papaya* leaves (MECpL) and *Carissa spinarum* leaves (MECsL) as antimicrobial agent against *S. aureus* using fractional inhibitory concentrations to determine the interactions. Using the same approach, an additive interaction was found for a number of combinations: ethanol extracts of *C. papaya* root (EECpR) and *C. spinarum* bark (EECsB), petroleum ether extract of *C. papaya* leaves (PECpL) and methanol extract of *C. spinarum* root (MECsR), and petroleum ether extract of *Carissa spinarum* leaves (PECsL) and ethanol extract of *C. papaya* seed (EECpS) as antimicrobial agents against *S. aureus*. PECsL and EECpS, EECpR and EECsB, and PECpL and MECsR were also found to have an additive interaction against *E coli*. However, combinations of EECpR and ethanol extract of *C. papaya* leaves (EECpL), PECpL and MECsR, PECsL and EECpS, and EECsR and EECpL exhibited antagonism when tested against *E. coli* and *S. aureus* respectively (Rubaka et al., 2014).

Interactions Between Carica papaya *and Many of the Herbs in Polyherbal Preparations*

A polyherbal preparation with *C. papaya* as a major herb component named Nefang has been reported for its potential health benefits. The polyherbal preparation comprises *Mangifera indica* (bark and leaf), *Psidium guajava*, *C. papaya*, *Cymbopogon citratus*, *Citrus sinensis*, and *Ocimum gratissimum* leaves. Tarkang et al. (2013, 2014a,b, 2015) conducted extensive studies into the biological properties of the polyherbal preparation and the constituents of the plants. The various plant components of Nefang, *C. papaya* leaves included, were individually shown to exhibit good antioxidant properties as a measure of therapeutic value in vitro. Additive interaction of the plant components in Nefang in vivo markedly reversed oxidative stress and enhanced the endogenous antioxidant enzyme system to ameliorate alterations in hepatorenal function markers (Tarkang et al., 2013). In vitro sensitivity assessment (on chloroquine-sensitive and multidrug-resistant *P. falciparum* strains) of Nefang component combinations (pairwise) for malaria using isobologram analysis at variable potency ratios showed that *C. papaya*/*O. gratissimum* and *C. papaya*/*C. sinensis* exhibited strong synergism with antiplasmodial

activities greater than five times that of Nefang (Tarkang et al., 2014a). The paired combinations interact in such a way that they enhance antiplasmodial activity better than the parent herbal drug (Nefang). These findings were separately confirmed in an in vivo antimalarial activity study on Nefang-treated BALB/c mice and Wistar rats preinfected with *P. berghei* and *Plasmodium chabaudi* (Tarkang et al., 2014b).

Herb–herb interaction or combination therapy has been given considerable attention in the treatment of typhoid. This has long been used in traditional medical practice. Combinations of parts of usually more than two plants are mixed in a decoction for patients. Nkuo-Akenji et al. (2001) and Oluduro and Omoboye (2010) extensively investigated the synergistic antibacterial potentials of different polyherbal formulations commonly prescribed by traditional medical practitioners against different species of *Salmonella* (*S. typhi, S. paratyphi,* and *S. typhimurium*) including the multidrug-resistant strains (reviewed in detail by Aliero and Ibrahim (2012)). Many of these herbal combinations, especially those that contained *C. papaya* as one of the important constituent plants, exhibited enhanced antibacterial activities against different species of *Salmonella* better than when they were tested alone.

CONCLUSION

C. papaya is an herbaceous laticiferous plant cultivated in most tropical and subtropical countries of the world. Asia (mainly India) is the largest producer. The plant produces latex rich in papain (a major proteolytic enzyme) and contains phenolics, flavonoids, and alkaloids as the major phytochemicals. These are responsible for the traditional and modern uses of *C. papaya* and its products as nutritional supplements and therapeutic agents under various disease conditions. The established pharmacological properties (antioxidant, antiinflammatory, immunomodulatory, and antimicrobial) of *C. papaya* make it a candidate in the treatment of a wide range of diseases (e.g., dengue fever, gastrointestinal infections, and periodontitis) and for wound healing. Studies have shown that *C. papaya* can interact with standard drugs and other herbal products to cause enhanced therapeutic efficacy, but can bring with it adverse effects or treatment failure. Therefore, patients and medical practitioners should exercise caution when using *C. papaya* and its products as medical remedies.

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Chapter 3.33

Passiflora (*Passiflora incarnata*)

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INTRODUCTION

Passion flower (*Passiflora incarnata*) is a member of the Passifloraceae. It has been given various names such as maypop, purple passion flower, true passion flower, wild apricot, and wild passion vine. It is the largest member of the Passifloraceae family with 16 genera and 650 species (Wiart, 2006). In fact, some of these species have long had applications as herbal tea. *P. incarnata* L. is a fast-growing perennial plant. It has large, intricate flowers with prominent styles and stamens and is one of the hardiest species of passion flower. In the southern United States, it is a common wildflower. The wild species readily available are *Passiflora edulis*, *Passiflora leschenaultia*, *Passiflora mollissima,* and *Passiflora subpeltata*. The constituents responsible for the typical odor of *P. incarnata* include limonene, cumene, α-pinene, prezizaene, zizaene, and zizanene (Buchbauer and Jirovetz, 1992).

Most of the species are tender evergreen tropical vines. *P. incarnata* is different from the other species of the genus as it is deciduous and can survive winter frosts. The reason it is also called maypop is as a result of its leaf buds opening in spring. Many species of *Passiflora* and their hybrids have beautiful ornamental flowers and, therefore, are cultivated. Additionally, approximately 60 varieties attract high market values as a result of the quality of their edible fruits. The traditional therapeutic values of different species of the genus are not confined to South America, since such values are also found in the Netherlands, Spain, and Italy (Patel et al., 2009; Patel et al., 2011). The literature reveals that different traditional claims made about extracts from *P. incarnata, P. edulis*, and *Passiflora alata* have so far been scientifically investigated, while the remaining species remain mostly untouched (Zucolotto et al., 2012). Many species of the genus have beautiful flowers and are widely grown by gardeners. Their diverse chemical composition makes them equally attractive for scientific research; this is especially true of pharmaceutical companies in their efforts to isolate bioactive lead compounds.

Recently, there has been an increase in the popularity of using nonvitamin nonmineral (NVNM) supplements in various communities, especially among young people. The main reason for their increased use is the belief that they can enhance energy, fitness, and weight loss, a belief that has its roots in print and electronic media. Sometimes such use has brought about unwanted side effects (Ayranci et al., 2005); therefore, only prescribed medication should be used. Different passiflora species can also be used as NVNM supplements either singly or combined with other herbs. The nutritious value of different *Passiflora* species has been reported by Corrêa et al. (2016). In this chapter we focus on the different phytochemical, pharmacological, nutritious, and toxicological aspects of *P. incarnata*.

PLANT HABITAT

Most *Passiflora* species are indigenous to South America, eastern Asia, southern Asia, and New Guinea. Nine species are native to the United States, the most northerly of which are found in Ohio, others in west California and south Florida. Australia and New Zealand are also rich in *Passiflora* species. Moreover, new species have been added to the genus in recent times. For example, *Passiflora pardifolia* and *Passiflora xishuangbannaensis* have only been known by the scientific community since 2006 and 2005, respectively. Some species of *Passiflora* have been naturalized beyond their native ranges. For example, blue passion flower (*Passiflora caerulea*) now grows wild in Spain. The purple passion fruit (*P. edulis*) and its yellow relative *Passiflora flavicarpa* have been introduced in many tropical regions as commercial crops.

PHYTOCHEMISTRY

Passiflora has a rich variety of phytochemicals (Table 3.33.1), but the main components are different types of flavonoid glycosides. However, different species have different concentrations.

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00049-7

TABLE 3.33.1 List of Isolated Secondary Metabolites From *Passiflora incarnata*

Group	Name	References
Flavonoids	Isovitexin, vitexin, isoorientin	Glotzbach and Rimpler (1968), Schilcher (1968)
	Schaftoside, isoschaftoside, isovitexin-2″-O-glucopyranoside and isoorientin-2″-O-glucopyranoside	Qimin et al. (1991)
	Isovitexin, isoorientin, schaftoside, isoschaftoside, swertisin	Rehwald et al. (1994)
	Isoscoparin 2″-O-glucoside	Rahman et al. (1997)
	Apigenin, chrysin, kaempferol, luteolin	Zanoli et al. (2000)
Alkaloids	Harman, harmol, harmine, harmalol, harmaline	Poethke et al. (1970)
γ-Benzopyrone derivative	Maltol	Aoyagi et al. (1974)

Plants primarily comprise C-glycosylflavones coupled with apigenin, chrysin, and luteolin (Zanoli et al., 2000). In 1968, researchers isolated several compounds from the leaves and stems of *P. incarnata* L., and isovitexin, vitexin, and isoorientin from other species (Glotzbach and Rimpler, 1968; Schilcher, 1968). Rehwald et al. (1994) identified some of the flavonoids including isovitexin, isoorientin, schaftoside, isoschaftoside, and swertisin. Similarly, Qimin et al. (1991) identified various glycoside flavonoids such as schaftoside, isoschaftoside, isovitexin-2″-O-glucopyranoside, and isoorientin-2″-O-glucopyranoside. A highly sensitive high-performance liquid chromatography (HPLC) method was developed by Rehwald et al. (1994) for the qualitative and quantitative analysis of flavonoids. The concentration of isovitexin was observed to be highest in *P. incarnata* in comparison with other species of the genus.

Similarly, β-carboline alkaloids have been found in *P. incarnata*. Poethke et al. (1970) found various indol alkaloids in the plant—namely, harman, harmol, harmine, harmalol, and harmaline. However, their concentration must be very small because these β-carboline alkaloids were not detected when studied in some commercial material from *P. incarnata*. When cultivated in a greenhouse the vegetative parts of the plant exhibited 0.012% harman and 0.007% harmine. On the other hand, the concentration of these alkaloids in the same plant when grown in a field was a little bit different (0.005% harman while harmine was not detected at all). Similarly, Aoyagi et al. (1974) isolated maltol, a γ-benzopyrone derivative, from dry extract of *P. incarnata*.

ESSENTIAL OIL COMPOSITION

In 1994 Buchbauer and Jirovetz (1992) explored the chemical composition of essential oil of *P. incarnata* using various analytical tools such as gas chromatography (GC), gas chromatography/ mass spectroscopy (GC/MS), and gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR). These tools used stem distillation for oil extraction. The very comprehensive determination of oil composition brought about by these techniques led to the identification of more than 160 different compounds and thus revealed the very complex chemical nature of the plant's essential oil. The results showed that the major components were carvone (8.1%), benzyl alcohol (4.1%), linalool (3.2%), transanethole (2.6%), p-ionone (2.6%), and two fatty acids (palmatic acid 7.3% and oleic acid 6.2%) (Fig. 3.33.1). The major component, carvone, has recently been associated with multiple therapeutic effects that are antimicrobial, antidiabetic, anticonvulsant, and antispasmodic in nature (Castiglione et al., 2017).

PHARMACOLOGY

Analgesic Effect

In light of the traditional use of *P. incarnata* as a means of treating addiction the butanol fraction of leaves was studied for antinociceptive effects in animal models (Ingale and Kasture, 2012). The authors tested the effects in eye-wiping and formalin tests at intraperitoneal doses of 150 and 300 mg/kg. The results revealed a marked antinociceptive effect

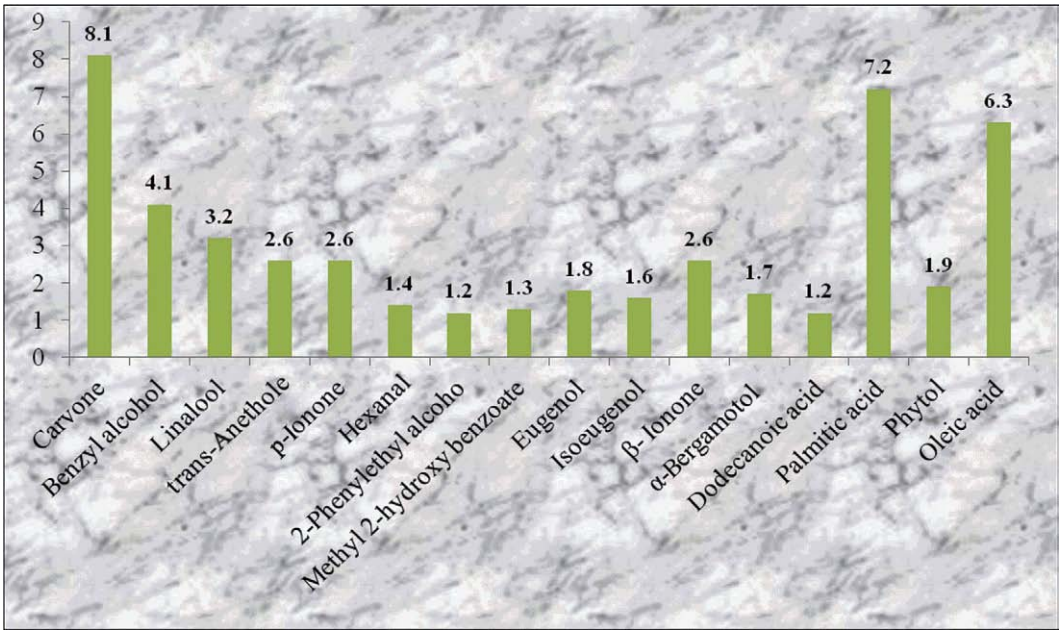

FIG. 3.33.1 Major chemical composition of essential oil of *Passiflora incarnata.*

of the leaves in both tests when injected intraperitoneally. The antinociceptive butanol of the plant's leaves was strongly antagonized by the pretreatment of naloxone, while no effect on atropine treatment was found in the formalin test. Thus, this implies that phytochemicals in the plant extract interfered with central-acting pain mediators.

Recently, Aman et al. (2016) studied the effect of plant methanolic extract in streptozotocin-induced diabetic rats in a neuropathic nociceptive model. At doses of 200 and 300 mg/kg, it showed significant antinociceptive effects. Investigation of the mechanism showed naloxone and pentylenetetrazole treatment to be antagonistic. The mechanism was suggested as being mediated through the opioidergic and GABAergic signaling pathways.

ANTIANXIETY AND ANTICONVULSANT EFFECTS

In various animal models assessing the plant's anxiolytic-like effect the plant extract showed marked antianxiety effects at test doses. Such activity can be attributed to the various secondary metabolites of the plant such as chrysin which mediates its effect through modulation of γ-aminobutyric acid (GABA) A receptors (Patel et al., 2009). Clinical studies in humans endorsed the animal studies. When it was administered to preoperative patients for control of anxiety the plant extract consumed as tea showed positive effects on the quality of sleep (Movafegh et al., 2008). Similarly, the extract caused anticonvulsant-like effects in pentylenetetrazole-induced seizures in mice probably due to interference with benzodiazepine receptors (Nassiri-Asl et al., 2007).

Effects on Withdrawal

P. incarnata has traditionally been used to treat addiction to cannabis or cannabis products. Keeping this in mind, Dhawan et al. (2002b) studied the effect of benzoflavones isolated from the plant in vivo. When benzoflavones (10 and 20 mg/kg twice daily) were administered in combination with cannabinoids (delta-9-tetrahydrocannabinol; 10 mg/kg twice daily), they brought about a significant reduction in tolerance to and dependence on cannabinoids. The experiment lasted 6 days, but even when studied for the effect of acute administration a similar situation was observed.

On the basis of traditional uses of the plant in tobacco addiction, benzoflavones isolated from *P. incarnata* were evaluated at doses of 1–20 mg/kg (Dhawan et al., 2002a). They were injected in combination with hydrogen tartrate (2 mg/kg). Significant changes in nicotine withdrawal symptoms were recorded in the overall 14-day treatment period. The effect was dose dependent and high doses elicited more pronounced effects in the reversal of nicotine withdrawal symptoms and behavioral status of experimental rodents.

Similarly, bioactive secondary metabolites (benzoflavones) of *P. incarnata* were studied for possible effects on alcohol withdrawal (Dhawan et al., 2002c). The authors used both pure compounds and plant extract for comparison. The results

showed a marked reduction in addiction to alcohol. It was also found that plant extract in chronic treatment had more significant control over addiction than did benzoflavones.

Aphrodisiac Action

The aphrodisiac effect of *P. incarnata* was investigated in animals (Dhawan et al., 2003b). The effect of plant methanolic extract on male mice exhibited significant aphrodisiac behavior at 75, 100, and 150 mg/kg per os (p.o., orally). Maximum effects were shown at a dose of 100 mg/kg p.o which was reflected by the maximum number of mountings after 90 min of treatment, more than at the high dose of 150 mg/kg p.o. This might be due to the extract's sedative-like effects at the high dose.

ANTIASTHMATIC AND ANTICOUGH EFFECTS

P. incarnata leaf extract significantly inhibited acetylcholine-induced bronchospasms in guinea pigs. This can be attributed to α-adrenoceptor function reported after excessive or continuous administration of an α-receptor agonist (Dhawan et al., 2003a). Additionally, when leaf extract was used as a remedy to sulfur dioxide–induced coughs in mice, marked antitussive effects were observed at 100 and 200 mg/kg p.o. (Dhawan and Sharma, 2002).

ANTI-*HELICOBACTER PYLORI*

P. incarnata extract showed moderate inhibitory activity against *Helicobacter pylori* (Masadeh et al., 2014). Thus, it could be effective in the treatment of ulcers.

ANTIDIABETIC EFFECT

P. incarnata leaf extract had an antidiabetic-like effect when injected at 100 and 200 mg/kg in diabetic mice. Pretreatment improved oral glucose tolerance, regaining of weight, urine glucose, liver glycogen, and overall lipid profile (Gupta et al., 2012) (Fig. 3.33.2).

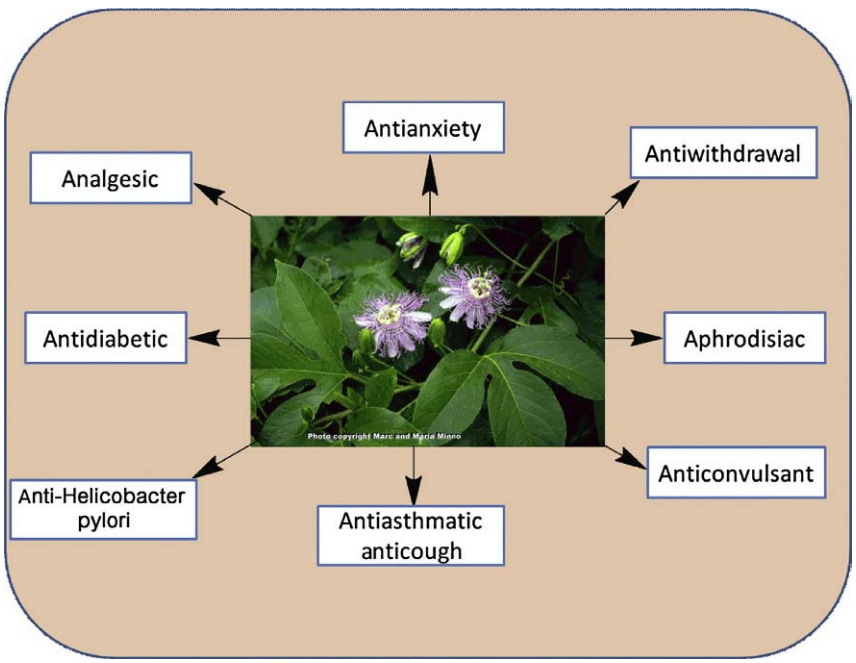

FIG. 3.33.2 Various pharmacological effects of *Passiflora incarnata.*

NUTRITIOUS VALUE

Since *P. incarnata* has traditionally been used as an herbal tea, its nutritious value needs to be explored. Carbohydrates supposedly in the form of raffinose, sucrose, D-glucose, and D-fructose are found in the chemical composition of the plant (Patel et al., 2009). Raffinose is composed of galactose, glucose, and fructose. It is only 20% as sweet as sucrose. α-Galactosidase is the enzyme that hydrolyzes raffinose to sucrose, but it is found in the human intestine. As a result, its breakdown occurs in the large intestine via resident bacteria and leads to excessive production of gas and thus flatulence. However, food technologists can take advantage of this quality of raffinose by using it as a food additive to reduce calories (Nishizawa-Yokoi et al., 2008). Other carbohydrates found in the plant include sucrose, D-glucose, and D-fructose which are instant sources of energy for both humans and plants. These carbohydrates act as primary metabolites in plants.

The main phytochemicals of *P. incarnata* are flavonoids (Table 3.33.1) which have long been known for their nutritious value. Since fruit, vegetables, tea, and wine are the primary dietary sources of flavonoids for humans, they have been extensively investigated for their health-supportive properties (Yao et al., 2004). Apigenin, chrysin, kaempferol, and luteolin are the flavonoids found in *P. incarnata* and freely found in dietary supplements (He et al., 2016). Similarly, flavonoids with sugar attachments are also frequently found in dietary components and may add to *P. incarnata*'s nutritious level. Steroids, such as stigmasterol and sitosterol, are present in the plant and can be used as precursors/intermediates in the manufacture of semisynthetic vitamin D3 and human hormones such as progesterone, androgens, estrogens, and corticoids.

Similarly, the composition of essential oil is very complex with approximately 150 compounds identified by GC/MS. They are nutritionally rich and include carvone (8.1%), fatty acids, palmitic acid (7.1%), oleic acid (6.3%) and numerous terpenoids.

TOXICOLOGY

P. incarnata has been approved by the US Food and Drug Administration as a safe herbal sedative. Overall, the literature also supports its safety. Boll et al. (2014) studied the effect of the plant (30 and 300 mg/kg) during gestation and lactation. The results showed the plant has a strong antioxidant effect without any maternal reproductive toxicity. However, the plant has shown significant neurological effects. Therefore, in combination with drugs acting on the central nervous system it should be used with care. Fisher et al. (2000) reported a clinical case in which a 34-year-old female was hospitalized following self-medication with *P. incarnata* at recommended doses as a consequence of severe nausea, vomiting, drowsiness, and ventricular tachycardia. This clearly suggests that only prescribed herbal products should be used to prevent any unwanted event.

In conclusion, pharmacological studies have strongly validated the plant's wide traditional uses for the treatment of various disorders. Moreover, as a result of the plant's versatile chemical background for such applications as nutrition, it can safely be recommended for large-scale formulation as an herbal supplement.

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Chapter 3.34

Pineapple (*Ananas comosus*)

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INTRODUCTION

The botanical name of pineapple is *Ananas comosus*. It is considered an herbaceous, tropical, and monocot perennial plant. The size of the plant ranges from approximately 1–2 m tall and wide. Its leaves are spiral in arrangement and on the terminal ends has flowers which then produce edible fruit. The stem at its center is about 25–50 cm long. A mature pineapple plant has about 60–80 leaves, each being sword shaped. After bananas and citrus fruits, pineapple is the third most produced fruit in the world (Bartholomew et al., 2003).

TAXONOMY

A. cosmosus belongs to the family Bromeliaceae which is further classified into three subfamilies, Tillandsioideae, Bromeliodeae, and Pitcarniodeae. *A. cosmosus* belongs to the subfamily Bromeliodeae (Bartholomew et al., 2003; Elfick, 2007).

This subfamily, Bromeliaceae, has about 2794 species and consists of 56 genera. It has the ability to adapt to a wide range of environmental conditions from sun to shade, from humid to very dry conditions, and also from hot tropical to cold subtropical conditions. The fruits are capsulated or berry-like and composed of bared, plumose seeds (Purseglove, 1975).

The *Ananas* genera, due to its characteristics, is considered to hail from Bromeliaceae. It is very unique in its shape and structure, consisting of broad leaves and fruits, and varies from medium to large in size. The unique characteristics of pineapple distinguishes it from other monocots. The taxonomical classification of pineapple went through different stages and modifications at different times, finally the classification proposed by Coppens in 2003 was internationally accepted (Bartholomew et al., 2003; Gilmartin and Brown, 1987).

There are around 30 cultivars of pineapple worldwide which are grown in different environmental conditions in tropical regions, but for the sake of commercial ease these various cultivars are classified into four classes, named as "Red Spanish," "Pernambuco," "Queen," and "Smooth Cayenne." Multiple factors contribute to the effective production of pineapple including, rainfall, soil type, nutrient requirements, drainage, and temperature. The most favorable temperature for growth is estimated to be 18–32 °C. Pineapple does not require more water compare to other fleshy fruits as it can be grown in the soil that does not have abundant of water from irrigation or rain; 5 cm^3 water per month is required for optimum growth (during both autumn and spring an average of 115 cm^3 rainfall is optimum for pineapple). Sandy and loamy soil, having a pH range from 4.5 to 6.5 with good water drainage makes soil perfect for pineapple production, for this purpose pineapple fields are deliberately built on slopes to provide good water drainage system (Black, 1962; Claude et al., 1987; Morton and Dowling, 2013).

DIVERSE ORIGINS

According to many researchers, pineapple originates from South American countries such as Columbia, Brazil, and Paraguay, and was exported to other parts of the world through travelers and historians who disseminated information regarding pineapple—especially the Portuguese and Spanish. Mutations in pineapple plants such as size of fruits, seedless fruits, and increased sweetness have occurred in the time since their discovery (Collins, 1960; Purseglove, 1975).

By the 16th century pineapple had already reached most parts of the world including the Philippines, China, India, African coasts, Holland, and England. The dispersion of the pineapple throughout the world is a result of its characteristic survival qualities in terms of different environmental conditions and droughts, and also the way it produces propagules with ease in undercultivated environments Table 3.34.1 (Collins, 1960; Morton and Dowling, 2013; Purseglove, 1975).

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00050-3

TABLE 3.34.1 Top 10 Pineapple-Producing Countries (Pariona, 2017)

No.	Country	Production (thousands of metric tons)
1.	Costa Rica	26,853
2.	Brazil	2483
3.	Philippines	2458
4.	Thailand	2209
5.	Indonesia	1837
6.	India	1571
7.	Nigeria	1420
8.	China	1386
9.	Mexico	771
10.	Columbia	643

COMMERCIAL USE OF PINEAPPLE

Pineapple is substantially produced because of its fruit; bearing good taste and flavor it is popularly used in different cuisines and by the food industry. Currently, pineapple is produced in almost in every corner of the world and due to its multiple applications, especially its biological importance, makes it incredibly well known worldwide. Annual worldwide production of pineapple is approximately 24.8 million tons Table 3.34.2 (Medina and García, 2005; Rohrbach et al., 2003).

TABLE 3.34.2 Pineapple's Nutritional Value, According to the US Department of Agriculture (USDA, 2016)

Nutrient	Unit	Value per 100 g
Water	g	86
Energy	Kcal	50
Energy	KJ	209
Protein	g	0.54
Total lipid	g	0.12
Carbohydrate by difference	g	13.12
Total sugars	g	9.85
Calcium	mg	13
Potassium	mg	109
Magnesium	mg	12
Phosphorus	mg	8
Vitamin C	mg	47.8
Niacin	mg	0.500
Pantothenic acid	mg	0.213

NUTRITIONAL VALUE OF PINEAPPLE

The overall composition of pineapple indicates that water is its major component, at 81%–86%, while total solids are 13%–19%. The solid portion is around 85% carbohydrate, mostly sucrose, glucose, and fructose, with the remaining 15% composed of other essential nutrients. Table 3.34.3 summarizes the constituents in both pineapple waste and pulp (Hulme, 1971; Rasid and Hosain, 1987).

Pineapple, along with its sweet taste, is very rich in essential nutrients including potassium and calcium, vitamin C, copper, folate, glycans, fiber, and other essential elements. All these ingredients make pineapple a good candidate for part of a balanced nutritional diet. One of the most favorable aspects of its composition is that it contains a minimal amount of fat and sodium, but contains high amounts of carbohydrate. The nutritional value of pineapple is summarized in Table 3.34.3 according to dietary reference intakes (DRI)/daily value (DV) (Farid Hossain et al., 2015; Mateljan, 2006).

MEDICINAL IMPORTANCE OF PINEAPPLE

Pineapple is used in different foods. Historically, pineapple has been used for medicinal purposes in many cultures and as time has passed increasing information has come to light about the nutritional value and beneficial ingredients of pineapple, making it even more popular among the masses. Therefore, in this chapter some of the medicinally significant ingredients of pineapple fruit and extracts are discussed along with their mechanisms of action.

BROMELAIN FROM PINEAPPLE

The chief medicinal component of pineapple is bromelain which was identified in the late 19th century. Its therapeutic potency was revealed in 1957 when for the first time it was found that pineapple was one of its major sources. Bromelain is a protein extract which can refer to either the two digestive enzymes called proteolytic enzymes present in it, or can refer to the combination of all enzymes and other ingredients in it. It can be found both in the fruit and stem of the pineapple plant and therefore is named accordingly as fruit bromelain and stem bromelain, respectively. The enzymatic composition of both types of bromelain also varies. Pineapple fruit is widely used as food source, therefore the major extraction source for bromelain is the stem of the pineapple plant, which is the cheaper source since it is the nonedible part of the plant (Heinicke and Gortner, 1957).

Although possessing much potential as a medicinal agent, bromelain has only been approved, in the form of the drug "NexoBrid," for topical use in 2012 by the European Medicines Agency (EMA, 2012) for removing dead cells from the skin surface.

TABLE 3.34.3 Nutritional Value of Pineapple per 165 mg

Pineapple fresh chunks[a] or one cup = 165 g				
Nutrients	**DRI/DV (%)**	**Amount**	**Nutrient density**	**WHF rating**
Vitamin C	105	78.8mg	22.9	Excellent
Manganese	77	1.53mg	16.7	Excellent
Copper	20	0.18mg	4.4	Very good
Vitamin B6	11	0.13mg	2.4	Good
Vitamin B1	11	0.18mg	2.3	Good
Fibre	9	2.31mg	2	Good
Folate	7	29.7mg	1.6	Good
Pantothenic acid	7	0.35	1.5	Good

DRI; *DV*; *WHF*, World's healthiest foods.
Ratings: excellent is for a DRI/DV value greater than or equal to 75% or a density that is greater than or equal to 7.6 V; very good is for a DRI/DV value greater than or equal to 50% or a density that is greater than or equal to 3.4; good is for a DRI/DV value greater than or equal to 25% or a density that is greater than or equal to 1.5.

[a] *One chunk or one cup is equivalent to 165 g, therefore, the nutrients are calculated on the basis of 165 g.*

Apart from this, no other established uses have been approved by the official agencies. Here we discuss the wide scope and potential biological activities of bromelain observed in different research studies (EMA 2012; Pavan et al. 2012).

According to biochemical studies, crude bromelain is composed of two proteolytic enzymes containing sulfhydryl proteases (proteases containing a thiol group). These enzymes possess a common cysteine amino acid residue in their catalytic site and possess a common mechanism where the cysteine moiety acts as a nucleophile and attacks electrophiles on the substrate. Apart from the proteases they contain glucosidase, phosphatases, cellulase, peroxidase, carbohydrates, and glycoproteins. As mentioned earlier, the source for both types of bromelain is different, therefore, the enzymes they possess are also different, known as EC.3.4.22.32 (stem bromelain) and EC.3.4.22.33 (fruit bromelain). The pH range for the enzymatic action of these enzymes is 5.5–8 (Bhattacharyya, 2008; Maurer, 2001; Taussig and Batkin, 1988).

ORAL ABSORPTION AND BIOAVAILABILITY OF BROMELAIN

One of the key features of bromelain is its ability to be absorbed through the gastrointestinal tract without much hindrance or degradation. About 12 g/day of bromelain can be absorbed safely with no side effects. When given orally about 40% of the high molecular-weight ingredients successfully reach the blood stream (Rowan and Buttle, 1994).

According to the findings of Castell et al. (1997), bromelain does not lose its proteolytic activity in plasma. When checked, the bromelain in rabbits was found to be active even in the presence of the two native antiproteolytic glycoproteins of the blood, α-antichymotrypsin and α-macroglobulin. Most of the research conducted on bromelain's biological effects is done through in vitro assays which do not require bromelain to pass through the pharmacokinetic stages for reaching its target, but rather allows it to make direct contact with its target. However, when the results of these studies were compared with in vivo data, differences were noted, the possible reason for these being that in the in vivo assays bromelain cannot successfully reach its target since it is found in combination with many other ingredients. Another possible reason for the reduction in the physiological activity of bromelain is that it may lose potency during the extraction process, or storage, since proteolytic enzymes are not temperature stable (Marcelo et al., 2000; Rowan and Buttle, 1994).

BROMELAIN'S EFFECT ON BLOOD CLOTTING

The intrinsic and extrinsic pathways of blood coagulation play a vital role in preventing blood loss by forming clots. During certain pathological conditions these clotting factors can cause serious problems to individuals and lead to deadly diseases like thrombosis and embolism. Drugs that help in breaking down these clots are called anticoagulants. The anticoagulating effect of bromelain obtained from pineapple was first identified in 1972 when it showed an anticoagulant effect in 17 out of 20 volunteers after oral administration. This was further studied in animal models with bromelain found to be very effective. Bromelain plays an active role in blood fibrinolytic activity, decreasing the concentration of active fibrin which is a protein involved in clotting. At elevated concentrations bromelain increased activated partial thromboplastin time and prothrombin time. When investigating the mechanism of action, bromelain was found to be active in fibrinolysis—activating the conversion of plasminogen to plasmin which plays a role in degrading fibrin (Castell et al., 1997; Lorkowski, 2012; Seifert et al., 1979; Winter, 1990).

BROMELAIN'S EFFECT ON CARDIOVASCULAR DISEASES

Angina pectoris and ischemic attacks are lethal complications resulting from a compromised blood supply to the heart due to thrombus formation. Similarly, in thrombophlebitis a blood clot blocks the blood supply in one or more veins. The effect of bromelain in these conditions has been studied and found to be effective at eliminating the clot by interfering with fibrinogen, thereby facilitating a free flow of blood through the blood vessels (Livio et al., 1978).

When given orally to 14 patients with angina pectoris, at doses ranging from 400 mg to 1 g, bromelain was found to eliminate all symptoms. Bromelain was found to be active in the skeletal muscles' reperfusion process after ischemic disease, while supplements of bromelain were also found to attenuate the symptoms that contribute to diabetic and cardiovascular complications (De-Guili and Pirotta, 1978; Gläser and Hilberg, 2006; Norred and Francis, 2001; Taussig and Batkin, 1988).

BROMELAIN'S EFFECT ON OSTEOARTHRITIS

Osteoarthritis, commonly known as joint pain disease, is a degenerative disorder. Patients feel moderate to severe pain in joints both during activity and after rest, especially in the morning. Patients often limit their activities because of severe pain. According to the research conducted by Akhtar et al. the analgesic effect of bromelain was compared with known analgesics

like diclofenac in osteoarthritis patients and pain relief in more than 100 patients was observed. They found a considerable improvement in pain reduction in both types of treatments after 6 weeks. Bromelain as a supplement enhances pain relief and can be employed in parallel to nonsteroidal antiinflammatory drugs known as NSAIDS (De-Guili and Pirotta, 1978).

BROMELAIN'S EFFECT ON IMMUNOGENICITY

The wide range of therapeutic effects of bromelain can be utilized for the purpose of supplementary therapies of autoimmune diseases and inflammatory conditions. Bromelain not only has the ability to enhance the attachment of cell surface molecules to immune cells like T cells, natural killer cells, and macrophages, but also enhances the secretion of interleukin-1, interleukin-6, and alpha tumor necrosis factors. In autoimmune diseases bromelain has the ability to attenuate the activation of CD4 T cells (immune cells) and thus suppress immune activation (Akhtar et al., 2004; Engwerda et al., 2001a,b; Nieper, 1978; Secor et al., 2009).

BROMELAIN'S EFFECT ON DIARRHEA

Some pathogens like *Escherichia coli* and *Vibrio cholera* cause diarrhea through their endotoxins in the gastrointestinal tract, particularly in the intestines. Bromelain has been found to counter the action of these endotoxins. It exerts this effect by interacting with certain intestinal secretory pathways like guanosine-cyclic monophosphates, adenosine-cyclic monophosphates, and also Ca^{+}-dependent signaling**.** According to another proposed mechanism, bromelain exerts its effects by preventing the attachment of *E. coli* to the surface glycoprotein receptors on the intestinal surface, known as an antiadhesion effect (Barth et al., 2005; Contreras et al., 2009; Eckert et al., 1999; Mynott et al., 1996).

BROMELAIN'S EFFECT ON TUMORS

Different pathways regulate the proliferation and metastasis of cancerous cells, bromelain has been found to interfere with these pathways, especially the one leading to malignancy (Chobotova et al., 2010). Apart from this, its overall anticancer activity is due to its antiinflammatory, immunomodulatory, and hemostatic systems. Bromelain was found to be very effective in reducing the number of cancerous cells in different research studies conducted on the skin papillomas cells of mice, gastric carcinoma cells, and glioblastoma cells. The main mechanism of action in these studies was through the enhancement of apoptosis, known as programmed cell death (Chandler and Mynott, 1998; Desser et al., 1993, 1994; Hale and Haynes, 1992; Lehmann, 1996; Mynott et al., 1997; Tysnes et al., 2001).

BROMELAIN'S EFFECT ON CONTRACTION OF UTERINE MUSCLES

During labor, uterine contraction plays a vital role in helping push out the baby, and is also helpful in expelling blood during the menstrual cycle. During the final term of pregnancy the hormone oxytocin is released naturally by the pituitary gland which helps with uterine contraction. In order to find natural or synthetic stimulants for the hormone oxytocin, much research work has been done to combat the complications associated with childbirth (Bàez et al., 2007; Taussig et al., 1985).

Nwankudu et al. (2014) conducted research to find out the effect of pineapple on uterine contraction. They took out the uterine muscles of rats and studied their behavior with different drugs like acetylcholine and atropine among others, along with fresh pineapple juice. They observed a similar contraction of the uterine muscle due to pineapple juice and acetylcholine. Pineapple caused the uterine contraction just like the known drug acetylcholine. The mechanism of acetylcholine is already known which acts on muscarinic receptors in uterine; So the possibility is that pineapple may also cause contraction by agonistic activity on M2 and M3 muscarinic receptors (Salleh and Ahmad, 2013; Sembulingam and Prema, 2009).

Therefore, to confirm this hypothesis they gave atropine and pineapple together. Because atropine is the antagonist of mucarinic receptors and giving both together pineapple should not produce the contraction of the uterine if the effect is because of muscarinic receptors but actually it still produced the same effect and contracted the uterine that means the effect is not because of muscarnic but may be Histamine H2 receptors which are also abundant in uterine muscles. However, they did not perform any test to find out if the effect is because of histamine receptors (Aucelio, 1988; Choppin et al., 1999; Kitazania et al., 2007).

CONCLUSIONS

The demand for pineapple is increasing day by day, as is evident by the increase in its worldwide consumption. Its sweet flavor makes it a popularly consumed fruit. It is rich in many essential nutrients including, vitamins B and C, manganese, folate, and crude fibers. In addition to its importance as a phytonutrient, its most clinically interesting ingredient is bromelain which has a myriad of biological importance including improving skin complications, acting as an anticoagulant, counteracting cardiovascular disease, as an analgesic, enhancing the immune system, reducing gastric complications, the contraction of uterine muscles during labor, and as a tumor suppressor. All these properties contribute to its versatile biological importance and increase in popularity in the food and cosmetic industry. However, the pharmaceutical industry is yet to capitalize on its wide range of potential therapeutic effects.

Until now bromelain has only been approved for use against one medical condition, as discussed earlier in the chapter, despite possessing many biological activities. There are multiple reasons for this. First, the most important challenge is that the chief biological ingredient of pineapple, bromelain, is a mixture of extracts and not a single ingredient. Therefore, combining these extracts into a single medication is a rigorous task. Second, the biological ingredients of bromelain are prone to decomposition in the gastric environment. Third, the extract contains multiple ingredients which vary in their mechanisms and cause side effects like diarrhea and gastric discomfort. Finally, the mechanisms of action for many components are yet to be identified. Therefore, future research should focus on all these challenges which are currently hindering bromelain's bioavailability. More importance should be given to the identification of mechanisms and the pharmacokinetic profiles of all its ingredients, as well as the identification of effective dosages and dosage forms, things which are extremely important for the enhancement of the therapeutic benefits of pineapple and its safe consumption.

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Chapter 3.35

Red Algae

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INTRODUCTION

Edible algae have long been an important part of the human diet in Asian countries (China, Korea, and Japan) due to their nutritional and functional properties (Suleria et al., 2015). As mentioned in several reviews, an algae-rich diet is associated with a lower risk of developing numerous pathologies such as cancer, hyperlipidemia, cardiovascular disease (CVD), and neurodegenerative diseases (Cornish et al., 2015; Lordan et al., 2011; Wan-Loy and Siew-Moi, 2016). For instance, the rates of the three major causes of death (cancer, heart disease, and stroke) in Japan are lower than in the West (Brown et al., 2014). Algae are served in 21% of Japanese meals and the daily consumption of algae is about 5.3 g/day in Japan (Matsumura, 2001). These are the reasons the consumption of algae in Western countries has been gaining attention and little by little introduced into the diet. However, they are already widely used in the food industry as emulsifiers, stabilizers, and gelling and thickening agents, using agar and carrageenan from red algae and alginate from brown algae (de Jesus Raposo et al., 2015; Suleria et al., 2015). In this context, edible algae are novel sources of potentially nutraceutical compounds that can complement human nutrition. Among these compounds, the high content of proteins, the presence of polyunsaturated fatty acids (PUFAs), insoluble and soluble dietary fibers and polyphenols, as well as essential compounds such as vitamins and minerals are of major interest (Holdt and Kraan, 2011; Wells et al., 2017). Most of these compounds are present in higher amounts in algae than in terrestrial food, although these values may vary depending on algae species, water temperature, wave exposure, harvest time, and location (Rioux et al., 2017).

Red algae (Rhodophyta) have a higher protein content than green or brown algae (Hamed et al., 2015), which makes them more interesting from the nutritional aspect. They are also a source of vitamins, especially vitamin C and B family members, and are rich in iodine, a very important mineral for human metabolism and growth since it is involved in the production of thyroid hormones (MacArtain et al., 2007; Valente et al., 2015). Omega-3 (ω-3) PUFA is an important constituent in red algae composition, which plays an essential role in human health by reducing the risk of developing heart disease and decreasing the levels of low-density lipoprotein (LDL) cholesterol (Mohamed et al., 2012). The nutritional values of some red algae have been well documented in recent reviews (Hamed et al., 2015; Taboada et al., 2013; Wells et al., 2017) and much evidence has been gathered indicating these marine organisms could be promising for functional food. Moreover, extracts and bioactive compounds of red algae have important biological properties that are antitumor, antioxidant, and antimicrobial in nature (Rocha de Souza et al., 2007; Rodrigues et al., 2015). Therefore, both red algae extract and its bioactive compounds have been studied for its use as a food supplement in animal feed (Ragaza et al., 2015; Valente et al., 2015) and for possible effects of its implementation; this is a field undergoing continuous growth and development.

SOURCE AND BIOAVAILABILITY

Red algae contain a great variety of structurally diverse compounds including PUFAs, vitamins, carotenoids, polyphenols, dietary fibers, polysaccharides, proteins, and minerals, all of which can be attractive for use in functional food and nutraceutical products (Hamed et al., 2015; Holdt and Kraan, 2011; Wells et al., 2017).

Polyunsaturated Fatty Acids

The consumption of PUFAs (namely, fatty acids belonging to the ω-3 and ω-6 family) have demonstrated several human health benefits (Simopoulos, 2002), since they are involved in the regulation of several biologic processes (Lee et al., 2016).

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Although the overall lipid content of algae is generally low, their proportion in PUFAs is high (Lordan et al., 2011). Red algae are particularly rich in the ω-3 fatty acids—eicosapentaenoic acid (EPA) and α-linolenic acid—and the ω-6 fatty acids—arachidonic acid (AA) and linoleic acid—along with relatively high levels of oleic and palmitic acids (Dawczynski et al., 2007). The amount of red algae in PUFAs can vary between 8% and 63% (Graeve et al., 2002; Li et al., 2002). Red algae have been shown to be an interesting, viable, and sustainable source of PUFAs.

Carotenoids

Carotenoids are known to be active agents in the fight against cancer and in macular degeneration of retin (Hamed et al., 2015). However, they are also widely explored by the food industry due to their color and antioxidant properties which means the shelf life of food products could be increased (Baky and El-Baroty, 2013; Derner et al., 2006). The composition of carotenoids present in red algae can be divided into two groups, the unicellular group contains β-carotene and zeaxanthin, and the macrophyte group contains α-carotene and lutein (Takaichi, 2011). Several studies have demonstrated that red algae are an interesting source of carotenoids since it has been possible to observe the occurrence of α- and β-carotene, lutein, zeaxanthin, as well as small amounts of α- and β-cryptoxanthin (Bjørnland and Aguilar-Martinez, 1976; Palermo et al., 1991). For instance, previous studies with the edible red alga *Gracilaria corticata* (0.431 mg/g) (Khairy and El-Sheikh, 2015) and another *Gracilaria* sp. (5.4 mg/100 g) (MacArtain et al., 2007) revealed an interesting content of β-carotene compared with values reported by USDA (2010) for some vegetables such as spinach (5.63 mg/100 g), red chilli (0.534 mg/100 g), and broccoli (0.361 mg/100 g).

Proteins, Peptides and Amino Acids

Proteins of marine origin have been the subject of several studies due to their bioactive potential and functional properties (Ibañez et al., 2012). The protein content in algae varies according to phylum. Brown algae (3%–15% dry weight) have the lowest protein content compared with red and green algae (10%–47% dry weight) (Cian et al., 2015). Protein–pigment complexes such as phycobiliproteins which are mainly found in blue-green and red algae are one of the most important groups of marine proteins (Hamed et al., 2015; Ibañez et al., 2012). Phycobiliproteins have revealed interesting antioxidant properties suggesting that may be useful in the prevention or treatment of numerous diseases. Generally, 1%–10% dry weight of algae biomass is composed of these kinds of compounds (Burtin, 2003). These proteins contain pigments that can be extracted (mainly from red algae). One of the most important products already extracted from red algae (e.g., *Porphyra* sp., *Corallina elongata*, *Palmaria palmata*) is R-phycoerythrin which has a high commercial value due to the sophisticated techniques used for its extraction. This protein has demonstrated great biotechnological potential and can be used in several applications. It is used in food science, immunodiagnostic, cosmetic, therapeutic, cell-labeling, and analytical processes (Fleurence, 2003; Rossano et al., 2003). The proteins present in macroalgae contain all the essential amino acids (i.e., leucine, valine, and methionine). For example, they are abundant in the red alga *P. palmata* and their values are close to those reported for ovalbumin (Conde et al., 2013). Moreover, these amino acids are rich in residues of aspartic acid and glutamic acid. Cian et al. (2014) found that the amount of aspartic acid plus glutamic acid was 22.7 g/100 g protein in several red algae. Similar results were obtained by other investigators for *Pyropia acanthophora* (27 g/100 g protein), *Hypnea charoides* (20.8 g/100 g protein), and *P. palmata* (15.5–27.4 g/100 g protein) (Cian et al., 2014; Galland-Irmouli et al., 1999). However, the interest in marine proteins may not be directly associated with the proteins themselves, but rather with the possibility of these originating bioactive peptides (Ibañez et al., 2012). Several marine bioactive peptides have been identified and their involvement in several biological functions such as antihypertension, immunomodulatory, antithrombotic, anticancer, and antimicrobial activities, and in their use as nutrients have been described (Kim and Wijesekara, 2010). For instance, Fitzgerald et al. (2012) isolated and characterized peptides derived from the red alga *P. palmata*, which showed a capacity to inhibit the activity of renin.

Polysaccharides and Fibers

Algae polysaccharides such as agar, carrageenans, and alginates have proven to be valuable bioactive ingredients in functional foods, possessing several benefits for human health (Hamed et al., 2015). Their total dietary fiber content can vary between 25% and 75% dry weight, these values being higher than the fiber content of most fruit and vegetables (Jiménez-Escrig and Sánchez-Muniz, 2000). The red species has the greatest variety of carbohydrates among the macroalgae, which includes sulfated galactans (agars and carrageenans), xylans, and floridean starch (Hamed et al., 2015). These polysaccharides have

been widely used in the food, pharmaceutical, cosmetic, paper, and textile industries as stabilizers and as emulsifying and gelling agents. Their ability to bind water and form a gel has led to many industrial applications (Wijesekara et al., 2011). For instance, the red algae *Chondrus crispus* is traditionally used for the extraction of carrageenan, a highly sulfated polysaccharide, widely used in France in the production of blancmange (Perez et al., 1992).

Vitamins

Algae are a rich source of vitamins such as vitamin B1, B2, B12, and C (Norziah and Ching, 2000; Taylor, 2011). However, their vitamin composition may vary according to numerous factors including geographical area, growth stage, season, and environment. Ortiz et al. (2006) reported that 100 g algae were able to supply more than the daily vitamin A, B2, B12, and C. For example, vitamin B12 is found in the red algae *P. palmata* and *Porphyra tenera*, and a higher vitamin B12 content has been measured in one *Porphyra* sp. (134 μg B12/100 g dry weight) (Mabeau and Fleurence, 1993). This alga has long used to prevent scurvy caused by vitamin C deficiency (Hamed et al., 2015).

SUPPLEMENTS BASED ON RED ALGAE

According to numbers presented in 2012 by the UN Food and Agriculture Organization (FAO), about 7 million tons of fresh algae are produced annually, half are destined for direct human consumption, and the remainder for food, pharmaceutical, cosmetic, and other applications (FAO, 2012). Although not all marine algae are consumed, many of them are included in human food, since they are rich in micronutrients (vitamins, minerals), proteins, amino acids, carbohydrates, etc. making them of interest for functional food or nutraceuticals. For instance, red algae have been shown to be a rich source of sulfated polysaccharides such as alginates, agar, and carrageenans, which are important in the food industry as additives with gelling and thickening properties (de Jesus Raposo et al., 2015). These additives are commonly used in water-based gelling desserts, low-calorie jellies, flan puddings, chocolate milk, dairy products, ice cream, soy milk, cheeses, processed and canned meat, beer, sauces, seasonings, jams, jellies, and other processed foods (Pereira et al., 2013). Red algae belong to a number of genera — *Gelidium*, *Pterocladiella*, *Gelidiella*, and *Gracilaria* — and are rich sources of agar, which is widely used in the food industry for the confection of gelatins that provide faster solidification than those of animal origin. In addition, its colloidal and gelling properties allow them to be used as an ingredient (E406) in the confection of pie fillings, toppings, etc. (Pereira, 2011). Other studies reported the higher antioxidant potential of these products after the addition of red alga *Porphyra umbilicalis* to meat products and cereals (Cofrades et al., 2008; López-López et al., 2009). Gupta and Abu-Ghannam (2011) and Mohamed et al. (2012) demonstrated the use of β-carotene isolated from red algae in the enrichment of chicken eggs, making the yolk more attractive visually. This is done by adding β-carotene to chicken feed, thus obtaining eggs with oranger yolks. Although this strategy for food enrichment is not recent, it is increasingly used for human health purposes through functional foods (nutraceuticals) (Gupta and Abu-Ghannam, 2011).

CLINICAL AND SCIENTIFIC EVIDENCE IN SUPPORT OF RED ALGAE COMPOUNDS

Prevention and Treatment of Cardiovascular Disease

CVD is an enormous health and economic burden worldwide, being one of the main causes of mortality in developing countries (Benjamin et al., 2017). Consumption of fish and seafood products has revealed a beneficial effect in the prevention of CVD, mainly due to PUFA content (Raatz et al., 2013). The intake of PUFAs (mainly ω-3) is associated with some metabolic effects such as reduction of blood pressure, triglyceride concentration, platelet aggregation, heart arrhythmias, and vasoconstriction, and hence helps prevent CVD (Juturu, 2008). Red algae are the richest source of n-3 PUFAs among the macroalgae (van Ginneken et al., 2011) suggesting that their intake can help reduce the risk of CVD. Matanjun et al. (2010) observed that the red alga *Kappaphycus alvarezii* mediated antihyperlipidemic and antioxidation effects in vivo. It influenced high-density lipoprotein cholesterol (HDL-C) and LDL-C concentrations, by increasing and decreasing them, respectively. Moreover, the authors verified that the most abundant fatty acid in *K. alvarezii* was the ω-3 fatty acid eicosapentaenoic acid, which may be related to the effects observed. The red alga *Porphyra yezoensis* also demonstrated its ability in a study to increase the HDL-C level 1.6 times and reduce the atherogenic index in 48.0% of patients compared with the control group (Ren et al., 1994). Chan et al. (2014) fed high-cholesterol/high-fat (HF) Sprague-Dawley rats with different percentages (5% and 10%) of *Gracilaria changii* powder. This diet led to a significant reduction in total cholesterol (−39.19%), LDL-C (−36.36%), and triglyceride content (−25.45%) compared with a control group. The authors suggested the effects brought about by *G. changii* may be related to its high dietary fiber content (61.29%). In a clinical trial, patients

with ischemic heart disease were administrated carrageenan. Prophylactic administration of this sulfated polysaccharide mediated a significant decrease in total plasmatic cholesterol and LDL-C levels compared with control groups (Sokolova et al., 2014). Angiotensin-converting enzyme (ACE) is one of the most relevant targets in the prevention of hypertension, since it is responsible for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor (Wijesekara and Kim, 2010). Fractions of *Porphyra yezoensis* when administrated in hypertensive rats induced a hypotensive effect mediated by the inhibition of ACE (Suetsuna, 1998). Of the 26 red algae, the species *Lomentaria catenata*, *Lithophyllum okamurae*, and *Ahnfeltiopsis flabelliformis* exhibited the most potent ACE inhibitory activities (IC_{50}: 12.21, 13.78, and 13.8 μg/mL, respectively) (Cha et al., 2006). These studies demonstrate that red algae can be an interesting source of compounds that can help prevent CVD.

Antiinflammatory, Analgesic, and Antiulcer Properties

Inflammation is associated with the pathogenesis of several diseases such as diabetes, cancer, CVD, and neurodegenerative diseases. It is characterized by redness, heating, swelling, pain, and loss of function of the affected area (Oishi and Manabe, 2016). Red algae have been demonstrated here to be an interesting source of bioactive compounds with antiinflammatory, analgesic, and antiulcer properties (Bhatia et al., 2015; García Delgado et al., 2013; Robertson et al., 2015). Lipid extracts obtained from *Porphyra dioica*, *P. palmata*, and *C. crispus* downregulated proinflammatory responses brought on by lipopolysaccharide (LPS) in human macrophages. It has been suggested that the potent effect mediated by these extracts could result in the incorporation of bioactive compounds such as pigments and n-3 PUFAs into complex lipid structures promoting synergistic effects (Robertson et al., 2015). The edible red alga *Porphyra vietnamensis* demonstrated a capacity to reduce the inflammatory process in an in vivo test of paw edema, abdominal writhes, and comparable ulcers. It showed a reducing potential comparable with that of omeprazole (commercial drug) (Bhatia et al., 2015). Accordingly, *G. changii* extract also showed a capacity to reduce several inflammatory markers and reduced gastric lesion sizes by >99%, making it comparable with that of omeprazole (Shu et al., 2013). In addition, red alga *Dichotomaria obtusata* extract demonstrated a capacity to inhibit mouse ear edema and reduced abdominal writhes (García Delgado et al., 2013). Silva et al. (2010) studied the antiinflammatory and antinociceptive properties of lectin extracted from *Pterocladiella capilacea*. This protein exhibited a capacity to inhibit inflammatory hyperalgesia in classical pharmacological tests suggesting its activity is mediated by peripheral actions. Moreover, a sulfated polysaccharide fraction isolated from the edible alga *Gracilaria caudata* helped reduce neutrophil migration and cytokine concentration in an in vivo model, leading to reduction in the hypernociceptive and inflammatory response (Chaves et al., 2013). Actually, red algae show great potential for the treatment of peripheral painful, inflammatory, and ulcer conditions suggesting their promise for incorporation in food products.

Potential Use as a Prebiotic

Recently, there has been an increase in interest in prebiotics as functional ingredients, since these compounds have a capacity to stimulate the growth/activity of beneficial gut microbiota and thereby bring concomitant health benefits for humans and animals (O'Sullivan et al., 2010). As a result of their polysaccharide and dietary fiber content, algae have aroused the interest of the scientific community because of their potential use as prebiotics (O'Sullivan et al., 2010). Liu et al. (2015) verified that inclusion of the red alga *C. crispus* in the diet of rats promoted numerous prebiotic effects (namely, the composition of gut microbial communities, health of the gut, and immune modulation). Moreover, incorporation of this alga in the feed of Lohmann Brown Classic laying hens enhanced their performance, egg quality, and overall gut health (Kulshreshtha et al., 2014). Muraoka et al. (2008) observed that the red alga *Porphyra yezoensis*, commonly known as Nori, contains interesting quantities of glycerol galactoside (GC), which are used as the main substrate for fermentation by bifidobacteria. Since GC is not digested by digestive enzymes nor absorbed in the small intestine, it has been suggested that GC may be used as a prebiotic. Moreover, low-molecular-weight polysaccharides extracted from red alga belonging to *Gelidium* induced significant increases in bifidobacterial populations exhibiting its potential use as a prebiotic (Ramnani et al., 2012). All in all, there is a lot of evidence in support of the use of red algae as a prebiotic promoting health benefits.

Prevention of Obesity

Obesity is a metabolic disorder characterized by excessive fat accumulation, a condition that contributes to the development of several diseases such as CVD, diabetes, cancer, and osteoarthritis (Lange et al., 2015). Red algae have demonstrated

interesting antiobesity effects that could contribute to prevention/treatment of this condition. Kang et al. (2016a) included the edible red alga *Gelidium amansii* (1%–3%) in mice fed a high-fat diet. After 12 weeks of treatment the authors observed a decrease in body weight, accompanied by a decrease in blood glucose, serum insulin, cholesterol, and triglyceride levels. Similar effects were induced by *Gelidium amansii* (GAE) and *Plocamium telfairiae* (PTE) extracts in a high-fat diet fed to C57BL/6J obese mice model. GAE promoted expression of lipid metabolic factors and reduced weight gain (Kang et al., 2017), whereas PTE mediated a reduction in body weight, fatty liver, and levels of triglyceride and glucose (Kang et al., 2016a,b).

CONCERNS ABOUT THE CONSUMPTION OF ALGAE

Despite the fact that algae are nutritionally rich and several benefits stem from their consumption, there are some risks that must be taken into account when introducing them into human food. Algae are known to accumulate metals and heavy metals, such as cadmium, arsenic, mercury, lead, and iodine, which are harmful to human health. Large amounts of iodine, in particular, are built up. Red algae showed a higher tendency to accumulate these types of compounds. However, the concentrations of these elements vary depending on the geological location of the algae (Holdt and Kraan, 2011; Rubio et al., 2017). One of the main issues is the high content of iodine present in some species of algae, such as *Laminaria* spp. Consequently, regular consumption of these can lead to thyroid malfunctions (Bouga and Combet, 2015). Desideri et al. (2016) stated that consuming seaweed with an iodine concentration >45 mg/kg dry weight can result in iodine intake above the recommended daily dose (1.1 mg/day) (EFSA, 2006). Another issue that has caught the attention of researchers is the high content of inorganic arsenic observed in some species of algae, especially from the Sargassaceae, such as *Sargassum fusiforme* which contains extremely high levels of this compound (41.6–117 mg/kg dry weight) (Almela et al., 2006; Khan et al., 2015). This metal has been associated with higher incidence of several types of cancer and has known negative effects on the human nervous system (Holdt and Kraan, 2011; Wells et al., 2017). The Joint FAO/WHO Expert Committee on Food Additives have already defined a provisional tolerable weekly intake (PTWI) for heavy metals, including cadmium (0.007 mg/kg body weight), mercury (0.004 mg/kg body weight), lead (0.025 mg/kg body weight), and arsenic (0.015 mg/kg body weight) (FAO/WHO, 2011). However, it is of the utmost importance that undesirable metals are quantified (especially in algae) and, in general terms, safe levels for the consumption of algae without presenting risks to human health are established.

CONCLUSIONS AND FINAL REMARKS

Several compounds isolated from red algae show great promise to be added in functional foods and nutraceutical products. Although several studies have reported that these compounds have interesting biological properties suggesting their possible use in drugs to treat several diseases (cancer, CVD, neurodegenerative diseases, etc.), these compounds may well be more useful and important in preventing these diseases from developing (as demonstrated in this chapter). By incorporating them in the human diet, especially in Western countries, there should be a positive impact on human health. However, this should be balanced against some concerns about the build-up of metals as a result of their consumption. Hence, utmost importance should be given to ensuring their safety and quality.

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Chapter 3.36

Rhodiola (*Rhodiola rosea* L.)

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INTRODUCTION

Rhodiola rosea L. (syn. *Sedum roseum* (L.) Scop.) (Crassulaceae), also known as golden root, rose root, or Arctic root, is a perennial plant with shoots reaching 35 cm in height (Khanna et al., 2017; Qian et al., 2011; Shikov et al., 2014). Since its yellow flowers smell like roses the name of the species is referred to as *rosea*. Cosmonaut plant is another attribution to *R. rosea* since it was believed its consumption was a means of protecting Russian cosmonauts from the harmful effects of ionizing radiation (Arora et al., 2013). Widely found at high altitudes in European and Asian highlands, the dried roots and rhizomes of *R. rosea* are commonly used in Chinese, Tibetan, and Russian folk medicine; it is also used in Eastern Europe (Shikov et al., 2014).

Several immunomodulators are known to be derived from plants and have long served as immunity enhancers (Ji et al., 2009). Mostly known as an adaptogen, *R. rosea* is a precious medicinal plant which boosts resistance to the detrimental impacts of numerous stressors (Arora et al., 2013). *R. rosea* has been used for many conditions such as depression, anxiety, fatigue, anemia, impotence, infections, cancer, nervous system disorders, and headache. Boosting physical endurance, stress resistance, attention span, memory, work productivity, and resistance to high-altitude sickness, the plant is also considered a tonic and stimulant (Iovieno et al., 2011; Wal and Wal, 2013). Lately, many significant commercial preparations such as drinks, food additives, and commercial pharmaceutical preparations sold worldwide contain *Rhodiola* spp. extract (Evstatieva et al., 2010). These preparations have biological properties that are antiallergenic and antiinflammatory in nature and have been found to increase mental alertness. Besides several other therapeutic applications, they can be found as dietary supplements in the marketplace (Chiang et al., 2015; Tolonen et al., 2003; Yousef et al., 2006).

Plants belonging to the *Rhodiola* genus contain polyphenols such as flavonoids, proanthocyanidines, tyrosol, cinnamyl alcohol, glycosides, organic acids, essential oils, sugars, fats, alcohols, and proteins (Chiang et al., 2015; Elameen et al., 2010; Gupta et al., 2012; Ma et al., 2011a, b; Ioset et al., 2011; Kobayashi et al., 2008; Kucinskaite et al., 2007; Panossian et al., 2010). The pharmacological effects of *Rhodiola rosea* boost durability, stimulate the central nervous system, and enhance work performance. The plant also has cardioprotective, neuroprotective, and hepatoprotective effects, and its immunotropic, antiinflammatory, and antiviral properties have been thoroughly examined (Bawa and Khanum, 2009; Chan, 2012; Kelly, 2001; Panossian et al., 2010; Skopinska-Rozewska et al., 2008). The traditional use, phytoconstituents, and pharmacological properties of *R. rosea* are given in Fig. 3.36.1.

TRADITIONAL USE OF *RHODIOLA ROSEA* L.

The rhizomes and aerial parts of *R. rosea* are edible, and have formed part of the diet in Greenland, North America, and Alaska (Ghiorghita et al., 2015). On the other hand, *R. rosea*'s use as an adaptogen is well documented in the folk medicines of European and Asian countries (Kylin, 2010; Shikov et al., 2014). In *Materia Medica* (Linne, 1749), *Rhodiola* root has been recommended for the treatment of hysteria, headaches, hernias, and discharges. In Middle Asia, tea prepared from *R. rosea* is used as a remedy against colds and influenza. It is recommended for the treatment of cancer and tuberculosis in Mongolia (Brown et al., 2002). There are reports of roots being utilized against scurvy, and *R. rosea* decoctions were used to wash human hair to stimulate hair growth and reduce baldness in Norway. They were also used as a stimulant and astringent in France and Sweden (Alm, 2004). The plant is considered a symbol of fertility in Siberia and has been used to improve fertility (Ghiorghita et al., 2015; Saratikov and Krasnov, 1987). In traditional Russian medicine, *R. rosea* is utilized to enhance physical endurance and work productivity as well as to treat infections, nervous and gastrointestinal system diseases, fatigue, depression, anemia, and impotence (Shikov et al., 2014). *R. rosea* was first recommended by

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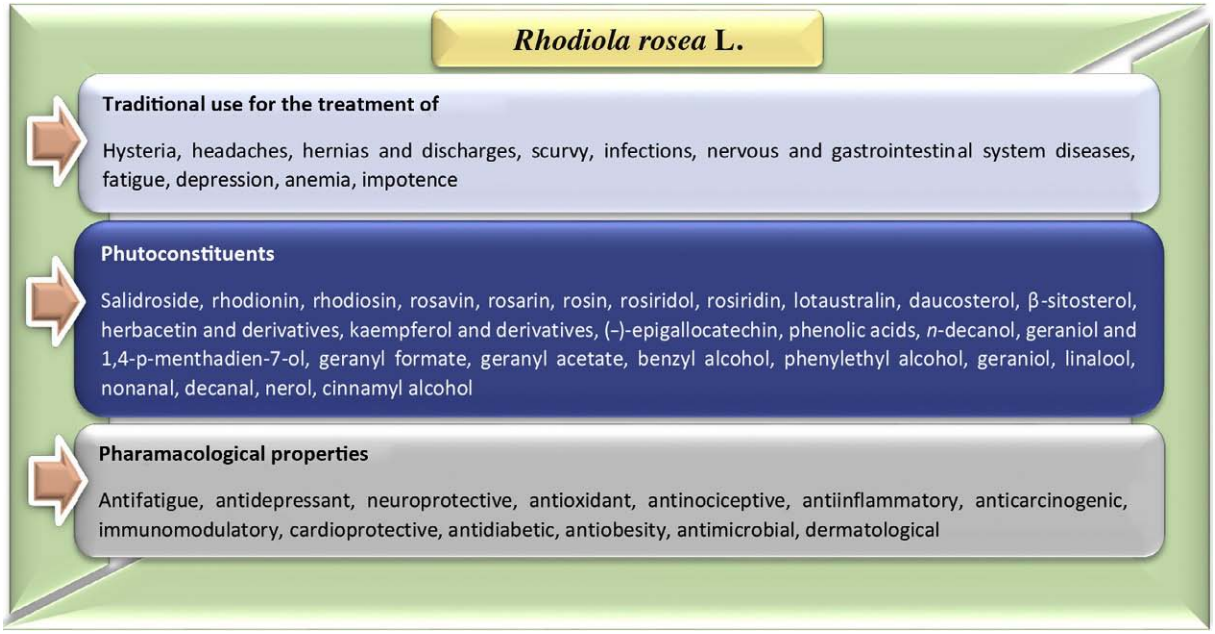

FIG. 3.36.1 Traditional uses, phytoconstituents, and pharmacological properties of *Rhodiola rosea* L.

the Pharmacological Committee of the Ministry of Health of the Union of Soviet Socialist Republics in 1969 for use as a stimulant against fatigue. It can also be used for the treatment of mental diseases and for the correction of neurological side effects associated with psychopharmacological therapy (Panossian et al., 2010; Saratikov and Krasnov, 1987). It has also been reported that *R. rosea* extract is readily available in pharmacies in Russia without a prescription and is recommended as a central nervous system stimulant and as an adaptogen by internal administration of 5–10 drops, 2–3 times daily, for 20 days (Sokolov, 2000) (Shikov et al., 2014). The European Medicines Agency (EMA) approved its use to relieve symptoms related to stress in a community herbal monograph on *Rhodiola rosea* L. (Shikov et al., 2014). *R. rosea* extract has been produced industrially for use as an adaptogen (Panossian et al., 2010).

PHYTOCONSTITUENTS OF *RHODIOLA ROSEA* L.

The rhizome of *Rhodiola* is the most important part of the plant and has been shown to have diverse secondary metabolites and various medicinal properties. Phytochemical analyses revealed the constituents of *Rhodiola* extract as salidroside (rhodioloside), rhodionin, rhodiosin, rosavin, rosarin, rosin, rosiridol, rosiridin, lotaustralin, daucosterol, and β-sitosterol (Akgul et al., 2004; Khanna et al., 2017; Kobayashi et al., 2008; Wang et al., 1992). Moreover, flavonoid-type compounds including herbacetin-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-D-L-rhamnopyranoside, kaempferol-3-*O*-β-D-glucopyranoside-(2 → 1)-β-D-xylopyranoside, and herbacetin-8-*O*-β-D-glucopyranoside were obtained from the roots of *R. rosea* by countercurrent chromatography (Ma et al., 2013). According to high-performance liquid chromatography/electrospray ionization/mass spectrometry (HPLC/ESI/MS) analysis, *R. rosea* rhizomes have been found to contain phenylethanoid-type compounds, proanthocyanidins composed of (−)-epigallocatechin and its 3-*O*-gallate esters (Yousef et al., 2006). On the other hand, flavonoids have been found to be the main constituents of the aerial parts of the plant as well, but no phenylpropanoids were detected. In a study conducted using liquid chromatography/mass spectrometry (LC/MS), 15 flavonoids were identified (Petsalo et al., 2006). The phenylpropanoid-type compound rosavin, which was not detected in 21 other *Rhodiola* species, has been reported as the characteristic feature of *R. rosea* (Kurkin et al., 1986; Ma et al., 2011a,b; Yousef et al., 2006). GC/MS analysis revealed that dried rhizomes contained essential oil mainly composed of monoterpene hydrocarbons, monoterpene alcohols, and straight chain aliphatic alcohols. The most abundant volatile compounds were identified as *n*-decanol, geraniol, and 1,4-*p*-menthadien-7-ol in the essential oil. Moreover, geranyl formate, geranyl acetate, benzyl alcohol, phenylethyl alcohol, and geraniol were detected as the compounds responsible for the rose-like odor. Other compounds including linalool and its oxides, nonanal, decanal, nerol, and cinnamyl alcohol are also involved in the flowery odor given off by the plant's rhizome (Rohloff, 2002).

As a result of the plant's diverse secondary metabolites and various medicinal properties, bioactivity and phytochemical studies have demonstrated that three classes of secondary metabolites including phenylethanoids (salidroside, *p*-tyrosol),

phenylpropanoid glycosides (rosarin, rosavin, rosin), and monoterpenoids (rosiridin) are responsible for the plant's biological effects (Li et al., 2006). In assessing the quality of raw drugs from *R. rosea*, the criteria put into use are salidroside, its aglycone tyrosol, and the content of these compounds (Chiang et al., 2015; Linh et al., 2000). Since the naturally occurring ratio of these compounds in *R. rosea* root is approximately 3:1, the numbers of extracts can be standardized to at least 3% rosavins and 0.8% salidroside in clinical studies (Iovieno et al., 2011; Kelly, 2001).

PHARMACOLOGICAL EFFECTS OF *RHODIOLA ROSEA* L.

Antifatigue and Adaptogenic Effects

Since a great many people suffer from stress, exhaustion, and burnout the medical community has little choice but to cope with these problems (Edwards, 2013). Normalizing the physiologic responses to various stressors, enhancing work performance, and boosting stress tolerance of the body are possible thanks to substances called adaptogens (Darbinyan et al., 2007; Grace et al., 2009). *R. rosea* has been classified as an adaptogen as it has the ability to boost resistance to various chemical, biological, and physical stressors, which has the effect of enhancing human performance (Chan, 2012; Chiang et al., 2015; Mattioli and Perfumi, 2007; Petkov et al., 1986; Spasov et al., 2000).

The antifatigue effects of salidroside and its effective dose have been examined. Based upon body weight a total of 120 normal male Kunming mice were randomized into five groups (four salidroside intervention groups and one control group). While the control group received distilled water the four intervention groups received various doses of salidroside (60, 180, 360, 720 mg/kg) for 15 consecutive days, respectively. Prior and subsequent to a swimming test the levels of lactate, serum urea nitrogen, muscle and liver glycogen, longest swimming time, and hemoglobin were designated. Compared with the control group the swimming time was prolonged and the contents of hemoglobin and muscle and liver glycogen were raised, but the serum lactate level diminished significantly by means of various doses of salidroside, especially at the 180-mg/kg dose. Salidroside has been demonstrated to possess remarkable dose-dependent, antifatigue properties in mice (Li et al., 2008a,b).

As a result of increased reactive oxygen species (ROS) production, intense exercise boosts oxygen consumption and brings about oxidative stress (Kan et al., 2013). There is a possibility that exogenous antioxidants prevent oxidative damage as these antioxidants remove the ROS generated during exercise (Wen et al., 2011; Xu and Li, 2012). It has been reported that salidroside not only enhances exercise tolerance and improves the liver glycogen levels of rats after tiring exercises such as swimming, but also decreases malondialdehyde (MDA) levels and increases antioxidant enzyme effects including catalase, superoxide dismutase (SOD), and glutathione peroxide in the liver tissue of Sprague-Dawley rats (Xu and Li, 2012). According to the results of these studies, while *Rhodiola* plants increase work performance and resistance to stress, salidroside is efficient at inhibiting oxidative stress after intense exercise (Chiang et al., 2015).

The pharmacological properties of *R. rosea* appear to be exerted centrally and peripherally affecting not only monoamine and opioid synthesis but also transport and receptor activity, which are transmuted by the hypothalamic–pituitary–adrenal system. Physical and mental capacity during stress and mental performance in subjects suffering burnout and fatigue seem to have been improved with *R. rosea* extract in several clinical studies (Edwards, 2013). *R. rosea* has also been suggested as a way of treating asthenic or lethargic conditions caused by intense physical or intellectual strain (Brown et al., 2002; Iovieno et al., 2011). According to a recent double-blind, placebo-controlled study, *R. rosea* extract displayed an antifatigue effect which boosts mental performance, especially the capacity to concentrate, and decreases the cortisol response to rousing stress in patients with stress-related fatigue. This study was carried out with 60 patients given a dose of 576 mg/day,who were assessed on day 1 and after 28 days of medication (Olsson et al., 2009). Research has also revealed that *Rhodiola* extract is useful in treating asthenic conditions, which are caused by intense physical or intellectual strain (Gupta et al., 2010).

Shanely et al. (2014) examined the effects of *R. rosea* supplementation on muscle damage, delayed onset of muscle soreness (DOMS), plasma cytokines, and extracellular HSP72 (eHSP72) induced by exercise in professional runners completing a marathon. Forty-eight experienced marathon runners were randomized into two groups. Under double-blind conditions the first group of 24 runners received 600 mg *R. rosea* extract per day, while the second group received placebo for 30 days both before, on the day of, and 7 days after the marathon. Blood samples were taken, and vertical jump and DOMS were evaluated the day before, 15 min postmarathon, and 1.5 h postmarathon. DOMS was also assessed 7 days postmarathon. There was no difference between *R. rosea* and placebo groups (3.87 ± 0.12 h and 3.93 ± 0.12 h, respectively) in terms of marathon performance. After the marathon, vertical jump diminished but did not differ between groups. While there was a marked increase in postmarathon DOMS, no difference was observed in the pattern of change between groups. There was an increase in myoglobin, creatine phosphokinase, aspartate aminotransferase, alanine aminotransferase, interleukin (IL)-6,

IL-8, IL-10, monocyte chemotactic protein-1, granulocyte-colony-stimulating factor, C-reactive protein, and eHSP72 after the marathon, but there was no difference between *R. rosea* and placebo groups. As a consequence, *R. rosea* supplementation for 30 days before running a marathon did not modulate decrease in muscle function or modulate increase in muscle damage, DOMS, eHSP72, or plasma cytokines in experienced runners after the marathon (Shanely et al., 2014).

ADAPT-232 extract—a standardized combination of *R. rosea*, *Schisandra chinensis* (Turcz.) Baill., and *Eleutherococcus senticosus* Maxim.—was given as a single dose to enhance mental performance of exhausted individuals involved in stressful cognitive tasks. Split into two parallel groups, a double-blind, placebo-controlled, and randomized pilot study was conducted. Forty healthy females (from 20 to 68 years old) participated in the pilot study; although otherwise healthy they reported having been under a lot of strain for a long time while enduring some psychologically stressful conditions. Moreover, to prepare the participants for the d2 test of attention (d2), which was employed to evaluate cognitive function, a Stroop color word test was utilized. Having been randomized into two separate groups the participants received different tablets. While the first group (n = 20) was given a single tablet of ADAPT-232 (270 mg), the second group (n = 20) was given placebo. By means of the d2 test the effects of the extract were assessed both before and 2 h after treatment. A remarkable difference in mental performance between the two treatment groups was observed. Compared with the placebo group, the attention, speed, and accuracy of participants belonging to the ADAPT-232 group enhanced quickly under stress. Moreover, better accuracy, quality of work, and degree of attention seemed to be observed in participants receiving ADAPT-232 extract during stressful cognitive tasks. Apart from a few negligible negative side effects such as drowsiness and cold in the extremities, no serious ones were reported in either treatment group (Aslanyan et al., 2010).

To ascertain the effects of *R. rosea* standardized extract on the competency of people with a background of fatigue and stress to perform mental work, a randomized, double-blind, placebo-controlled, parallel group clinical study with an extra nontreatment group has been carried out. Another objective of this study was to examine possible difference between two distinct doses. The first dose was the standard mean dose according to well-known medicinal use as an adaptogen, while the second dose was 50% higher. Certain physiological parameters such as pulse rate, systolic blood pressure, and diastolic blood pressure were evaluated as well. The study was performed on a very similar population consisting of 161 participants aged from 19 to 21 years. Initial data on all groups were quite similar and there was no remarkable difference pursuant to any parameter. Antifatigue index mean values of the groups were 1.0385 and 1.0195 after taking 2 and 3 capsules, respectively. Although no significant difference between the two dosage groups was spotted, this was statistically significant for both doses (Shevtsov et al., 2003).

Randomized, double-blind, placebo-controlled trials were conducted on *R. rosea* standardized extract to reveal its beneficial effects on healthy subjects under stressful conditions. In the first study, fatigue and mental performance were evaluated. This study comprised 56 young, healthy physicians on night duty who were treated with a 170-mg/day dose of *R. rosea*. During the first 2 weeks, there was a statistically marked improvement in fatigue and mental performance in the treatment group. However, such an improvement appeared to be lost by 6 weeks, possibly due to a short-term effect (Darbinyan et al., 2000). In the second randomized study, 40 medical students who were under stress due to taking exams for 20 days were either given a low dose of *R. rosea* (100 mg/day) or placebo. The physical fitness, mental fatigue, psychomotor performance, and general well-being of subjects who received *R. rosea* improved considerably. Moreover, their sleep patterns improved, they needed less sleep, had greater mood stability, and felt motivated to study (Spasov et al., 2000). When 120 adults, consisting of 83 women and 37 men who were from 50 to 89 years old, received *R. rosea* extract at two different dosages in combination with vitamins and minerals in a 12-week monitoring study, physical and cognitive deficiencies appeared to improve significantly (Fintelmann and Gruenwald, 2007; Iovieno et al., 2011).

In another experiment, 101 subjects showing symptoms of general life stress were given 200 mg *R. rosea* extract twice daily for over 4 weeks. Seven evaluatory questionnaires completed by the subjects revealed that there was a clinically important improvement in stress symptoms, inability, functional impairment, and overall therapeutic effect. Moreover, improvements were observable for 3 days after treatment and lasted up to 1 and in some cases 4 weeks. In a recent multicenter study, 101 patients with chronic fatigue symptoms were treated for 8 weeks with *R. rosea* extract twice daily. All the criteria significantly improved after day 7. Furthermore, this improvement continued to last longer at each stage in the course of treatment. To a clinically relevant degree, *R. rosea* extract is a reliable and efficient option as a remedy to improve general life stress symptoms (Edwards, 2013).

ANTIDEPRESSANT EFFECT

R. rosea showed its multitarget properties and regulated cell response to stress by means of affecting the neuroendocrine, neurotransmitter receptor, and molecular networks that are involved in the beneficial efficacy on mood (Amsterdam and Panossian, 2016).

The inhibitory properties of methanol extract and *R. rosea* water extract against monoamine oxidases (MAOs) can be revealed using a microtiter plate bioassay. Bioassay-guided fractionation and isolation procedures led to 12 compounds being isolated. Methanol extract and water extract displayed inhibitions of 92.5 and 84.3% on MAOA and 81.8 and 88.9% on MAOB, respectively at a concentration of 100 μg/ml. Rosiridin has been identified as the most active component inhibiting MAOB by over 80% at a concentration of 10^{-5} M. The results showed that roots of *R. rosea* possess an antidepressant effect by inhibiting MAOs (Diermen et al., 2009).

According to *in vivo* preclinical studies, *R. rosea* increased the level of 5-hydroxytryptamine (5-HT) and induced neural stem cell proliferation and differentiation in the hippocampus of rats with depression, therefore protecting injured hippocampal neurons (Qin et al., 2008). *R. rosea* has been reported to reduce corticotrophin-releasing factor production (Lishmanov et al., 1987; Maslova et al., 1994) and exerted an effect on the opioid system by stimulating opioid peptide biosynthesis and activating central and peripheral opioid receptors (Lishmanov et al., 1987, 1997). *R. rosea* has been shown to enhance serum β-endorphins and moderate opioid peptide release. Such a moderate release protected high levels of opioid and catecholamine, therefore enhancing stress tolerance without damaging the central nervous system or the cardiovascular system (Iovieno et al., 2011; Lishmanov et al., 1987; Maslova et al., 1994).

Experimental animal models and clinical studies have revealed that nicotine decreases depression and nicotine cessation causes depressive-like symptoms. It has been demonstrated that *R. rosea* extract suppressed the withdrawal symptoms of nicotine in mice (Titomanlio et al., 2014). In addition, salidroside treatment reduced damage to the blood–brain barrier and decreased the proinflammatory cytokine level i in rats having focal cerebral ischemia-reperfusion injury (Han, 2013; Khanna et al., 2017).

In another study, induction of nicotine dependence was carried out by subcutaneous injection of rats with 2 mg/kg nicotine 4 times per day for 14 days. An experimental group was orally treated with *R. rosea* extract. Following nicotine withdrawal, rats were investigated for behavioral features. Dose-dependent reduction of immobility by treating with *R. rosea* at 10-, 20-, and 40-mg/kg doses was found to be significant in a forced swimming test. The outcome of this research demonstrated a notable increase in 5-HT level and a remarkable enhancement of serotonin receptor 1A, indicating the involvement of serotonin in the beneficial effects of *R. rosea* (Mannucci et al., 2012).

The potential effect of *R. rosea* on modulatory stress responses has been examined by using an experimental model of binge eating in rats. After a stressful procedure, rats exposed to both food restrictions and stress ended up with binge-eating disorder. *R. rosea* extract at a dose of 10 mg/kg markedly reduced binge eating and in some cases stopped it completely . There was also a stress-induced increase in serum corticosterone levels at the 20-mg/kg dose. Salidroside, the active principle of *R. rosea,* decreased or inhibited binge eating in a dose-dependent manner, while rosavin did not (Cifani et al., 2010).

Panossian et al. (2008) investigated the antidepressant properties of *R. rosea* root extract, its combination with piperine-containing extract, and rhodioloside, rosavin, rosin, rosarin, tyrosol, cinnamic alcohol, cinnamaldehyde, and cinnamic acid, isolated from *R. rosea* by using the Porsolt behavioral despair test. *R. rosea* root extract in a dose-dependent way increased the swimming time of rats at 20 mg/kg, showing stronger antidepressant activity than either imipramine or *Hypericum perforatum* L. extract. Rhodioloside and tyrosol were found to be the active constituents of the extract, while rosavin, rosarin, rosin, cinnamic alcohol, cinnamaldehyde, and cinnamic acid were not active. A mixture of rhodioloside, rosavin, rosarin, and rosin exhibited a greater effect than did individual compounds, indicating a synergistic effect. *Rhodiola* combined with piperine changed the pharmacokinetic profile of rhodioloside and rosavin (Panossian et al., 2008). In other research the anxiolytic and antidepressant properties of a single dose of salidroside were evaluated in mice and it was found that salidroside increased fear memory and showed promise for use in curing mood disorders (Khanna et al., 2017; Palmeri et al., 2016).

To assess the antidepressant activity of salidroside an olfactory bulbectomized (OBX)-induced hyperactivity in rats model was used. Salidroside, at doses of 20 and 40 mg/kg for 2 weeks significantly suppressed hyperactivity in an open field test and reduced immobility time in a tail suspension test and a forced swimming test. Long-term treatment with salidroside significantly reduced the levels of tumor necrosis factor (TNF)-α and IL-1β in the hippocampus. Western blot analysis revealed that salidroside markedly enhanced the expression of glucocorticoid receptor and brain-derived neurotrophic factor in the hippocampus. In addition, salidroside alleviated expression of corticotropin-releasing hormone in the hypothalamus and noticeably decreased corticosterone levels (Yang et al., 2014).

The antidepressant properties and potential mechanisms of salidroside were investigated in OBX rats. The administration of salidroside at doses of 20 and 40 mg/kg markedly reduced proinflammatory cytokine levels including IL-1β and IL-6, inhibited activation of nuclear factor-kappa B (NF-κB), and normalized monoaminergic system changes in the prefrontal cortex (PFC) of OBX rats (Zhang et al., 2016).

After chronic mild stress lasting 6 weeks the effect of *R. rosea* hydroalcoholic extract (standardized in 3% rosavin and 1% salidroside) was investigated by assessing behavioral and physiological parameters, locomotor and exploratory activities, body weight gain and estrous cycle length in female rats. Following the first 3 weeks of stress induction, *R. rosea* extract was applied at 10-, 15-, and 20-mg/kg doses by gavage once a day for the remaining 3 weeks. As the reference treatment, the antidepressant drug fluoxetine was administered at a dose of 10 mg/kg. *R. rosea* extract treatment completely restored sucrose intake, moving behavior, weight gain, and dysregulation of estrous cycle. The results demonstrated that long-term administration of *R. rosea* extract inhibited behavioural and physiological changes induced by chronic exposure to mild stressors (Mattioli et al., 2009).

The antidepressant properties of salidroside were investigated in a lipopolysaccharide (LPS)-induced neuroinflammation model in mice in response to previous reports that depressive-like behavior may develop due to inflammation (Haapakoski et al., 2016). Salidroside noticeably reduced the levels of serum proinflammatory cytokines and neurotransmitters including norepinephrine and 5-HT in the prefrontal cortex, and enhanced the expression of brain-derived neurotrophic factor and tropomyosin-related kinase B (Zhu et al., 2015). A clinical study was conducted on 57 patients having mild to moderate depression. Patients receiving *Rhodiola* extract for 12 weeks had odds of 1.4 of improvement with fewer side effects (30%) than patients on sertraline therapy who had odds of 1.9 of improvement and increased side effects (63%) (Khanna et al., 2017; Mao et al., 2015a,b).

The effect *R. rosea* had on anxiety treatment has been examined in ten patients diagnosed with generalized anxiety disorder. Patients received 340 mg of *R. rosea* extract daily for 10 weeks. At the end of the experiment, patients reported a marked decrease in Hamilton Anxiety Rating Scale scores (Bystritsky et al., 2008; Iovieno et al., 2011).

Darbinyan et al. (2007) conducted a double-blind, placebo-controlled, randomized pilot study on 89 patients with mild to moderate depression. Patients were split in groups to receive placebo or *R. rosea* extract at doses of 340 or 680 mg/day. The properties of *R. rosea* extract were evaluated using the Hamilton Anxiety Rating Scale for depression. Patients receiving *R. rosea* reported a marked decrease in depressive symptoms (Darbinyan et al., 2007; Sarris et al., 2011).

According to the literature, *R. rosea* extract combined with tricyclic antidepressants brought about a significant decrease in the side effects of antidepressants and an improvement of psychopathological symptoms in patients with depression (Brichenko et al., 1986; Brichenko and Skorokhodova, 1987; Iovieno et al., 2011).

MEMORY-ENHANCING EFFECT

Hydroalcoholic extract obtained from the roots of *R. rosea* brought about a remarkable decrease in alpha-2 and beta-1 waves in all brain areas in mice at a dose of 100 mg/kg. Moreover, spectral theta power was reduced in the frontal cortex, hippocampus, and reticular formation, while delta power decreased only in the frontal cortex. The results were found to be similar to the effects of tramadol (Dimpfel, 2013; Mattioli et al., 2012).

In another study the potential activity of hydroalcoholic extract of *R. rosea* roots on learning and memorizing processes was examined in rats. When given in a single dose (0.1 mL), *Rhodiola* extract has been found to enhance learning and retention after 24 h by making use of the maze method with negative reinforcement. After treating with the same dose of the extract for 10 days, long-term memory significantly improved in memory tests. The fact that the extract has no significant effect on learning and memory was observed in the other two doses tested (0.02 and 1.0 mL per rat) (Petkov et al., 1986).

The activities of a standardized commercial *Rhodiola* extract on learning and memory processes were carried out in naive rats and rats with memory impairment induced by scopolamine. The number of contingent stimuli, the number of unconditional stimuli, and the number of intertrial crossings were used as criteria. Compared with the control group, the number of avoidances during the learning session and memory retention test increased in naive rats that had been cured with standardized *Rhodiola* extract. Rats having scopolamine-induced memory disability that had been cured with *Rhodiola* extract displayed an increase in the number of avoidances compared with the scopolamine group during the learning session and memory tests. *Rhodiola* extract has been found to display a beneficial effect on learning and memory processes in naive rats and rats with memory impairment induced by scopolamine (Vasileva et al., 2016).

The effect of *R. rosea* in the treatment of 200 patients over 60 years of age diagnosed with mild cognitive impairment (MCI) has been assessed. They were equally divided into four groups receiving piracetamum (1600 mg/day), *R. rosea* (2 capsules/day), or galantamine (16 mg/day). Patients in the last group were not treated. The Mini Mental State Examination (MMSE) scale was used for evaluation after 1 and 2 years of treatment. The results of treated and nontreated groups revealed that early treatment of MCI retards the transition to dementia and long-term treatment shows a marked improvement (Podea et al., 2013).

To assess the effects of *R. rosea* supplementation on mental and physical performance, hormonal and oxidative stress biomarkers were also investigated in a randomized double-blind trial which included healthy male students receiving *R.*

rosea extract (600 mg/day) or placebo. The students performed psychomotor tests for simple and choice reaction time, which are involved in the Vienna Test System, both before supplementation (term I) and after 4 weeks of supplementation (term II). These students also underwent a VO2 peak test. To measure the hormonal profile, the biomarkers of oxidative stress, and muscle damage, blood samples were collected before and after the test. The reaction time and total response time were shortened by ingestion of *R. rosea*. Compared with the placebo group, there was a greater relative increase in the number of correct responses in the *R. rosea* extract group. After receiving *R. rosea*, there seemed no change in endurance exercise capacity and hormonal profile. While *R. rosea* ingestion increased plasma total antioxidant capacity, it did not have any effect on other measured parameters. The results of certain psychomotor tests in young, healthy, and physically active men can be enhanced; however, chronic *R. rosea* ingestion does not have any influence on physical performance. However, since the improvements in mental performance, at least in our study, appear not to be related to changes in cortisol release or antioxidant activity of *R. rosea* extract, the particular mechanisms liable for these effects still need to be clarified (Jowko et al., 2016).

There have been experimental studies into the potential use of *R. rosea* as a selective estrogen receptor modulator in preventing and curing certain diseases such as fatigue, stress, depression, cognitive decline, memory impairment, cardiovascular disease, osteoporosis, and cancer which are related to menopause. The relationship between estrogen decline and menopause-related health risks as well as the molecular mechanisms that lie behind estrogenic effects have also been examined. Many mechanisms have been revealed including activation of intracellular signal transduction pathways by binding to estrogen receptors, ER-α–mediated activation of endothelial nitric oxide synthase (NOS) with increased nitric oxide release, and antiinflammatory effects counteracting TNF-α by inhibiting NF-κB and protecting osteoblasts from hydrogen peroxide. Many findings have indicated that *R. rosea* should be examined as a potential selective estrogen receptor modulator to prevent, delay, or mitigate the cognitive, psychological, cardiovascular, and osteoporotic conditions related to menopause (Gerbarg and Brown, 2016).

NEUROPROTECTIVE EFFECT

According to studies conducted on the protective effect *R. rosea* has on the central nervous system, it has been shown that *R. rosea* has therapeutic potential for treating neurodegenerative diseases via suppression of oxidative stress, neuroinflammation, excitotoxicity, and antagonism of oncogenic p21-activated kinase (Nabavi et al., 2016). Oral administration of *R. rosea* extract at a dose of 500 mg/kg suppressed the expression of proinflammatory factors—inducible nitric oxide synthase (iNOS), IL-1β, and TNF-α—in the kidney and prefrontal cortex of mice. In the same study it was also reported that salidroside suppressed the LPS-induced expression of iNOS and cytokines in BV2 cells (Lee et al., 2013). However, in another study *R. rosea* exhibited cytotoxic effects at 100 mg/mL in cultured primary cortical neurons. *R. rosea* extracts have been reported to exhibit antioxidant effects, but do not display neuroprotective effects in primary cortical neurons (Shen et al., 2013).

Panossian et al. (2014) investigated the potential mechanism of action of *R. rosea* extract on the genes, interactive signaling pathways, and molecular cellular networks associated with emotional behavior as well as psychological, neurological, cardiovascular, metabolic, endocrine, and gastrointestinal disorders. *R. rosea* upregulated the expression of 336 genes and downregulated a further 295 genes. On the other hand, 1052, 1062, and 1057 genes were deregulated by salidroside, triandrin, and tyrosol, respectively. *R. rosea* displayed multitargeted properties by regulating the cellular response. The constituents isolated including salidroside, triandrin, and tyrosol exhibited either a similar or different effect from that of the crude extract (Panossian et al., 2014). *R. rosea* extract at doses of 1.5, 3, and 6 g/kg improved the serotonin level in the hippocampus of depressive rats induced by chronic mild stress. A dose of 1.5 g/kg of *R. rosea* repaired injured neurons in the hippocampus by improving neural stem cell proliferation at the hippocampus (Chen et al., 2009a,b).

R. rosea extract and its phenylpropanoid glycoside–type component salidroside displayed a protective effect on human cortical neurons aginst oxidative stress and prevented the apoptosis of glutamate-induced cells in the human cortical cell line HCN 1-A (Chiang et al., 2015; Palumbo et al., 2012). Salidroside isolated from *R. rosea* has been investigated for its antioxidant potential against 1-methyl-4-phenylpyridinium (MPP^+)-induced cell damage in rat adrenal pheochromocytoma PC12 cells. Salidroside in both a dose-dependent and time-dependent way inhibited apoptosis, chromatin condensation, and lactate dehydrogenase release by PC12 cells by partial activation of the PI3K/Akt pathway (Zhang et al., 2012). In a similar study, salidroside noticeably inhibited cell apoptosis and alleviated the collapse of mitochondrial membrane potential in PC12 cells under MPP^+ exposure. Moreover, NO increases induced by MPP^+, overexpression of neuronal nitric oxide synthase (nNOS) and iNOS, and accumulation of ROS and intracellular Ca^{2+} have been reported to be inhibited by salidroside (Li et al., 2011a,b). In cultured nerve growth factor–differentiated PC12 cells, salidroside relieved apoptotic cell death and hydroxyl peroxide–induced

cell viability loss in a dose-dependent way which has been suggested to be modulated by the extracellular signal-related kinase (ERK) signaling pathway (Yu et al., 2010). Moreover, dopaminergic neurons were protected in a dose-dependent way against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/MPP^+-induced toxicity by salidroside application, which attenuated the production of ROS and NO, regulated the Bcl-2/Bax ratio, decreased cytochrome-C and Smac release, inhibited caspase-3, -6, and -9 activation, and reduced the aggregation of α-synuclein (Wang et al., 2015). Salidroside markedly inhibited hydroxyl peroxide–induced apoptosis and necrosis in cultured cortical neurons of the rat. Salidroside also prevented cerebral ischemic injury in Sprague-Dawley rats which was induced by middle cerebral artery occlusion and reperfusion (Chiang et al., 2015; Shi et al., 2012). In another study the molecular mechanism behind salidroside on neuroprotection was found to be via AMP-activated protein kinase (AMPK)/sirtuin 1 (SIRT1)/FoxO1 signaling since salidroside reversed the reduction in AMPK, enhanced SIRT1 deacetylase activity, and suppressed the increase in Ace-FoxO1 induced by kainic acid. In other words, salidroside inhibited the status epilepticus induced by kainic acid and decreased the incidence by suppressing oxidative stress (Si et al., 2016). In addition, by reducing neurotoxin-induced ROS and calcium levels, salidroside has been demonstrated to protect primary cortical cells and DA neuronal cell lines from ER stress by preventing 6-hydroxydopamine–induced cytotoxicity; therefore, it has been suggested for therapeutic use in Parkinson disease (Tao et al., 2016).

A bioassay-guided fractionation and isolation method was carried out on *R. rosea* rhizome extract to identify the active constituents of *R. rosea* possessing an acetylcholinesterase inhibitory effect. Chloroform, ethyl acetate, *n*-butanol, and water fractions were prepared from the ethanol extract of the rhizomes. Silica gel and Sephadex LH-20 chromatography were used to separate the chloroform fraction. One compound demonstrating anticholinesterase activity was identified as hydroquinone (Wang et al., 2007).

ANTIOXIDANT AND ANTIAGING EFFECTS

R. rosea extract has been found to have the capacity to inhibit intracellular ROS production and increase antioxidant enzyme activity including catalase, SOD, glutathione peroxidase, and glutathione reductase. According to indirect evaluation of the intracellular redox status in keratinocytes, *R. rosea* extract promoted the effect of transplasma membrane oxidoreductase in a time-dependent and dose-dependent manner (Calcabrini et al., 2010). Water and alcohol extracts prepared from *Rhodiola* rhizomes have been found to reduce the percentage of early and late apoptotic cells; inhibit apoptosis, tert-butyl hydroperoxide (tert-BHP)-induced DNA single-strand breaks, and free radical production; decrease mitochondrial transmembrane potential; and improve U-937 human macrophage antioxidant levels (Kanupriya et al., 2005). To assess the effect of water extract obtained from *R. rosea* root on lifespan and antioxidant enzymes, the budding yeast *Saccharomyces cerevisiae* has been used. *R. rosea* extract reduced the survival of exponentially growing yeast cells under oxidative stress induced by H_2O_2, but increased the viability and reproduction success of cells in the stationary phase. *R. rosea* extract did not markedly influence catalase activity and decreased SOD activity demonstrating its positive action on the survival of yeast cells without activation of major antioxidant enzymes (Bayliak and Lushchak, 2011). It has also been reported that *R. rosea* extract protected human osteosarcoma–derived 143B cells or the human fibroblast cell line IMR-90 from ultraviolet light, paraquat, and hydrogen peroxide (H_2O_2), but did not significantly activate antioxidant response and heme-oxygenase-1 expression levels or improve the levels of antioxidant defenses (Schriner et al., 2009). In another study conducted on adult fruit flies(*Drosophila*) fed with diets of yeast paste supplemented by *R. rosea* for the duration of their lives, *R. rosea* has been reported to markedly decrease mortality by increasing the survival rate by 3.5 days in male flies and 3.2 days in female flies (Jafari et al., 2007). In preclinical investigations, *R. rosea* extract displayed an antioxidant effect and reduced lipid peroxidation (Chiang et al., 2015; Gupta et al., 2007).

According to antioxidant activity studies conducted on the secondary metabolites of *R. rosea*, salidroside, tyrosol, and oligomeric proanthocyanidins have attracted attention. Salidroside decreased serum advanced glycation endproduct levels induced by D-galactose in C57BL/6J mice. The compound has been found to reverse aging effects in immune and neural cells, repair motor activity, improve memory latency time, increase lymphocyte mitogenesis and release of IL-2, as well as induce expression of glial fibrillary acidic protein and neurotrophin-3 (Mao et al., 2010a). Moreover, salidroside has been reported to reverse senescence-like phenotypes in an oxidant-challenged model by altering expression of p53, p21, and p16 in the 2BS human fetal lung fibroblast cell line (Mao et al., 2010b). Salidroside has also been reported to be a protective agent against oxidative stress in human erythrocytes by increasing cell survival and preventing human erythrocytes from undergoing eryptosis or erythroptosis. It is said to have an inhibitory action on H_2O_2-induced intracellular ROS production in human erythrocytes. The use of salidroside as an erythropoiesis-adjuvant agent against anemia and malhypoxia has also been demonstarted (Qian et al., 2011, 2012; Tuck and Hayball, 2002). Similarly, tyrosol—*R. rosea*'s aglycone—displayed an antioxidant effect by scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals with 4.7 mg/mL of the IC_{50} value

(Ko et al., 2011). Oligomeric proanthocyanidins from *R. rosea* also exerted an antioxidant effect by scavenging DPPH, OH, and O_2; enhancing SOD and glutathione peroxidase activities; and decreasing the MDA content in serum, heart, liver, and brain tissues of mice (Zhou et al., 2014).

ANTINOCICEPTIVE AND ANTIINFLAMMATORY EFFECTS

Hot plate, Randall–Selitto, and formalin tests have been conducted to assess the antinociceptive activity of *R. rosea* in male Wistar rats. Oral administration of *R. rosea* extract increased latency reaction in the hot plate test at doses of 50 mg/kg and 100 mg/kg and provided a marked increase in pressure reaction in the Randall–Selitto test at a dose of 50 mg/kg. *R. rosea* extract displayed a remarkable analgesic effect by inhibiting thermal pain, mechanical hyperalgesia, and pain behavior induced by formalin (Doncheva et al., 2013). *R. rosea* extract has also been shown to inhibit cyclooxygenase-1 (COX-1), COX-2, and phospholipase A2 (PLA2) *in vitro* and showed significant and dose-dependent antiinflammatory activity in carrageenan-induced and nystatin-induced paw edema and in a formaldehyde-induced arthritis model in rats at a dose of 250 mg/kg (Bawa and Khanum, 2009). *R. rosea* water extract caused an increase in the production of IL-6 and TNF-α in peripheral blood mononuclear cells via phosphorylated IkB and the transcription factor NF-κB, demonstrating its immunostimulatory properties. Furthermore, *R. rosea* induced the production of NO and LPS in RAW 264.7 cells (Mishra et al., 2006). In a clinical research study, serum C-reactive protein level and creatinine kinase expression were inhibited by *R. rosea* extract after administration to healthy untrained volunteers indicating the protective effect on muscle tissue as well as the plant's antiinflammatory properties (Abidov et al., 2004). Ethanol extract of *R. rosea* on its own or in combination with B-vitamins provided a marked decrease in the nociceptive response in a synergistic manner, suggesting these combinations could be applied clinically for pain therapy (Montiel-Ruiz et al., 2013).

The antiinflammatory and antinociceptive potential of salidroside, the main compound of *R. rosea*, has also been investigated in several in vivo and in vitro studies. It has been demonstrated that salidroside acts as an inflammation suppressor by reducing the ratio of concanavalin-A–induced aspartate transaminase to alanine transaminase suppressing the release of proinflammatory cytokines via NF-κB downregulation. Salidroside also decreased the severity of liver injuries by alterating T-cell (CD3+) and T helper cell (CD4+) distribution by regulating CXCL-10 (Hu et al., 2014). In another study, administering salidroside an hour before LPS infusion has been shown to markedly decrease inflammatory cell infiltration, reduce myeloperoxidase activity in mammary tissue, and in a dose-dependent way decrease TNF-α, IL-1β, and IL-6 levels (Li et al., 2013). Similarly, salidroside attenuated serum TNF-α, IL-1β, and IL-6 concentrations in mice challenged with LPS. A marked increase in survival rate was detected in mice treated with salidroside before or after LPS challenge in a murine model of endotoxemia. Salidroside has been reported to downregulate LPS-induced nuclear transcription factor-B and activation of DNA-binding and extracellular signal-related kinase (ERK)/mitogen-activated protein kinase signal transduction pathway production in RAW 264.7 macrophages (Guan et al., 2011). Moreover, pretreatment with salidroside (120 mg/kg) prior to LPS administration increased mouse myeloperoxidase activity in lung tissue and lowered protein level and total number of cells, neutrophils, and macrophages in bronchoalveolar lavage fluid in BALB/c mice (Guan et al., 2012).

Salidroside reduced D-galactosamine–induced and LPS-induced increases in serum aspartate aminotransferase and alanine aminotransferase activities, as well as TNF-α and serum NO levels. Hepatic glutathione, superoxide dismutase, catalase, and glutathione peroxidase activities were also improved. The compound attenuated the levels of malondialdehyde and reduced the number of necrotic regions, caspase-3 expression, and hypoxia-inducible factor-1α in liver tissue (Wu et al., 2009). These results were supported by other studies which revealed the inhibitory properties of salidroside in the production of TNF-α, IL-6, and IL-1β; in NF-κB DNA-binding activation after LPS challenge; and in the downregulating effect on the phosphorylation of LPS-induced NF-κB p65, p38, ERK, and c-jun NH(2)-terminal kinase (Choi et al., 2002; Guan et al., 2011; Li et al., 2013).

The effect of salidroside has been investigated in the treatment of experimental sepsis in C57BL/6 male mice induced by cecal ligation and puncture. Salidroside was injected intraperitoneally at a dose of 50 mg/kg an hour after the operation. Survival of mice, bacterial clearance in blood, peritoneal lavage fluid, serum cytokine level, and histology of the lung were examined. The apoptosis of immune cells in the spleen and thymus were also evaluated. As a result if administering salidroside the survival of septic mice was prolonged, proinflammatory responses were inhibited, and bacterial clearance was increased. Salidroside also improved pathologic status in the lung and inhibited the apoptosis of immune cells. Salidroside exhibited a protective activity in cecal ligation and puncture-induced sepsis by suppressing proinflammatory responses, increasing bacterial clearance, and preserving adaptive immunity (Liu et al., 2015).

Salidroside has been reported to improve inflammatory status and memory loss through the SIRT1/NF-κB signaling pathway in a D-galactose–induced Alzheimer model in rats (Gao et al., 2016). *Rhodiola* has also been demonstrated to have an effect on endotoxemia caused by production of an endotoxin-like LPS by Gram-negative bacteria. It is known that bacterial

infections can cause sepsis leading to TNF-α, IL-6, IL-8, and IL-1β increase and finally multiorgan failure. Salidroside reduced the apoptosis of immune cells in the spleen and thymus and suppressed proinflammatory immune response in the sepsis model (Kurt et al., 2007; Liu et al., 2015). To investigate the antiinflammatory effects of salidroside, human mast cell line-1 was treated with phorbol-12-myristate-13-acetate plus A23187. Salidroside decreased proinflammatory cytokine levels and expression of NF-κB (Yang et al., 2016).

In previous research, salidroside has been demonstrated to reverse the UVB effect in a dose-dependent way in human dermal fibroblast cells by suppressing proinflammatory cytokines and levels of UVB-induced MMP-1, therefore increasing cell viability (Mao et al., 2015a,b).

ANTICARCINOGENIC EFFECT

In a study by Huo et al. (2013), Weikang Keli, a Chinese herbal formula, composed of roots of *Codonopsis pilosula*, rhizomes of *Atractylodis macrocephala*, rhizomes of *Curcuma aeruginosa*, rhizomes of a *Pinellia* sp., *Actinidia chinensis*, and *Rhodiola rosea*, was used for gastric cancer therapy in clinical treatment. It was found to induce patterns of autophagy in SGC-7901 cells, including intracellular vacuole formation, microtubule-associated protein 1 light-chain 3 conversion. In the *in vivo* study, administration of Weikang Keli once daily for 25 days noticeably decreased tumor volumes (by 50%) (Huo et al., 2013). *R. rosea* rhizome extract inhibited the division of HL-60 cells which led to induction of apoptosis and necrosis and to significant reduction in their survival. No chromosome aberrations or micronuclei were observed by applying the extract (Majewska et al., 2006). *R. rosea* water and hydroalcoholic extract inhibited proangiogenic factors in tumor-induced angiogenesis (Bany and Skurzak, 2008). In another study, *R. rosea* extract and salidroside markely decreased tumor-induced angiogenesis in mice in a similar way (Skopinska-Rozewska et al., 2008). Proliferation of A549 human alveolar adenocarcinoma cells, pp38 protein expression, ROS formation, and TGF-β–induced tumor invasion were all inhibited by salidroside treatment (Wang et al., 2014).

IMMUNOMODULATORY EFFECT

Rhodiola has been reported to increase IL-6 production and activate B-cells leading to antibody generation (Mishra et al., 2006). *R. rosea* water extract markedly increased the levels of tetanus toxoid-specific immunoglobulin in rats and responses of ovalbumin-induced antibody. Tetanus toxoid and ovalbumin in combination with complete Freund's adjuvant or *R. rosea* water extract induced a marked delayed-type hypersensitivity response. In an adjuvant-induced arthritis model the extract did not suppress swelling (Mishra et al., 2010). *R. rosea* water extract was reported to display adjuvant and immunopotentiating effects. Dietary supplementation of the major component salidroside enhanced $CD3^+$, $CD4^+$, the delayed-type hypersensitivity response, as well as the production of antikeyhole limpet hemocyanin (anti-KLH) IgG, anti-KLH IgG 1, and anti-KLH IgG2-α in aged rats (Lu et al., 2013). Asthmatic reactions were reportedly inhibited by intraperitoneal administration of salidroside before an ovalbumin challenge. Salidroside significantly suppressed the translocation of NF-κB and decreased p38 phosphorylation. Salidroside alleviated intercellular adhesion molecule 1 and IL-6 expression by regulating the effects of cells stimulated by proinflammatory cytokines including p38 and NF-κB in BEAS-2B (Yan and Choi, 2014).

The immunomodulatory and anticancer effects of a homogeneous polysaccharide (RRP-ws) obtained from *R. rosea* have been tested in in vitro and in vivo experiments using sarcoma 180 (S-180) cells. A direct cytotoxic effect of RRP-ws on the growth of S-180 cells was revealed in the in vitro experiment. RRP-ws inhibited tumor growth; enhanced relative spleen/thymus indexes and body weight; enhanced the release of IL-2, TNF-α, and interferon-γ (IFN-γ) in serum; and increased the ratio of $CD4^+/CD8^+$ on peripheral blood T-lymphocyte in S-180 tumors transplanted in mice (Cai et al., 2012).

CARDIOPROTECTIVE EFFECT

Stress-induced cardiac damage can be shown to be prevented by *R. rosea*, which provided an antiarrhythmic effect and modulated coronary flow and contractility in the postischemic period in the animal study (Maslova et al., 1994; Zhang et al., 1998). *R. rosea* inhibited stress-induced catecholamine production and cAMP elevation in the myocardium, reduced blood pressure, and prevented cardiac damage in animals (Li et al., 2006; Lishmanov et al., 1997; Maslova et al., 1994). By inhibiting caspase-3, caspase-9, cleavage of poly (ADP-ribose) polymerase and Bax, nuclear condensation in H9c2 cells, cytochrome-C release, and JNK activation (Sun et al., 2012; Zhao et al., 2013), *R. rosea* extract and its components salidroside and tyrosol prevented oxidative stress in cardiovascular and cerebrovascular diseases (Chiang et al., 2015).

ANTIDIABETIC EFFECT

Streptozotocin (STZ)-induced diabetes mellitus is known to cause heart failure. *R. rosea* ethanol extract enhanced peroxisome proliferator–activated receptor (PPAR)-δ expression and cardiac output in STZ-diabetic rats. Twenty-one days after treatment with *R. rosea* ethanol extract, cardiac output increased while diabetic parameters were not altered. As a result of treatment with *R. rosea* ethanol extract the enhanced phosphorylation level of cardiac troponin-I was modulated. Study results showed that *Rhodiola* extract has the capacity to enhance STZ-diabetic rat cardiac output (Cheng et al., 2012).

Salidroside has been shown to stimulate glucose uptake in differentiated L6 rat myoblast cells in a dose-dependent manner. According to Western blotting analyses, salidroside enhanced the degree of phosphorylation in AMPK and acetyl-CoA carboxylase (ACC). Furthermore, salidroside induced insulin-mediated Akt activation and glucose uptake which was shown to be specifically inhibited by compound C. It has been demonstrated that AMPK activation is involved in the effects salidroside has on the activation of glucose transport and insulin sensitivity, which suggests salidroside has promise as a candidate for developing an antidiabetic agent (Li et al., 2008a,b).

Daily administration of salidroside to mice at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg for 28 days demonstrated its protective properties against diabetes-induced oxidative stress and hypoglycemic effects. It further demonstrated its capacity to reduce the levels of fasting blood glucose, total cholesterol, triglyceride, and MDA. Moreover, the levels of serum insulin, SOD, and glutathione peroxidase were reported to increase as did catalase activities (Li et al., 2011a,b).

EFFECTS ON OBESITY AND HYPERLIPIDEMIA

R. rosea has been demonstrated to inhibit the effect of lipase in isolated mouse plasma *in vitro* and in the gastrointestinal tube of mice in vivo indicating its beneficial properties in treating or preventing lifestyle-related disorders including hyperlipidemia and exogenous obesity (Kobayashi et al., 2008). During adipogenesis, *Rhodiola* extract induced the activity of SOD in a dose-dependent manner, which results in a low level of ROS. *R. rosea* extract inhibited proline dehydrogenase, glucose-6-phosphate dehydrogenase, lipid accumulation, and ROS production in 3T3-L1 preadipocytes. Moreover, SOD activity in cells treated with *R. rosea* extract markedly increased during differentiation of 3T3-L1 preadipocytes. The antiadipogenic properties of *Rhodiola* extract have been assumed to disrupt proline-mediated energy generation and antioxidant enzyme response, therefore inhibiting lipid accumulation. One of *R. rosea*'s compounds, tyrosol, markedly increased the SOD effect during differentiation of 3T3-L1 preadipocytes at 1.0 mg/mL concentration (Lee et al., 2011).

The effect *Citrus aurantium* fruit extract (6% synephrine) combined with *R. rosea* root extract (3% rosavins, 1% salidroside) have on diet-induced obesity alterations has been assessed in adult male Sprague-Dawley rats. Animals were fed a high-fat diet (60% fat) for 13 weeks. *C. aurantium* (5.6 mg/kg) and *R. rosea* (20 mg/kg) were administered either alone or in combination for 10 days. The combination brought about a 30% decrease in visceral fat weight and an increase in hypothalamic norepinephrine and dopamine in the frontal cortex, suggesting their beneficial effect in the treatment of obesity by regulating central monoamine pathways (Verpeut et al., 2013).

ANTIMICROBIAL EFFECT

The effect a standardized extract combination composed of *Rhodiola rosea* L., *Schisandra chinensis* Turcz. Baill., and *Eleutherococcus senticosus* Maxim., namely ADAPT-232, had on two parallel groups of patients suffering from acute nonspecific pneumonia has been investigated in a double-blind, placebo-controlled, randomized, phase III study. Standard treatment with cephazoline, bromhexine, and theophylline was given to the patients. The first group of 30 patients additionally received the ADAPT-232 mixture, whereas the second group of 30 patients received a placebo, twice a day for 10–15 days. At the end of the experiment, it was found that adjuvant therapy with ADAPT-232 exhibited a positive effect on the recovery of patients by shortening the acute phase of the illness, increasing the mental performance of the patients, and improving their quality of life (Narimanian et al., 2005).

In in vitro and in vivo experiments, salidroside has been shown to have antiviral properties against coxsackievirus B3, the main cause of viral myocarditis (Wang et al., 2009). Salidroside has been demonstrated to restore mRNA expression of IFN-γ, IL-10, TNF-α, and IL-2 in coxsackievirus B3 and increase serum lactic dehydrogenase, aspartate transaminase, and creatine kinase effects in infected mice (Anggakusuma and Hwang, 2010).

In vitro antiviral properties of flavonoids isolated from *R. rosea* against influenza have been evaluated using two influenza viral strains, H1N1 (A/PR/8/34) and H9N2 (A/Chicken/Korea/MS96/96). Gossypetin displayed the most

potent inhibitory effect on neuraminidases from *Clostridium perfringens* and recombinant influenza virus A (rvH1N1). Kaempferol has also been found to have antiviral properties against both influenza viruses, H1N1 and H9N2. However, such properties were found to be dependent on the position and number of hydroxy groups on the flavonoid structure (Jeong et al., 2009).

BENEFICIAL EFFECTS ON SKIN DISORDERS

Previous resarch has revealed the positive effect *R. rosea* extract has in skin care by modulating melanogenesis and facilitating wound healing. *R. rosea* extract/L-carnosine–associated compound (RCAC) treatment has been demonstrated to provide a barrier function by reducing transepidermal water loss clinically. RCAC treatment also brought about proopiomelanocortin peptide release and regulated the levels of neuropeptides and cytokines produced by keratinocytes exposed to ultraviolet radiation (Dieamant et al., 2008).

R. rosea hydroalcoholic and acetone extract has been found to exhibit an antityrosinase effect. The acetone extract and its hydrolysate have also been shown to inhibit melanin synthesis in mouse melanoma cells B16F0 (Chen et al., 2009b; Chiang et al., 2014). c-AMP response element binding protein (CREB) phosphorylation, AKT and glycogen synthase kinase-3 beta activation, melanocortin 1 receptor (MC1R), microphthalmia-associated transcription factor (MITF), and tyrosinase-related protein 1 (TRP-1) expressions were all inhibited by *R. rosea* extract and its hydrolysate. The results demonstrated that *R. rosea* extract can act as a tyrosinase inhibitor by regulating the CREB/MITF/tyrosinase pathway in B16F0 (Chiang et al., 2012, 2014; Hearing, 2011; Kondo and Hearing, 2011).

Studies investigating the active constituents of *R. rosea* have shown that tyrosol and its glycoside, salidroside, also exerted antimelanogenesis effects. Salidroside was demonstrated to have the capacity to inhibit the tyrosinase effect and melanin content in a dose-dependent way in B16F0 cells. Salidroside also inhibited UVB-induced hyperpigmentation in brown guinea pig skin by decreasing the numbers of DOPA-positive melanocytes in the basal layer and melanin synthesis in melanocytes (Peng et al., 2013). Tyrosol and salidroside treatment of B16F0 cells reduced the melanin content and inhibited tyrosinase activity. While tyrosol suppressed α-MSH–induced MC1R and TRP-1 expression, salidroside had no effect on this. MITF or TRP-2 expression was also unaffected by tyrosol or salidroside. Molecular docking revealed that the compounds brought about metal-coordinating interactions between copper ions and tyrosinase (Wen et al., 2013).

SAFETY AND TOLERABILITY

R. rosea extract should be taken 30 min before meals on an empty stomach for proper absorption and early in the day so as not to interfere with sleeping (Panossian et al., 2009). *R. rosea* extract has been reported to be safe and well tolerated at a dose of 200 mg (equivalent to 300–1000 mg of *R. rosea* roots and rhizomes) twice daily for 4 weeks against general life stress symptoms. The safety of *R. rosea* extract has been investigated in several preclinical and clinical studies indicating it has a very low level of toxicity and possesses an excellent safety profile (Brown et al., 2002; Hung et al., 2011; Ishaque et al., 2012; Kelly, 2001; Panossian and Wagner, 2005; Panossian et al., 2010; Saratikov and Krasnov, 1987).

Following intraperitoneal injection of liquid extract to mice, LD_{50} was found to be 28.6 mL/kg (equivalent to approximately 3360 mg/kg or approximately 235 g for a 70-kg human). There is a wide safety margin since the recommended clinical dose is 200–600 mg/day (Brown et al., 2002). No toxicity has been reported as a result of salidroside administration (1000 mg/kg, equivalent to 50 mL/kg of extract). Oral administration of either the extract at a dose of 1 mL/kg or salidroside at a dose 20 mg/kg for 14 days has been reported as safe in rabbits. After intraperitoneal or intravenous injection, salidroside did not affect arterial blood pressure in rabbits (10–40 mg/kg). The LD_{50} values for *p*-tyrosol were found to be 2700 mg/kg and 7079 mg/kg for oral administration to mice and rats, respectively and 1700 mg/kg for intraperitoneal injection in mice. No toxic signs were reported after 3 months of oral administration of 200 mg/kg of *p*-tyrosol in rats and 10 mg/kg in dogs (Saratikov and Krasnov, 2004). In addition, *R. rosea* extract did not show any toxic effect on *Artemia salina* at a 1000-mg/mL concentration (Montiel-Ruiz et al., 2012). In reverse mutation, chromosomal aberration, and mouse micronucleus assays, salidroside did not exhibit genotoxicity up to 5000 μg/plate, 2000 μg/kg, and 1500 mg/kg, respectively (Zhu et al., 2010). Oral administration of *p*-tyrosol (2500 mg/kg) did not cause mutations in rat sexual and somatic cells (Saratikov and Krasnov, 2004; Shikov et al., 2014).

At high doses, observed side effects are uncommon and mild, including allergy, irritability, insomnia, fatigue, and unpleasant sensations. *R. rosea* extract (standardized over 2% rosavin) at doses of 1.5–2.0 g have been reported to increase irritability and insomnia in some individuals. In case side effects occur a smaller dose with gradual increases has been suggested (Iovieno et al., 2011). No drug interactions (even with warfarin and theophylline) have been reported for *R. rosea*;

therefore, it can be of value for patients taking multiple drugs (Panossian et al., 2009). Concomitant use of *R. rosea* with tricyclic antidepressants has been shown to possess a favorable antidepressant effect with reduced side effects (Brichenko et al., 1986; Brichenko and Skorokhodova, 1987). According to a study performed on 40 patients with pulmonary fibrosis a 10 mL daily injection of *R. rosea*, in combination with oral prednisone therapy, markedly enhanced partial pressure of oxygen, maximum amount of pulmonary ventilation, pulmonary diffusion, and total efficacy of the patients and at the same time decreased symptom scores. However, it has been suggested that in vivo preclinical studies should be conducted first to clarify its possible side effects before clinical trials (Li and Kan, 2017).

No information regarding the safety of *R. rosea* supplementation during pregnancy and breastfeeding is available, therefore *R. rosea* is not recommended for pregnant women or during lactation (Iovieno et al., 2011).

CONCLUSIONS

R. rosea, golden root, has a long history as an important medicinal plant and has reportedly been utilized for several purposes in Asian and European folk medicine. *R. rosea* extract has been shown to augment immunity, exert anticarcinogenic effects, and improve health via its antiviral and antioxidant properties. Studies have also shown that *R. rosea* and its major constituents display a wide range of biological properties that are adaptogenic, antifatigue, antidepressant, antioxidant, antiinflammatory, antinociceptive, and anticancer in nature. They also augment immunity and prevent cardiovascular, neuronal, liver, and skin disorders. It has also been demonstrated that *R. rosea* extract can help avoid depression, stress, and other lifestyle disorders and can help increase lifespan (Chiang et al., 2015; Khanna et al., 2017).

The principal application of *R. rosea* has clearly been for the treatment of asthenic conditions. Indeed, preclinical and clinical research has provided encouraging evidence of its antidepressant and adaptogenic potential, either on its own or in combination with monoamine modulation. Concomitant intake of *R. rosea* with SSRIs and SNRIs might be beneficial in avoiding side effects such as poor memory, sexual dysfunction, and weight gain (Iovieno et al., 2011). Nevertheless, the extract's mechanism of action needs further study to verify its effectiveness as a clinical treatment for the abovementioned disorders (Chiang et al., 2015; Khanna et al., 2017).

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Chapter 3.37

Saw Palmetto (*Serenoa repens*)

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Serenoa repens, also known as *Sabal serrulata* or saw palmetto, is a plant belonging to the Arecaceae botanical family (Capasso et al., 2006), native to the southeastern United States. It is a bush that grows in swampy places near the coast and that can reach heights of about 2 or 3 m. One of its main features is its extraordinary longevity: it is said that some plants in Florida are 500 to 700 years old (Tanner and Mullahe, 1999). *S. repens* leaves, which develop from a large underground trunk (Magri et al., 2008), are characteristic and similar to those of the palm; its name stems from its similarity to the palm tree. Its flowers are white, grouped in clusters, and appear in the spring. Fruits have different coloring depending on the maturation period: in May–June they are green and they become yellow in mid-August; then they change to blue–black when they are mature in September (Tanner and Mullahe, 1999). For its use in phytotherapy, partially dried ripe fruits are treated to obtain a liposterolic extract (Magri et al., 2008). The *S. repens* phytocomplex comprises glycerides, free fatty acids, phytosterols, organic acids, polysaccharides, tannins, flavonoids, and essential oils (Capasso et al., 2006).

The use of *S. repens* as a medicinal plant is not recent. As early as the 1800s the scientific literature described its therapeutic properties, particularly for the treatment of prostate disorders or as an aphrodisiac (Tanner and Mullahe, 1999). Nevertheless, the knowledge of the therapeutic effects of *S. repens* seems to extend further back in time: indeed, Native American Indians were already using the herb to treat urological disorders (Chua et al., 2014; Magri et al., 2008).

Since it is commonly used in phytotherapy, it has drawn the attention of scientific research, which investigates popular and traditional uses of the herbs. Studies are focused on explaining the mechanisms of action underlying the effects of such plants, which of course could not be known in the past. Several studies demonstrating the curative effects of *S. repens* have investigated its action in prostate health. The main disorder for which *S. repens* is indicated is the benign prostatic hyperplasia (BPH).

BPH is very common in the elderly (Russo et al., 2016). At the basis of its pathogenesis coexist different factors, including static components (in particular, gland growth due to the diffusion of testosterone in prostatic cells and its conversion to dihydrotestosterone, or DHT) and dynamic components (relative to the tone of prostatic smooth muscle). Pharmacological therapy for BPH consists in the use of molecules, which act as alpha-1 blockers or inhibitors of 5-alpha-reductase that convert testosterone to DHT (Plosker and Brogden, 1996) to treat lower urinary tract symptoms (LUTS) (Russo et al., 2016).

Currently, the use of medical herbs to treat BPH is widespread worldwide. France is the principal European country making use of phytotherapy; in Asia, Africa, and India, phytotherapy is considered the first line of intervention in BPH (Chua et al., 2014; Levin and Das, 2000). In the United States the number of people making use of phytotherapy for this kind of problem is growing: it is estimated that approximately one in three people with prostate disorders uses phytotherapy remedies (MacDonald et al., 2012). The most commonly known and used remedy is *S. repens*.

To explain the therapeutic effect of *S. repens* in BPH treatment, a number of mechanisms of action have been proposed. In particular, *S. repens* has the capacity to inhibit in vitro the proliferation of cancer prostate cell lines and human prostate cells by stimulating apoptosis mechanisms (Minutoli et al., 2013). At the same time, it also has the capacity to inhibit 5-alpha reductase (Chua et al., 2014; Magri et al., 2008; Plosker and Brogden, 1996) by impeding the binding of DHT and [H]methyltrienolone to their cytosolic receptors in prostate cells (Capasso et al., 2006; Magri et al., 2008; Plosker and Brogden, 1996); this latter mechanism, however, has not been demonstrated in vivo (Capasso et al., 2006).

In vivo studies showed that the administration of *S. repens* extract (160 mg for 1–3 months) is better than placebo in ameliorating the pathological condition in patients with BPH. In particular, the herb has the capacity to decrease dysuria, nucturia, and urinary frequency during the day and to increase the peak urinary flow rate (Plosker and Brogden, 1996). In

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addition, *S. repens* has the capacity to reduce the accumulation of mast cells in rats and decrease the levels of tumor necrosis factor (TNF) and interleukin-1 (IL-1). This antiinflammatory activity contributes to the therapeutic effect *S. repens* has on prostatic disorders (Magri et al., 2008).

In clinical comparisons between *S. repens* and other drugs commonly used for the treatment of BPH, *S. repens* has been shown to have effects similar to finasteride (Plosker and Brogden, 1996).

Studies have demonstrated the synergic effect of *S. repens* in combination with selenium (Se) and lycopene (Ly) in improving BPH and associated conditions (Russo et al., 2016), such as reduced prostate weight and hyperplasia (Minutoli et al., 2013), by chemopreventive activity against several kinds of cancer including prostate cancer (Magri et al., 2008). In addition, this complex (*Serenoa*+Se+Ly) has a more pronounced effect than tamsulosin on both BPH symptoms and maximal urinary flow rate (Russo et al., 2016). On the other hand, this combination exerts interesting antioxidant and antiinflammatory effects (Minutoli et al., 2013) by inhibiting the activity of phospholipase A2, which results in decreased arachidonic acid and prostaglandin E2 synthesis (Magri et al., 2008).

Limited data on the pharmacokinetic properties of the bioactive compounds of *S. repens* are available, due to the large number of molecules that are pharmacologically active in the extract. However, it has been shown that the plasmatic mean peak of the drug concentration was 2.6 mg/L in 1.5 h, after extract (320 mg) was given orally (Plosker and Brogden, 1996).

The German Commission E recommends a daily dosage of 1–2 g raw drugs or 320 mg liposterolic extract containing 85%–95% fatty acid and sterols extracted using ethanol, hexane, or other organic solvents (Nathan and Scholten, 1999).

S. repens has been found to be safe and well tolerated (Plosker and Brogden, 1996). Few adverse effects have been recorded; those that have been recorded are bland and comparable with those of the placebo (Agbabiaka et al., 2009). Principal adverse effects included gastrointestinal disorders (such as nausea, diarrhea, abdominal pain) (Agbabiaka et al., 2009; Plosker and Brogden, 1996), decline in sexual desire, and the onset of headache and fatigue (Agbabiaka et al., 2009). However, in a comparative trial, hypertension, urinary retention, and back pain were reported as a result of *S. repens* administration (Plosker and Brogden, 1996). No interactions with drugs have been registered apart from an isolated case in which death as a result of cerebral hemorrhage was reported (Agbabiaka et al., 2009).

In conclusion, *S. repens* appears to be an effective remedy for the treatment of BPH and the management of associated complications. Its action is comparable with that of the synthetic pharmacological molecules currently used for this pathological condition, such as finasteride or tamsulosin. The added value of *S. repens* lies in its harmlessness and reduced side effects.

ABBREVIATIONS

BPH Benign prostatic hyperplasia
DHT Dihydrotestosterone
IL-1 Interleukin 1
LUTS Lower urinary tract symptoms
TNF Tumor necrosis factor

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Chapter 3.38

Scutellaria baicalensis Georgi

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SOURCES AND AVAILABILITY OF *SCUTELLARIA BAICALENSIS*

Scutellaria baicalensis Georgi is also known as baical skullcap, huangcen, huang lien, huangqin, hwanggum, hwang-keum, Koganebana, skullcap, senohgon, whang-geum, whangegum, wogon, and golden root. *Scutellaria grandiflora*, *Scutellaria lanceolaria*, and *Scutellaria macrantha* are all scientific synonyms of this plant. It is generally cultivated in the Korean peninsula, China, Japan, Mongolia, Siberia, and other parts of Russia. More recently, European countries have started to cultivate *S. baicalensis*. The detailed classification of *S. baicalensis* is shown in Table 3.38.1. Ger-Gen-Chyn-Lian-Tang (rhizome of *Coptidis chinensis* with roots of *Pueraria lobata*, licorice, and *S. baicalensis*) (Ho et al., 2012), Soshiho-tang (*Bupleurum falcatum*, licorice, panax ginseng, ginger, *Ziziphus jujuba*, and *Pinellia ternata* with *S. baicalensis*) (Lee et al., 2013), and shuanghuanglian (forsythia fruits and honeysuckle with *S. baicalensis*) (Zhang et al., 2013) contain *S. baicalensis* as a major constituent.

PHYTOCHEMISTRY OF *SCUTELLARIA BAICALENSIS*

Flavonoids are the principal bioactive phytoconstituents of *S. baicalensis* (Han et al., 2007). Total flavonoid content in the roots of skullcap may vary from 15% to 20% (where 12%–16% is baicalin, baicalein, norwoginin, oroxylin A, β-sitosterol and 3–4% is wogonoside); there are also predominant glycosides. The dry weight of roots is 10–15 g per plant (Han et al., 2007). The morphological detail of all parts of *S. baicalensis* is shown in Table 3.38.2. The major phytochemicals are baicalin (baicalein-7-glucuronide) (Huang et al., 2003; Jiang et al., 2014) and its aglycone, baicalein, as well as another glycoside named baicalein-7-O-glucoside (Yu et al., 2013), wogonoside (wogonin-7-glucuronide), and its aglycone, wogonin (Zhang et al., 2013), as well as another glycoside known as wogonin-5-O-glucoside (Yu et al., 2013), oroxylin A (5,7-dihydroxy-6-methoxyflavone) (Shih et al., 2009), and its 7-O-glucoside (Yu et al., 2013). The structures of baicalein, wogonin, and oroxylin A are shown in Fig. 3.38.1 which differ in their functional groups. Other compounds found in the roots of skullcap are neobaicalein, scutellarin, isoscutellarein (Liu et al., 2011), apigenin, salvigenin, chrysin (Wang et al., 2002), viscidulin-I and visdulin-III (Huang et al., 2003). There are also a number of chemical substances that are used for various medical and health purposes such as skullcap flavone I (Park et al., 2005) and II (Wang et al., 2011), 2′,3,5,6′,7-pentahydroxyflavanone (Kimura et al., 1982; Li et al., 2012), (2S)-5,7,2′,6′-tetrahydroxyflavanone (Wang et al., 2011), (2S)-5,7-dihydroxy-6-methoxyflavanone (Wang et al., 2011), 5,7,2'-trihydroxy-6,8-dimethoxyflavone (K36) (Wang et al., 2011), 6,2′-dihydroxy-5,7,8,6′-tetramethoxyflavone (Wang et al., 2011), 5,7,4′-trihydroxy-8-methoxyflavone (Wang et al., 2011), apigenin (as 6-C-glucoside 8-C-arabinoside) (Liu et al., 2011), luteolin and 6-hydroxyluteolin (Liu et al., 2011), carthamidin (as 7-O-glucuronic acid) and isocarthamidin-7-O-glucuronic acid (Liu et al., 2011). The main bioactive compounds are present in high levels in the roots but in lower levels in the aerial parts (stems and leaves) (Horvath et al., 2005; Liu et al., 2011; Makino et al., 2008).

HEALTH EFFECTS AND PHARMACOLOGICAL PROPERTIES OF *SCUTELLARIA BAICALENSIS*

The antiinflammatory properties of baical skullcap have been recognized through several studies. The methanolic extract of baical skullcap containing wogonin, baicalein, and baicalin has similar effects to prednisolone (Chung et al., 1995). Another study demonstrated that chloroform extract of baical skullcap had greater inhibitory action than indomethacin.

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TABLE 3.38.1 Binomial Classification of *Scutellaria baicalensis*

Group	Description
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Eudicots
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Subfamily	Scutellarioideae
Genus	*Scutellaria* (skullcap)
Species	*baicalensis*

TABLE 3.38.2 Morphological Characterization of *Scutellaria baicalensis*

Feature	Description
Nature	Perennial herb, herbaceous
Height	Can grow up to 0.3–1.2 m
Adaptation	Flourishes in sunny, grassy slopes, also in dry and sandy soils
Root (dried)	5–25 cm long, 0.5–3.0 cm in diameter, conical, twisted, or flattened root
Stem	Tetragonal, erect, branching near base, pubescent in the stem margins
Leaf	1.5–4.0 cm long, 5 mm wide; opposite, simple, with short petioles 2 mm long; limb lanceolate
Flowers	Racemed orientation, bluish purple in color
Floral character	Calyx campanulate, bilabiate, the superior lip with a crest at the back; corolla tube long, much longer than the calyx, enlarged towards the top, swelling at the base; limb bilabiate; four stamens, didymous, fertile, ascending under the superior lip; anthers ciliate; ovary superior
Fruit	A bunch of small tuberculate nutlets, nearly globular, leathery in appearance
Propagation	By seeds, during autumn

Baicalin showed greater inhibitory action than baicalein and wogonin (Lin and Shieh, 1996). Several scientific studies have been carried out into the antioxidant activities of *S. baicalensis* (Gao et al., 1999). *S. baicalensis* has been shown to have potential antioxidant actions signifying its potential use against certain skin diseases (Gabrielska et al., 1997). Baicalein depressed lipid peroxidation action in rat liver microsomes (Gao et al., 1995), while baicalin scavenged hydroxyl radicals and superoxide anions (Gao et al., 2000). Another report found that baicalein can directly halt some free radicals such as superoxide, hydrogen peroxide, and hydroxyl in cardiomyocytes (Shao et al., 1999), while wogonin and wogonoside showed little action (Gao et al., 2000; Kim et al., 2001; Shao et al., 1999; Tezuka et al., 2001).

Baical skullcap has been shown to have a positive response in immunological disorders and cancer research. It demonstrated antitumor and metastasis-preventing effects in rats with Pliss lymphosarcoma, a disease associated with platelet-mediated hemostasis disorder (Lim et al., 1999). Another study found the anticancer activity of *S. baicalensis* in laboratory animals (Razina et al., 1989).

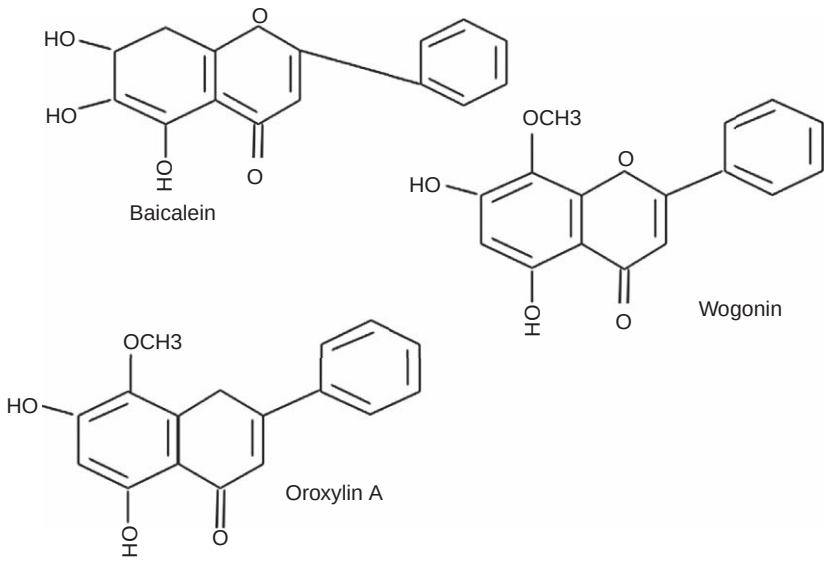

FIG. 3.38.1 Structure of baicalein, wogonin, and oroxylin A.

Much research has assessed the antimicrobial effects of baical skullcap. *S. baicalensis* has been found to have bacteriostatic and bactericidal effects at certain concentrations on selected oral bacteria. One of the major flavonoids, baicalin, demonstrated synergistic effects with β-lactam antibiotics against methicillin-resistant bacteria like *Staphylococcus aureus* (Chan et al., 2011). *S. baicalensis* has also been found to have significant antifungal activity against *Candida albicans* (Liu et al., 2000), *Cryptococcus neoformans*, and *Pityrosporum* in an herbal screening study (Blaszczyk et al., 2000).

There has also been research reporting the antiviral effect of *S. baicalensis*. Baicalin can avert reverse transcriptase activity in human T-lymphotropic virus-I (HTLV-I)–infected cells (Yang et al., 1995). Other flavones, such as isoscutellarein (Baylor et al., 1992) and isoscutellarein-8-methylether (Nagai et al., 1992) have revealed in vitro antiinfluenza activity.

It has been demonstrated in vitro that baicalin can prevent hydrogen peroxide production and oxidative stress induced by amyloid-β aggregation in SH-SY5Y cells (Nagai et al., 1995). *S. baicalensis* has been shown in animal research to have antinociceptive (reduced pain) effects (Yin et al., 2011). Baicalin flavonoid from baical skullcap has been shown to play a significant role in the inhibition of in vitro histamine and leukotriene release from mast cells (Martin and Dusek, 2002; Zhou et al. 2010). *S. baicalensis* has been shown to have antidiabetic activity by inhibiting α-glucosidase activity (Kim et al., 2010) and by reducing elevated insulin concentrations as a result of a high-fat diet (Nishioka et al., 1998). One study found the potential hypotensive effects of *Scutellaria* constituents in an animal model (Guo et al., 2009). In an animal trial, baicalin significantly altered cholesterol levels reducing serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B concentrations, and free fatty acid concentrations as well as increasing high-density lipoprotein cholesterol (HDL-C) and apolipoprotein AI levels (Huang et al., 2005; Waisundara et al., 2008). It has also been shown that baicalin can reduce weight gain and visceral fat mass following a high-fat diet (Huang et al., 2005). Other studies have shown in vitro that *S. baicalensis* has potential inhibitory effects against human immunodeficiency virus type-1 protease (HIV-1 PR) (Guo et al., 2009; You et al., 2008). Scientific research has proven the sedative activity of baical skullcap as it decreased slow-wave sleep (SWS) during the first 2 h of a period of light sleep without affecting rapid eye movement sleep (REMS), possibly mediated via antagonism of the interleukin-1 (IL-1) receptor (Li et al., 1993). *S. baicalensis* has also demonstrated its capacity to inhibit osteoclast differentiation in mouse monocyte (Chang et al., 2011). Another study revealed in vitro the estrogenic effects of *S. baicalensis* (Zhang et al., 2005). One of the major phytochemicals, baicalein, has been shown to have inhibitory action against thrombin and thrombin-induced calcium and plasminogen activators that cause arteriosclerosis and thrombosis (Chang et al., 2011).

INTERACTION BETWEEN *SCUTELLARIA BAICALENSIS* AND SOME SELECTED DRUGS/FOODS/SUPPLEMENTS

A drug interaction occurs when one drug affects the action of another drug as a result of concomitant administration of both drugs. This action could be synergistic (increased drug effect) or antagonistic (decreased drug effect) or a new effect can be produced that neither creates on its own. Generally, interactions exist between drugs and drugs (drug–drug interaction),

drugs and foods (drug–food interactions), and drugs and medicinal plants or herbs (drug–plant interactions). In this section, we highlight common interactions between *S. baicalensis* and selected drugs, foods, and/or supplements. *S. baicalensis* extract may ameliorate 5-fluorouracil-induced myelotoxicity (bone marrow suppression) according to an animal study (Zhang et al., 2005). In an in vitro study, *S. baicalensis* extract combined with cisplatin had an apoptotic effect on the human ovarian carcinoma cell line SKOV-3 and reduced the toxicity of cisplatin (Razina et al., 1987). Animal research has shown that baical skullcap extract may potentiate the antimetastatic effect of cyclophosphamide (Zhang et al., 2005). In laboratory research the wogonin constituent of *S. baicalensis* has been found to inhibit cytochrome P4501A2 and 2C19, which may affect the concentrations of drugs metabolized by these enzymes (Bonavida et al., 1990).

S. baicalensis has been shown to increase the action of metformin by elevating the hepatic activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); by increasing the level of plasma and pancreatic insulin; and by decreasing the level of plasma and hepatic TGs and cholesterol levels (Li et al., 2011) in streptozotocin-induced diabetic Wistar rats. Pharmacokinetic research has shown that baicalin, a constituent of baical skullcap, can decrease plasma concentrations of rosuvastatin, likely by stimulating organic anion-transporting polypeptide-1B1 (OATP1B1) activity, which transports rosuvastatin into the liver (Waisundara et al., 2008). An assay study has shown that baical skullcap may reduce the berberine content in berberine-containing herbs (Fan et al., 2008). In rats with pelvic inflammation, coadministration of garlic decreased the absorption of active constituents from *S. baicalensis* (He et al., 1998). In vitro, *S. baicalensis* and grape seed containing proanthocyanidins have been shown to have synergistic effects on scavenging reactive oxygen species (Shao et al., 2004; Zhou et al., 2008).

CONCLUSIONS

S. baicalensis is widely used nowadays in the treatment of a number of physiological conditions in both traditional and modern medicine. It contains many compounds with diverse therapeutic properties. Further studies are warranted to discover its potential for innovative and productive use in the field of medicinal and pharmaceutical sciences. Other therapeutic effects of *S. baicalensis* will have to be evaluated via cellullar and molecular mechanisms as well as drug–drug and drug–food interactions to get the desired physiological properties.

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The authors declare that they have no conflict of interest.

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Chapter 3.39

Spirulina

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INTRODUCTION

Of the many dietary supplements commercially available, *Spirulina* has become the focus of a great deal of food science and biochemistry research in recent years. Consisting of biomass formed by cyanobacteria of the *Arthrospira* genus, most commonly *Arthrospira platensis*, it has a nutritional content matched by very few other food products, as well as a high-protein, unsaturated fatty acid, mineral, and vitamin content. Since it can be produced and harvested with relative ease, *Spirulina* has been used specifically as a food supplement for centuries by populations residing near alkaline lakes, where it grows naturally. These regions include Lake Chad in Africa and Lake Texcoco in Mexico. In these regions, *Spirulina* is collected using fine nets and processed into an edible blue–green cake (Sotiroudis and Sotiroudis, 2013; Wan et al., 2016). Harvesting *Spirulina* is popular with the Kanembu population living along the shores of Lake Chad. Algae are collected in clay pots and drained with cloth. They are then dried under sunlight to allow lengthier storage and sold at local markets. Currently, it remains an important source of protein in those African regions lacking this nutrient (Belay, 2008).

As a result of the former classifications, *Arthrospira platensis* and *Arthrospira maxima*, a lot of confusion remains as to the correct designation of species that make up the commercial supplement. *Spirulina* remains the most common designation among both manufacturers and consumers, and most investigators into cyanobacteria worldwide label the genus as *Spirulina*. However, efforts to shift common academic labels back to *Arthrospira* have been pushed by peer-reviewed journals (Wan et al., 2016).

Spirulina has been extensively studied and strong connections between its consumption and therapeutic benefits in a wide array of pathologies have been established. These include hypercholesterolemia and glycemia, viral infections, cardiovascular and inflammatory diseases, and cancer (Deng and Chow, 2010).

Ever-increasing awareness of its functional and nutritional properties has led to worldwide popularity and it is now cultivated in many countries, both in greenhouses or outdoor ponds. Cultivated *Spirulina* is then used in human diets and animal feed, as a main component in fish and shrimp diets, and as a supplement for poultry and other land animals (Habib and Parvin, 2008).

BIOCHEMICAL COMPOSITION

It could be argued that biochemical analysis of *Spirulina* was responsible for the sudden rise in attention this product got from academics and the food industry (Habib and Parvin, 2008; Hosseini et al., 2013; Wan et al., 2016). It was labeled a "wonderful future food source" in 1967 by the International Association of Applied Microbiology when a complete nutritional analysis pointed up its exceptionally high protein content and balanced distribution of essential amino acids. This report launched a great number of studies into *Spirulina* for industrial purposes and resulted in its widespread popularity as a functional food and supplement (Habib and Parvin, 2008).

The general composition of *Spirulina* (percentage dry weight) can be summarized as: 50%–70% protein, 15%–25% carbohydrates, 6%–13% lipids, 4.2%–6% nucleic acids, and 2.2%–4.8% minerals (Belay, 2002; Habib and Parvin, 2008; Hosseini et al., 2013).

With protein making up the vast majority of its dry weight, *Spirulina* is a richer source of this macronutrient than many standards including meat, eggs, milk (dried), grain, and soybeans. It contains all the essential amino acids, with particularly high concentrations of leucine, valine, and isoleucine. Adding more value to its use as an alternative protein source, *Spirulina* protein is very digestible, with up to 90% absolute digestibility using pure casein as reference. This has been attributed to lack of a cell wall, which usually restricts digestibility of the more common vegetable protein sources

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00055-2

or, at least, warrants heat processing (Belay, 2008; Hosseini et al., 2013). Phycobiliproteins such as phycocyanin-C and allophycocyanin are present in significant quantities within the protein fraction of *Spirulina*. Phycocyanins make up about 15%–25% of the complete dry weight of the bacteria and have known antioxidant, antiinflammatory, and neuroprotective effects. Phycobiliproteins also have functional uses in biotechnology, with *Spirulina* now acting as a major source of these compounds. Their fluorescent properties and high water solubility make them ideal cell and macromolecule tags for high-sensitivity multicolor detection. *Spirulina*-borne phycocyanin is also gaining popularity as a food-coloring agent for products ranging from chewing gum to confectionery and dairy products (Hosseini et al., 2013; Romay et al., 2003).

A great deal of importance and scientific focus is being given to the polysaccharide content of *Spirulina*. Such content is believed to be the source of many of its most interesting bioactivities including its immunomodulatory, antiviral, and anticancer effects (Grzanna et al., 2006; Parages et al., 2012; Sotiroudis and Sotiroudis, 2013; Wan et al., 2016). It is also very complex with little information published regarding the structure of these polysaccharides. Hayashi and Hayashi (1996) performed some of the earliest research into characterizing *Spirulina* polysaccharides; they isolated a sulfated polysaccharide, calcium spirulan, and tested its antiviral properties and metastasis-inhibiting effects in mice. Further work by researchers allowed discrimination of the molecular structure (Lee et al., 1998). *Spirulina maxima* water extract has shown strong anticancer activity in an array of different human cancer cells (Oh et al., 2011).

The lipid content of *Spirulina* mainly comprises a saponifiable fraction (83% of total lipids) with an almost equal share (in weight) of polyunsaturated and saturated fatty acids (approx. 1.95 g/100 g powder *Spirulina*). There is a lot of γ-linoleic acid in *Spirulina*, constituting 30%–35% of total polyunsaturated fatty acids. *Spirulina* is very low on cholesterol, measuring as low as about 0.1 mg per 100 g making it a suitable alternative source of protein. Compared with other sources of balanced protein, *Spirulina* comes out very favorably for low-fat diets or when treating hypercholesterolemia (Belay, 2008; Tanticharoen, et al., 1994; Wan et al., 2016).

Spirulina is a rich source of vitamins, with a single dose of 20 g fulfilling the body's requirements for thiamine (B1), riboflavin (B2), and niacin (B3). It is also known as the richest whole-food source of methylcobalamin, a form of vitamin B12. While nearly two-thirds of this vitamin (64%) is present in inactive pseudovitamin B12 analogues, *Spirulina* still remains an important source as its consumption does not require heat processing. This feature is particularly desired in sources of vitamin B12, considering the thermosensitivity of cobalt-supplying molecules. *Spirulina* also bolsters a high amount of β-carotene, a precursor to vitamin A, with quantities reaching 352,000 IU per 100 g (Belay, 2008; Hosseini et al., 2013; Watanabe and Miyamoto, 2002). Further enriching the micronutrient content of these cyanobacteria are high levels of iron, calcium, phosphorus, and potassium (Belay, 2008).

In addition to its great potential for use as a food supplement, its powerful bioactive compounds have drawn the attention of the pharmaceutical sector. Phycocyanin-C, in particular, has displayed curative effects and anticancer activity under clinical conditions (Romay et al., 2003). It should also be mentioned that, similar to most natural sources of bioactive compounds, the chemical characteristics of *Spirulina* are subject to significant differences depending on culture conditions and harvest times. Research into how these conditions can influence the biochemical content of *Spirulina*, and how they can be made use of beneficially, is not included in this chapter, but should be taken into account when attempting to produce *Spirulina* and make use of its metabolites (Tanticharoen et al., 1994).

CLINICAL STUDIES

Ever since the first human studies involving dietary *Spirulina* performed by Nakaya et al. (1988), cyanobacteria have been recognized as a highly promising functional food and supplement as a result of their anticholesterolemic properties. Over the years, there have been numerous studies into *Spirulina* supplements and their introduction in foods as raw materials. The in vitro properties of *Spirulina* have been observed to varying degrees in in vivo studies, both in animals and humans.

CLINICAL STUDIES: PROINFLAMMATORY, IMMUNOMODULATORY, AND ANTIOXIDANT PROPERTIES

As a dietary supplement, *Spirulina*'s immune-enhancing effects are well known. Grzanna et al. (2006) attributed these effects to its high-molecular-weight polysaccharide fraction, while other authors have attributed them to increased secretion of inflammatory cytokines brought about by consuming phycocyanin-C, and hence immunoenhancement (Chen et al., 2014).

Selmi et al. (2011) evaluated the immunomodulatory capabilities of *Spirulina* in a sample of 40 senior citizen volunteers (50 years or older), obtaining clear results for 30 of them. *Spirulina* supplements were administered at a dose of six 500-mg tablets daily for 12 weeks. Monitored parameters included complete cell count and indoleamine 2,3-dioxygenase activity.

The researchers verified a steady increase in mean corpuscular hemoglobin count, volume, and concentration. An increase in indoleamine 2,3-dioxygenase activity and white blood cell count was also verified throughout the trial. The study concluded that *Spirulina* consumption had the potential to ameliorate anemia and immunosenescence in older subjects.

Having previously verified improved immunological functions in mice after consumption of a commercial *Spirulina* supplement (Immulina), Nielsen et al. (2010) performed two additional studies assessing how natural killer cell activity in healthy humans had been enhanced. They attributed previous bioactivity of this supplement to the presence of Braun-type lipoproteins. In their first study, ten individuals were supplied with 400 mg/day of the supplement for 7 days during which time peripheral blood samples were collected daily. A 40% increase in the death of K562 tumor cells by collected natural killer cells was reported. Different dosages of Immulina were administered in their second study, involving 11 individuals who were given 200 and 400 mg daily for 7 days. Increased mRNA expression of a natural killer cell marker was observed, accompanied by roughly proportional increases according to dosage.

Vázquez-Velasco et al. (2014) studied the influence of high-fat squid surimi diets supplemented by *Spirulina* and glucomannan on lipemia, liver glutathione status, antioxidant enzymes, and inflammation biomarkers in Zucker fa/fa rats. The authors reported that glucomannan enriched not only diet-induced hypocholesterolemia but also both antioxidant and proinflammatory effects, and the addition of 0.3% *Spirulina* retained these positive effects and reduced observable inflammation.

In an attempt to understand the value of *Spirulina* as a food-enriching raw material, Bolanho et al. evaluated the chemical composition, antioxidant potential, and sensory properties of several cookie formulations that had been supplemented by 2%–5% *Spirulina platensis* dry powder; there was also a *Spirulina*-free control. The addition of *Spirulina* contributed to an increase in protein content of up to 20% as well as an increase in total minerals and fiber content. Phenolic content and antioxidant capacity were also bolstered by the addition of 64% and 37% *Spirulina platensis* dry powder, respectively. However, sensory acceptance and physical properties (expansion coefficient, color, and hardness) were negatively impacted albeit to a small degree, upon the addition of *Spirulina*.

Abdel-Daim et al. (2015) evaluated the ameliorative effects *Spirulina platensis* and *Dunaliella salina* had on acetic acid–induced ulcerative colitis in rats. The use of *D. salina* was justified because of its markedly high concentrations of carotenoids, molecules with known antioxidant activity and the capacity to reduce colitis symptoms (van Poppel and Goldbohm, 1995). Equal dosages of *Spirulina* and *Dunaliella* (500 mg/kg body weight) were administered. Colonic lesions were examined, as were colon lipid peroxidation and oxidative stress markers including malondialdehyde, protein carbonyl, catalase, reduced glutathione, and superoxide dismutase. Inflammatory markers, tumor necrosis factor alpha, and interleukins (ILs) were also monitored. All colonic mucosal injury, biochemical, and histopathological results were attributed to the significant modulatory effects of the supplements, which brought about an increase in antioxidant enzyme activity and inhibition of lipid peroxidation and inflammation markers. Overall, *Spirulina* was shown to have the greater therapeutic potential of the two foods tested.

Having proven proinflammatory and antioxidant effects, *Spirulina* has shown its potential to prevent the pathogenesis of a number of diseases and thus become an important part of a healthy diet.

CLINICAL STUDIES: HYPOLIPIDEMIC, HYPOGLYCEMIC, AND HYPOTENSIVE EFFECTS

Mazokopakis et al. (2013) evaluated the effect of *Spirulina* supplementation on the lipid profile of 52 dyslipidemic patients given a daily intake of 1 g commercially available tablets for 12 weeks. The authors reported a significant decrease in the mean levels of triglycerides, low-density lipoprotein cholesterol, total cholesterol, and total to high-density lipoprotein cholesterol ratio.

Park et al. (2008) undertook a wide-ranging study of the bioactive capabilities of *Spirulina* and verified their significant cholesterol-decreasing capabilities, which resulted in increased plasma IL-2 and reduced IL-6 concentrations. Female subjects also demonstrated increased superoxide dismutase activity. The study involved 78 elderly (aged 60 and over) Korean individuals, randomly assigned either a dosage of 8 g/day *Spirulina* tablets or a placebo for 16 weeks.

Studies vary greatly in sample size, patient condition, and overall design, which has resulted in inconsistent responses to *Spirulina* supplementation. Nonetheless, cumulative data seem to demonstrate varying degrees of hypolipidemic and hypotensive responses in humans given *Spirulina*-supplemented diets.

CLINICAL STUDIES: ANTIATHEROSCLEROTIC ACTIVITY

As with other chronic diseases, much attention has been given to the role nutraceuticals and functional foods could play in the prevention of atherosclerosis in recent years. Natural products often reduce the risk of prolonged treatments and

incidence of side effects. As a result of rarely documented side effects, *Spirulina* has become a prime candidate for this type of therapeutic approach, although concrete research on its effects is still lacking (Wan et al., 2016).

CLINICAL STUDIES: HEPATIC HEALTH BENEFITS

Studies investigating the hepatic health benefits of *Spirulina* are relatively recent (Madrigal-Santillán et al., 2014). Murthy et al. (2005) were the first to demonstrate the hepatoprotective capabilities of carotenoids extracted from *Spirulina platensis*. The carotenoids isolated were mixed with olive oil and administered to Wistar rats at a dosage of 100 μg carotenoids per kilogram of body weight per day. Serum transaminases, serum alkaline phosphatase, total albumin, and total protein were measured to estimate the degree of hepatoprotection. It was concluded that carotene obtained from *Spirulina* extract provided greater hepatic protection than the synthetic β-carotene used as a control.

These findings triggered further research into the hepatic health effects of *Spirulina* and its compounds. Most findings relate to the evaluation of hepatic recovery/protection capacity upon administration of toxic compounds, such as mercuric chloride, cisplatin, and carbon tetrachloride. Bashandy et al. (2011) studied rats treated with mercury chloride and the consequent rise in hepatic toxicity parameters. They witnessed a decrease in said parameters after administration of *Spirulina*.

CONCLUSIONS

Spirulina is already a very popular and well-regarded dietary supplement. There is little need of research to promote its health benefits. Data regarding its nutritional value complement this, making *Spirulina* an excellent choice when formulating diets and combating malnutrition.

The bioactive potential of *Spirulina* is still being evaluated in preclinical studies using animal models. Nevertheless, these studies seem to indicate *Spirulina*'s strong antioxidant, anticancer, and antiviral properties as well as its capacity to combat obesity, diabetes, and inflammatory allergic reactions. It also shows great immunomodulatory, hypocholesterolemic, and hypoglycemic potential.

Consistent results regarding its bioactivities have led to increasing interest in evaluating the potential of *Spirulina* as a therapeutic food. Hypercholesterolemia, hyperglycemia, cardiovascular disease, and cancer should benefit greatly from treatments using nutraceuticals.

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Chapter 3.40

St. John's Wort (*Hypericum perforatum*)

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INTRODUCTION

As a result of having disease-curing or health-promoting properties, many plants have long been used in traditional medicine. *Hypericum perforatum* L. belongs to the Hypericaceae and is commonly known as St. John's wort (SJW). It has been used as a medicine in various oriental countries (Damlar et al., 2016). It is also known as Klamath weed, Goat weed, Tipton weed, and Enola weed (Klemow et al., 2011; Muenscher, 1946). The name *Hypericum* is derived from two Greek words *hyper* (over) and *eikon* (icon) indicating its historical use against evil spirits. However, researchers say it was used by ancient Greeks to treat mental disorders that they believed to involve demonic possession (Dugoua et al., 2006). It grows well in temperate open disturbed areas (Klemow et al., 2011). Its common name SJW derived from St. John the Baptist, whose birthday is in the same month (June) in which SJW blooms (Foster, 2000). It is a perennial broad-leaved herbaceous plant, erect, multistemmed, and grows to 80 cm in height (Gleason and Cronquist, 1991; Klemow et al., 2011).

SJW was recommended in the first century by Greek physicians (Galen, Dioscorides, Pliny, and Hippocrates) as a diuretic and wound healer, and as a treatment for menstrual disorders (Castleman, 2001; Foster, 2000; Redvers et al., 2001). It is also used in folk medicine for cuts, burns, skin ulcers, and topically for viral infections (World Health Organization, 2002). It contains naphthodianthrones, phloroglucinols, and flavonoids as the major bioactive compounds (Linde, 2009) which have been demonstrated to be an effective treatment for mild to moderate depression (Hubner and Kirste, 2001; Muldner and Zoller, 1984). Moreover, it influences the serotonergic system (Fornal et al., 2001) and suppresses proinflammatory cytokine levels (Tedeschi et al., 2003). Dried flower tips or aerial parts of *Hypericum perforatum* are widely used as crude drugs throughout the world, known as Hyperici herba.

The various beneficial effects brought about by various elements of SJW make it a suitable candidate for discussion. Thus, this chapter should increase knowledge about the distribution, phytopharmacology, interactions, toxicity, and market value of SJW.

DISTRIBUTION AND HABITAT

H. perforatum L. is a native of Europe (Klemow et al., 2011), but is widely distributed in Asia, Australia, North Africa, North and South America, and throughout the temperate areas of the world (Foster, 2000; Gleason and Cronquist, 1991) (Table 3.40.1). SJW commonly grows in grasslands, pastures, meadows, waste areas, roadsides, abandoned mines and quarries, and rangelands (Gleason and Cronquist, 1991; Klemow and Raynal, 1983). It is one of the first plants to invade open areas in natural sites disturbed by forest fires, logging, etc. The growth of SJW is inhibited under wet conditions, since it requires well-drained, coarse-textured soil for best growth. However, the requirement for water for proper growth varies, being higher in winter rains and lower in winter snow areas (Campbell, 1985).

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00056-4

TABLE 3.40.1 Distribution of St. John's Wort (SJW) Throughout the World

Serial no.	Country	Region of occurrence	References[a]
1	America	–	Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237)
2	Australia	–	Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237)
3	Canada	British Columbia, New Brunswick, Newfoundland, and Quebec	PLANTS Database (https://plants.sc.egov.usda.gov/core/profile?symbol=HYPE)
4	Chile	Chile	PLANTS Database (https://plants.sc.egov.usda.gov/core/profile?symbol=HYPE)
5	China	Gansu, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Shanxi, Sichuan, Xinjiang Uygur, and Yunnan	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237), Chinese Plant Names (www.eFloras.org), Flora of China Vol. 13 (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237) and Flora of North America Vol. 6 (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200014237)
6	Europe	–	Flora of Missouri Vol. 2 (www.eFloras.org), Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237), Flora of North America (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200014237)
7	India	Kumaun or North West India	Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237), Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237), Flora of North America (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200014237)
8	Kazakhstan	–	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237)
9	Kyrgyzstan	–	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237)
10	Mongolia	–	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237)
11	North Africa	–	Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237)
12	North America	Caribbean, Hawaii (Alaska; arid west; Atlantic and Gulf Coastal Plain; Eastern Mountains and Piedmont; Great Plains; Midwest; northcentral and northeast; western mountains, valleys, and coast)	PLANTS Database (https://plants.sc.egov.usda.gov/core/profile?symbol=HYPE)
13	Russia	–	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237)
14	Siberia	–	Flora of North America Vol. 6 (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200014237)
15	South Africa	–	Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200014237), Flora of Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014237)
16	United States	California, Kentucky, Michigan, North Carolina, Tennessee, Texas, Virginia, and West Virginia	Tropicos (http://www.tropicos.org/Name/7800012?tab=specimens)

[a] *Sources: Chinese Plant Names and Flora of China, vol. 13; Flora of China; Flora of Missouri, vol. 2; Flora of North America, vol. 6; Flora of Pakistan; Plants Database; Tropicos, plants.usda.gov (plant database) and e-flora.*

MARKET VALUE

The effective and safe use of SJW in treating depression makes it the fifth best-selling dietary supplement in the United States (Wollschlaeger, 2003). It is a sedative herb and currently used to treat depression, insomnia, and sleep disturbances (Khan et al., 2014). Various formulations of SJW are available nowadays, most of which are known to have antidepressant properties (Table 3.40.2). SJW has been shown to deliver better results, greater safety, and fewer side effects than the chemically derived available drugs treating depression (Beckman et al., 2000).

Recent data on the market growth rate of SJW are not available. However, McCutcheon (2017) pointed out that retail sales of SJW dietary supplements from natural sources in the United States remained relatively constant between 2012 and 2015. A similar trend can be observed for sales in mainstream multioutlet sources where sales and rankings from 2013 to 2015 have been consistent.

BIOACTIVE COMPOUNDS

H. perforatum is one of the most widely studied medicinal plants because of its chemical composition and pharmacological properties. The bioactive compounds present in *H. perforatum* can be mainly classified in different groups such as (1) phloroglucinols; (2) naphthodianthrones; (3) flavonoids, tannins, and related compounds; (4) phenylpropanoids and other simple phenolic compounds; (5) xanthones; and (6) volatile compounds (Ganzera et al., 2002; Hansen et al., 1999; Jurgenliemk and Nahrstedt, 2002; Nahrstedt and Butterweck, 1997; Patocka, 2003; Silva et al., 2005; Tatsis et al., 2007; Wurglics and Schubert-Zsilavecz, 2006; Zou et al., 2004). A detailed list of these compounds is given in Table 3.40.3. These constituents are reported to vary according to collection season, flower maturity, and genomic variations (Kosuth et al., 2003; Verma et al., 2008).

TABLE 3.40.2 List of Commercially Available Products Containing SJW as an Active Ingredient

Serial no.	Product name[a]	Price (per piece or packet) in US$	Uses	Manufacturing company
1	SJW	$13.49	Mood support, promotes health and eases symptoms, anxiety, and depression	Vitastrength, USA
2	SJW (LFI Labs)	$13.99	Enhances mood	21st century Healthcare Inc., USA
3	SJW herb	$16.82	Mood support	Nature's Way, USA
4	NeuroIgnite	$27.75	Clarification, focus, and memory	Havasu Nutrition, USA
5	One daily SJW	$10.97	Boosts levels of serotonin in the brain, promotes better mental health, encourages a more positive outlook	Solaray Nutraceutical, USA
6	SJW	$15.63	Promotes positive mood	Nature's Bounty, USA
7	SJW Plus	$21.99	Mood support, mood enhancement	Highest Sun, USA
8	SJW 700	$11.99	Supports mood	Herbal Secrets, UK
9	SJW extract Nervous system	$33.31	Promotes positive mood and emotional balance	Herb Pharm, USA
10	SJW extract	$18.28	Promotes positive mood	Nature's Plus, USA
11	SJW positive thoughts	$14.54	Promotes positive thoughts	Source Naturals, USA
12	Wild harvest SJW	$15.59	Promotes healthy mood	Oregon's Wild Harvest, USA
13	Tea (SJW, Vervain and Skullcap)	Rs. 2240/-	Depression and stress	Tea Tonix, Canada

[a] *Sources: amazon.in, amazon.com, amazon.uk, jet.com*

TABLE 3.40.3 Bioactive Compounds of SJW

Compound name	References
(a) Phloroglucinols	
Hyperforin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Silva et al. (2005), and Tatsis et al. (2007)
Adhyperforin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Silva et al. (2005), and Tatsis et al. (2007)
Hyperfirin	Tatsis et al. (2007)
Adhyperfirin	Tatsis et al. (2007)
(b) Naphthodianthrones	
Hypericin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Silva et al. (2005), and Tatsis et al. (2007)
Protohypericin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Silva et al. (2005), and Tatsis et al. (2007)
Pseudohypericin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Silva et al. (2005), and Tatsis et al. (2007)
Protopseudohypericin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Silva et al. (2005), and Tatsis et al. (2007)
Cyclopseudohypericin	Nahrstedt and Butterweck (1997)
(c) Flavonoids, tannins, and related compounds	
Quercetin	Butterweck et al. (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Zou et al. (2004), Silva et al. (2005), Tatsis et al. (2007), and Wei et al. (2009)
Quercitrin	Butterweck et al. (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Zou et al. (2004), Tatsis et al. (2007), and Wei et al. (2009)
Isoquercetrin	Butterweck et al. (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Zou et al. (2004), Silva et al. (2005), Tatsis et al. (2007), and Wei et al. (2009)
Hyperoside	Butterweck et al. (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Zou et al. (2004), Silva et al. (2005), Tatsis et al. (2007), and Wei et al. (2009)
Rutin	Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Zou et al. (2004), and Silva et al. (2005)
Miquelianin	Butterweck et al. (1997), Jurgenliemk and Nahrstedt (2002), Tatsis et al. (2007), and Wei et al. (2009)
Guaijaverin	Jurgenliemk and Nahrstedt (2002)
Avicurarin	Zou et al. (2004), Wei et al. (2009)
Astilbin	Butterweck et al. (1997), Jurgenliemk and Nahrstedt (2002), and Tatsis et al. (2007)
Quercetin 3-*O*-(2″-acetyl)-β-D-galactopyranoside	Jurgenliemk and Nahrstedt (2002)
Kaempferol	Silva et al. (2005)
Nicotiflorin	Silva et al. (2005)
I3,II8-Biapigenin	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Ganzera et al. (2002), Jurgenliemk and Nahrstedt (2002), Silva et al. (2005), and Tatsis et al. (2007)
Amentoflavone	Nahrstedt and Butterweck (1997), Hansen et al. (1999), Jurgenliemk and Nahrstedt (2002), and Silva et al. (2005)
Procyanidin B2	Patocka (2003)
Isoorientin	Jurgenliemk and Nahrstedt (2002)
Cyanidin 3-*O*-α-rhamnoside	Jurgenliemk and Nahrstedt (2002)
Catechin	Nahrstedt and Butterweck (1997)

TABLE 3.40.3 Bioactive Compounds of SJW—cont'd

Compound name	References
Epicatechin	Nahrstedt and Butterweck (1997)
Epigallocatechin	Wei et al. (2009)
(d) Phenylpropanoids and other simple phenolic compounds	
Chlorogenic acid	Nahrstedt and Butterweck (1997), Patocka (2003), Silva et al. (2005), and Tatsis et al. (2007)
Neochlorogenic acid	Jurgenliemk and Nahrstedt (2002), and Silva et al. (2005)
Chryptochlorogenic acid	Jurgenliemk and Nahrstedt (2002)
3-*O*-(*Z*)-*P*-coumaroylquinic acid	Jurgenliemk and Nahrstedt (2002)
3-*O*-(*E*)-*P*-coumaroylquinic acid	Jurgenliemk and Nahrstedt (2002), and Tatsis et al. (2007)
Caffeic acid	Barnes et al. (2001)
p-Coumaric acid	Barnes et al. (2001)
Ferulic acid	Barnes et al. (2001)
p-Hydroxybenzoic acid	Barnes et al. (2001)
Vanillic acid	Barnes et al. (2001)
Protocatechuic acid	Jurgenliemk and Nahrstedt (2002)
(e) Xanthones	
Mangiferin	Jurgenliemk and Nahrstedt (2002)
1,3,6,7-Tetrahydroxyxanthone	Patocka (2003), and Jurgenliemk and Nahrstedt (2002)
(f) Volatile compounds	
Caryophyllene oxide	Schwob et al. (2004), and Radusiene et al. (2005)
β-Caryophyllene	Schwob et al. (2004), and Radusiene et al. (2005)
Spathulenol	Schwob et al. (2004), and Radusiene et al. (2005)
1-Tetradecanol	Schwob et al. (2004)
β-Funebrene	Schwob et al. (2004)
1-Dodecanol	Schwob et al. (2004)
γ-Muurolene	Schwob et al. (2004)
Viridiflorol	Radusiene et al. (2005)

Phloroglucinols

Hyperforin is one of the major phloroglucinol compounds in *H. perforatum*; there are also small amounts of adhyperforin (Fig. 3.40.1A) (Nahrstedt and Butterweck, 1997). Phloroglucinols are relatively unstable, and few oxygenated analogues of hyperforin have been reported (Verotta et al., 2000). Hyperforin is the main compound responsible for the antidepressant activity of *H. perforatum*; it has other pharmacological effects (Adam et al., 2002; Butterweck et al., 1997; Wurglics and Schubert-Zsilavecz, 2006).

Naphthodianthrones

Hypericin is one of the major naphthodianthrones present in *H. perforatum*, and is also reported to have antidepressant properties (Butterweck et al., 1997). Other minor constituents include protohypericin, pseudohypericin, protopseudohypericin, and cyclopseudohypericin (Fig. 3.40.1B) (Ganzera et al., 2002; Hansen et al., 1999; Jurgenliemk and Nahrstedt, 2002; Nahrstedt and Butterweck, 1997; Silva et al., 2005; Tatsis et al., 2007). Hypericin content is reported to be no less than 0.08% (World Health Organization, 2002). These compounds also have antiretroviral and cytotoxic properties (Kosuth et al., 2003).

Flavonoids, Tannins, and Related Compounds

Flavonoids, biflavonoids, catechins, and anthocyanidins are the main phenolic compounds present in *H. perforatum*, comprising about 2%–4% of total compounds (Fig. 3.40.1C) (Patocka, 2003). The flavonol glycosides of quercetin are the major flavonoids; they include rutin, hyperoside, quercitrin, and isoquercitrin (Butterweck et al., 1997; Ganzera et al., 2002; Hansen et al., 1999; Jurgenliemk and Nahrstedt, 2002; Silva et al., 2005; Tatsis et al., 2007; Wei et al., 2009; Zou et al., 2004). A low number of flavonol aglycones such as quercitrin and kaempferol are also present. Flavans including catechin, epicatechin (Nahrstedt and Butterweck, 1997), epigallocatechin (Wei et al., 2009), flavan dimers, procynidin B2, and tannins have also been reported (Nahrstedt and Butterweck, 1997). *H. perforatum* extract has been reported to have two biflavonoid derivatives, I3,II8-biapigenin and amentoflavone (Ganzera et al., 2002; Hansen et al., 1999; Jurgenliemk and Nahrstedt, 2002; Nahrstedt and Butterweck, 1997; Silva et al., 2005; Tatsis et al., 2007).

Phenylpropanoids and Simple Phenolic Compounds

A low number of phenylpropanoids and other simple phenolic acids are present in the plant extract (Fig. 3.40.1D) (Nahrstedt and Butterweck, 1997; Patocka, 2003; Silva et al., 2005; Tatsis et al., 2007). All three derivatives of caffeoyl quinic acid—chlorogenic acid, cryptochlorogenic acid, and neochlorogenic acid—are reported. Simple phenolics include p-hydroxybenzoic acid and protocatechuic acid; there are others (Barnes et al., 2001).

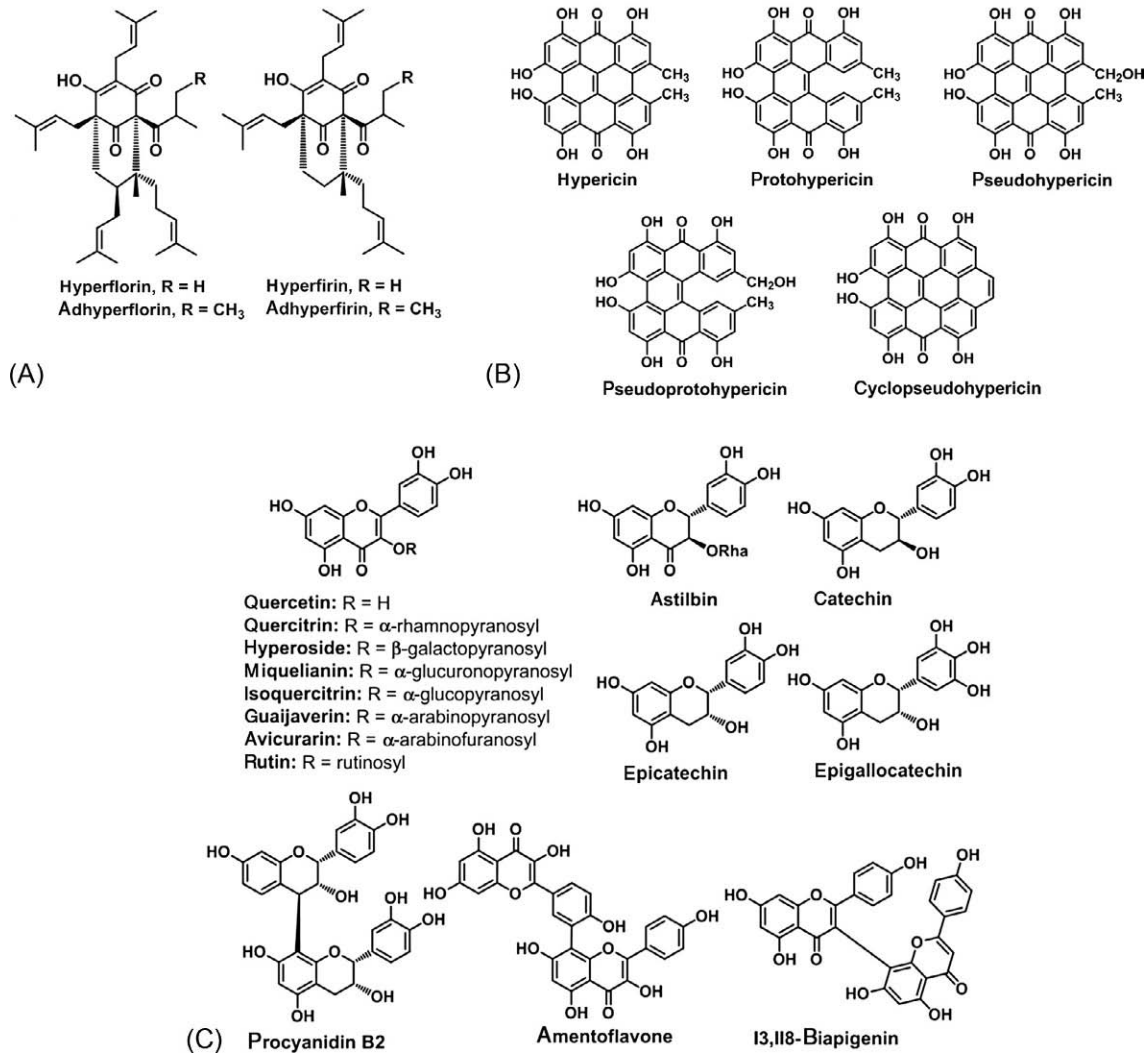

FIG. 3.40.1 Bioactive compounds detected in *Hypericum perforatum* [St. John's wort (SJW)]: (A) phloroglucinol derivatives, (B) naphthodianthrone derivatives, (C) flavonoid derivatives,

(Continued)

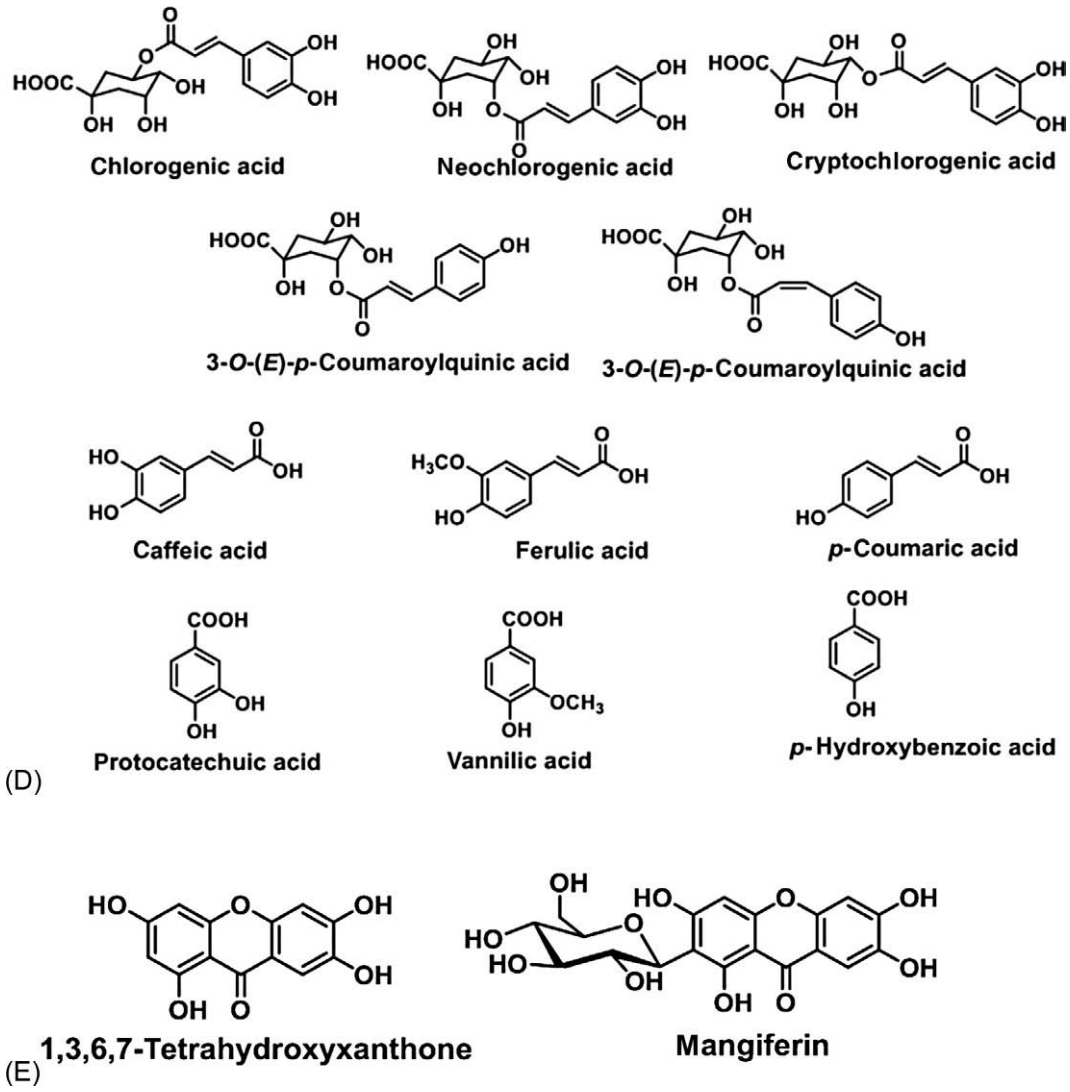

FIG. 3.40.1, CONT'D (D) phenylpropanoid derivatives and other simple phenolics, and (E) xanthone derivatives.

Xanthones

Mangiferin and 1,3,6,7-tetrahydroxyxanthone (Fig. 3.40.1E) are two widely reported xanthones present in *H. perforatum* (Jurgenliemk and Nahrstedt, 2002; Patocka, 2003).

Volatile Compounds

H. perforatum flower and leaf extract (brought about by hydrodistillation) has been reported to contain many volatile compounds such as caryophyllene oxide, β-caryophyllene, spathulenol, 1-tetradecanol, β-funebrene, 1-dodecanol, γ-muurolene, and viridiflorol (Radusiene et al., 2005; Schwob et al., 2004).

BIOACTIVITY

H. perforatum has long been used to treat a number of external and internal disorders. Externally, oily preparations of the plant are used to treat burning mouth syndrome, minor burns, contusions, eczema, hematomas, keloid scars, lumbago, nerve pain, psoriasis, rheumatism, snake bites, sunburn, tooth extraction, and general wounds (Barnes et al., 2001; Blaschek et al., 2008; Greeson et al., 2001; Istikoglou et al., 2010; Moffat, 2014; Patocka, 2003; World Health Organization, 2002). Internally, it is most commonly used for the treatment of anxiety, mild to moderate depression, mood disorders, and stress due to its effect on the nervous system. It soothes the mind and restores natural functioning of the brain (Barnes et al., 2001;

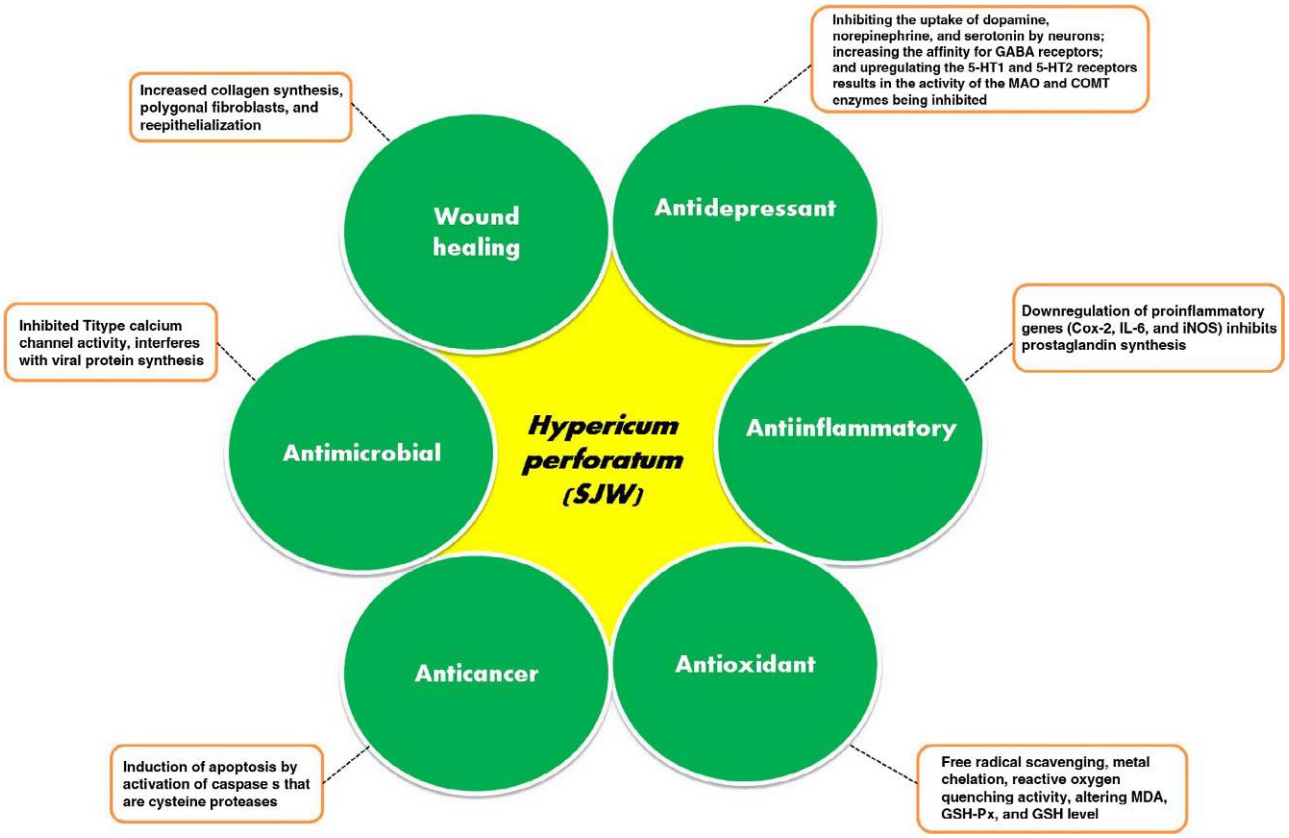

FIG. 3.40.2 Mechanisms underlying the various biological activities of *Hypericum perforatum* SJW. *COMT*, Catechol-*O*-methyltransferase; *COX-2*, cyclooxygenase-2; *GABA*, γ-aminobutyric acid; *GSH*, glutathione peroxidase; *GSH-Px*, glutathione peroxidase and lipid peroxidation; *5-HT1 and 5-HT2*, serotonin receptors; *IL-6*, interleukin-6; *iNOS*, inducible nitric oxide synthase; *MAO*, monoamine oxidase; *MDA*, methylenedioxyamphetamine.

Butterweck and Schmidt, 2007; Greeson et al., 2001; Patocka, 2003; Singh, 2017). SJW is the only herbal alternative to synthetic antidepressants available in the market (Wurglics and Schubert-Zsilavecz, 2006). It also possesses antibacterial, hypotensive, spasmolytic, stimulant (Chopra and Nair, 1956), antiviral (Bombardelli and Morazzoni, 1995; Panossian et al., 1996; Park et al., 1998), apoptotic (Hamilton et al., 1996; Lavie et al., 1999), sedative (Newall et al., 1996), antimalarial, diuretic, sedative, antifungal (Solujic et al., 1997), biochemical (Bork et al., 1999; Cellarova et al., 1997; Cott, 1997; Denke et al., 1999; Kleber et al., 1999), analgesic, antiinflammatory (Bukahri et al., 2004), anticancer, antioxidant, antitumor (Birt et al., 2009; Caraci et al., 2011), nootropic, antischizophrenic, anticonvulsant, and antidiabetic properties (Can et al., 2011; Can and Ozkay, 2012). It has also been found helpful in minimizing the symptoms of attention deficit hyperactivity disorder (ADHD), chronic fatigue syndrome (CFS), irritable bowel syndrome, obsessive–compulsive disorder (OCD), seasonal affective disorder (SAD), somatization disorder, and premenstrual syndrome (PMS) (Newall et al., 1996). It stops breast tenderness, cramps, and irritability in females with PMS (Singh, 2017). Other uses include capillary strengthening, unblocking of clogged arteries, cessation of smoking, decreasing uterine bleeding, treatment of AIDS, hepatitis C, loss of appetite, gastroenteritis, genital herpes, heart palpitations, hemorrhoids, hysteria, kidney and lung ailments, menopausal neurosis, migraines, neuralgia, social phobia, tiredness, trouble sleeping, and weight loss (Bombardelli and Morazzoni, 1995; Jat, 2013). Various biological activities have been tested using in vitro and in vivo models to identify underlying mechanisms (Fig. 3.40.2), some of which are now discussed.

Antidepressant Activity

H. perforatum extract has proven its efficacy as a dietary herbal supplement to treat mild to moderate depression (De Vry et al., 1999; Kasper et al., 2006, 2008; Linde and Knuppel, 2005; Linde et al., 1996; Müller et al., 2001; Schrader, 2000; Schrader et al., 1998; Uebelhack et al., 2004; Woelk, 2000). *H. perforatum* compounds such as hypericin and hyperforin have been reported as primarily responsible for antidepressant activity (Butterweck et al., 1997). Flavonoids, biflavonoids, phloroglucinols, naphthodianthrones, xanthones, proanthocyanidins, phenolic acid, essential oils, and other phenolic compounds have also been reported as responsible for antidepressant activity (Barnes et al., 2001; Hostettmann and Wolfender, 2005).

Gambarana et al. (1999) found that *H. perforatum* extract was able to protect rats against unavoidable stress. Similarly, it was observed to enhance exploratory activity and reduce aggressive behavior in mice (Okpanyi and Weishcer, 1987). Muldner and Zoller (1984) conducted a trial in women using standard hypericin extract and observed a significant improvement in symptoms of anorexia, anxiety, depression, dysphoric mood, hypersomnia, insomnia, loss of interest, obstipation, psychomotoric retardation, and feelings of worthlessness; it was found to have no side effects. Moreover, improved symptomology of depression was observed in children on using SJW extract (Hubner and Kirste, 2001). Numerous clinical trials have been performed using SJW extract; it has been found to possess a similar pharmacological profile to that of clinically effective antidepressants, but some side effects have also been detected at preclinical and clinical levels (Rodriguez-Landa and Contreras, 2003). In another study, *H. perforatum* was observed to be more effective than placebo (Kim et al., 1999) and equally effective as maprotiline (Harrer et al., 1994), amitriptyline, citalopram, fluoxetine, imipramine, paroxetine, sertraline (Linde et al., 1996), and bupropion (Nahrstedt and Butterweck, 1997). In addition, *H. perforatum* has been found to be more tolerable than other synthetic antidepressants with fewer side effects such as dizziness and tiredness (Nathan, 1999). It has also been observed to be a cost-effective alternative to synthetic antidepressants (Solomon et al., 2013). The mechanism of action underlying *H. perforatum* as an antidepressant is not fully understood. A number of theories have been put forward. One possible mechanism is inhibition of the uptake of dopamine (DA), norepinephrine (NE), and serotonin (5HT) from the synaptic cleft of interconnecting neurons (Butterweck, 2003; Neary and Bu, 1999) causing an increase in synaptic concentrations that might be associated with the increase in free intracellular sodium ion concentrations (Singer et al., 1999), which may help in improving mood and restoring emotional balance. A second probable mechanism is its increased affinity for γ-aminobutyric acid (GABA) receptors that block the binding of GABA (Baureithel et al., 1997; Chatterjee et al., 1998), resulting in decreased central nervous system (CNS) depression. Another mechanism is upregulation of 5-HT1 and 5-HT2 receptors in the frontal cortex. A possible fourth mechanism is the ability of *H. perforatum* to inhibit the activity of monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) (Thiede and Walper, 1994; Muller et al., 1997).

Antiinflammatory Activity

Inflammation is a complex response of vascular tissue to harmful stimuli (Kladar et al., 2015). There is demand for development of safer and more effective antiinflammatory drugs as a consequence of the severe side effects of steroidal and nonsteroidal antiinflammatory drugs; hence the interest of researchers in developing herbal drugs (Hyun and Kim, 2009; Shokrzadeh and Saeedi Sarvari, 2009). Studies carried out by different researchers have shown *H. perforatum* to be a promising antiinflammatory agent. Evidence of this is reduced levels of blood and bowel enzymes associated with colonic inflammation (Dost et al., 2009) and lower incidence of gastric ulcers (Cayci and Dayioglu, 2009), which have been observed in rats fed with SJW. Quercetin and I3,II8-biapigenin of oil extract have been observed to provide antiinflammatory effects in rats (Zdunic et al., 2009). Pseudohypericin has been reported to be the main contributor of the antiinflammatory potential of SJW, while hyperin has been found to be associated with the analgesic effect in mice (Huang et al., 2011). There are two likely mechanisms of antiinflammatory action of *H. perforatum*. One is *H. perforatum* extract may downregulate expression of proinflammatory genes such as cyclooxygenase-2, interleukin-6, and inducible nitric oxide synthase (iNOS), which play important roles in chronic inflammation (Tedeschi et al., 2003). Another possible mechanism may be the inhibition of prostaglandin (PG) synthesis by pseudohypericin and hyperforin (Bukahri et al., 2004; Hammer et al., 2007).

Antioxidant Activity

Antioxidants protect the human body against oxidative stress, which has the ability to generate many chronic conditions like Alzheimer disease, atherosclerosis, cancer, diabetes mellitus, ischemic heart disease, Parkinson disease, rheumatism, and even the aging process (Aruoma, 2003; Hemati et al., 2010). *H. perforatum* has been shown to have antioxidant potential by scavenging free radicals, metal chelating, and reactive oxygen-quenching activity (Zou et al., 2004). *H. perforatum* extract has been observed to be an effective antioxidant by altering brain malondialdehyde (MDA), glutathione peroxidase (GSHPx), and glutathione (GSH) levels in rats (El-Sherbiny et al., 2003). Moreover, Sanchez-Muniz et al. (2012) observed that standardized *H. perforatum* extract inhibited lipid peroxidation.

Wound-Healing Activity

The wound healing-activity of *H. perforatum* has been investigated widely. For instance, ointment formulations have been tested in in vitro, in vivo, and histopathological models (Süntar et al., 2010, 2011). The wound-healing activity of *H. perforatum* ointment was found effective in mice and rats. Although elastase activity was not reduced by the extract, collagenase activity was inhibited in vitro (Süntar et al., 2010, 2011).

A study into the wound-healing activity of *H. perforatum* extract was conducted on diabetic rats both topically and orally. The oral formulation was found to be more effective in healing diabetic wounds than topical treatment (Altiparmak and Eskitaşçıoğlu, 2017). Another interesting test used a microcurrent (inducing the flow of electrons) with a gel containing *H. perforatum* and *Arnica montana* to treat surgically induced wounds in rats (Castro et al., 2012). The combined treatment was found much more effective at healing wounds than when individually used (Castro et al., 2012). The leaves of *H. perforatum* were also investigated for their capacity to heal wounds in a rat model. The ointment was formulated for use against excision and incision wounds in rats. The treatment was found effective in reducing closure time, improving regeneration of tissue, and just as effective as the standard drug nitrofurazone (Mukherjee et al., 2000). Similar to the leaves, an oil formulation was extracted from the aerial parts of *H. perforatum* and applied to horse lesions. After 2 days of treatment, healing started and continued for up to 14 days; complete recovery ranged between 1 and 5 weeks (Elisabetta et al., 2017). Hypericin and phenytoin cream were investigated for their effectiveness at healing burn wounds in a rat model. The treatment increases reepithelialization of burn tissue and shortens the time of recovery (Sayar et al., 2014). Moreover, a rat model involving tympanic membrane perforation was used to investigate the curative role of an *H. perforatum* suspension in wound healing and was found effective (Yaşar et al., 2016). An increase in production and activation of fibroblast collagen cells was also recorded when *H. perforatum* extract was tested on chicken embryonic fibroblasts (Öztürk et al., 2007). All these findings suggest that *H. perforatum* is an effective treatment for wound healing.

There have been clinical trials into the wound-healing activity of *H. perforatum*. One involved testing ointment containing *H. perforatum* and *Achillea millefolium* on episiotomy wounds of 140 primiparous women. Both plants reduce pain and redness level, edema, and ecchymoris of episiotomy wounds (Hajhashemi et al., 2017). Another wound-healing study investigated the efficacy of *H. perforatum* by conducting a double-blind clinical trial on women who had undergone surgical childbirth. The treatment was found effective in wound healing, preventing scar formation, and relieving pain (Samadi et al., 2010). A patient with a stage-2 pressure ulcer wound in the sacrococcygeal area was treated with *H. perforatum* oil extract topically, twice daily for 14 days. Macroscopic and histopathological examination showed that the oil extract effectively healed the wound (Yücel et al., 2017).

Anticarcinogenic Activity

Cancer is characterized by overexpressed growth and division of cells infiltrating distant tissues and organs of the body. Cancer therapy often includes radiation, chemotherapy, and surgery (Boik, 2001), which affects both cancer cells and healthy cells (Milis-Torres et al., 2010). Anticancer research has shown that phytotherapy has a lot to offer. Researchers have observed that *H. perforatum* inhibits the proliferation of tumor cells and can treat both melanoma and nonmelanoma skin cancer cells. The anticancer mechanism of *H. perforatum* appears to involve inducing apoptosis by activating caspases (cysteine proteases), which trigger a cascade of events (proteolytic cleavage) in mammalian cells (Klemow et al., 2011).

Hyperforin and hypericin have been identified as the main components responsible for the anticarcinogenic activity of *H. perforatum*. The effect hyperforin has in inhibiting tumor growth and its cytotoxic activity have been examined against human cell lines (Hostanska et al., 2003; Schmitt et al., 2006) and reported to inhibit tumor growth (Schempp et al., 2002). In a study performed by Hostanska et al. (2003), hyperforin combined with the polyphenol, procyanidin B2, was proven effective against the growth of brain glioblastoma, leukemia cells, and normal human astrocytes. Hypericin has also been identified as a potent anticancer agent, limiting the growth of adenoma, carcinoma, glioma, leukemia, melanoma, mesothelioma, neuroblastoma, and sarcoma cells (Fox et al., 1998). It has also been found to show cytocidal activity against the growth of a human erythroleukemic cell line (K562) (Roscetti et al., 2004). Both hypericin and hyperforin compounds have been found to inhibit leukemia cell growth (Hostanska et al., 2003).

Antimicrobial Activity

H. perforatum has been shown to possess various important biological and chemical properties that can be used to treat a number of infectious diseases. For instance, hyperforin has been shown to exhibit antibacterial activity against *Staphylococcus aureus* (Brondz et al., 1982), while hypericin showed an inhibitory effect against penicillin-resistant *S. aureus* (PRSA) and methicillin-resistant *S. aureus* (MRSA) (Lopez-Bazzocchi et al., 1991). The antibacterial properties of this compound were discovered as a result of it inhibiting T-type calcium channel activity and possessing a cellular protective effect (Breyer et al., 2007). *H. perforatum* butanol extract has been observed to possess anti-*Helicobacter pylori* properties (Reichling et al., 2001), while ethanol extract exhibited an inhibitory effect on the growth of *Penicillium canescens*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus glaucus*, *Streptococcus mutans*, *Streptococcus sobrinus*,

Lactobacillus plantarum, and *Enterococcus faecalis* (Maskovic et al., 2011; Suntar et al., 2016). Methanol extract inhibited in vitro growth of *Escherichia coli*, *Proteus vulgaris*, *S. mutans*, *Streptococcus sanguis*, *Streptococcus*—oxford, and *S. aureus* (Barbagallo and Chisari, 1987).

Hypericin has been seen to act differently against enveloped and nonenveloped viruses; the compound has been shown to be effective only against enveloped virus (Diwu, 1995). The antiviral mechanism underlying hypericin probably involves photoactivation (American Herbal Pharmacopeia, 1997; Hudson et al., 1991). Degar et al. (1992) stated that hypericin inactivates enveloped viruses by interfering with viral protein synthesis, rather than affecting nucleic acids that are the target of antiviral nucleosides. Hypericin binds to the lipid membrane of the virus and absorbs photons, thus inhibiting viral fusion with cell membranes (Degar et al., 1992; Lenard et al., 1993). This is the reason hypericin inactivates enveloped viruses and not nonenveloped ones. Flavonoid and catechin-rich *H. perforatum* extract has been shown to inhibit the influenza virus (Mishenkova et al., 1975). In vitro inhibition of herpes simplex types 1 and 2, sindbis virus, poliovirus, and retrovirus as well as in vivo inhibition of murine cytomegalovirus (MCMV) and hepatitis C have been credited to *H. perforatum* (Bombardelli and Morazzoni, 1995; Newall et al., 1996; Sardella et al., 2008). It also possesses antiviral properties against poliovirus II, vaccinia virus (May and Willuhn, 1978), herpes viruses (Tang et al., 1990), vesiculostomatitis, Sendai viruses (Lenard et al., 1993), and duck hepatitis B virus (Moraleda et al., 1993). Hypericin has also been shown to inhibit human immunodeficiency virus (HIV). It does so by inhibiting the activity of HIV reverse transcriptase in vitro (Cohen et al., 1996; Weber et al., 1994). In vivo studies have also been conducted and SJW was found to be effective against Friend virus, LPBMS murine immunodeficiency viruses, MCMV, Ranscher leukemia virus, and sindbis virus (Hudson et al., 1991; Meruelo 1993; Stevenson and Lenard, 1993). There have been few studies into the antifungal activity of *H. perforatum*; one of them by Schempp et al. (1999) found *H. perforatum* had no effect against *Candida albicans*.

Other Activities

Different parts of SJW have been found to act on the immune system in different ways. For instance, the hydrophilic part of polyphenols stimulate the immune system by activating the mononuclear immune system, cellular immunity, and humoral immunity, while the lipophilic part exhibited immune-suppressive activity (Evstifeeva and Sibiriak, 1995). In another experiment, *H. perforatum* flavonoid-rich extract along with a cholesterol-rich diet was administered to rats (Zou et al., 2005). Rats treated with extract showed reduced levels of total cholesterol, triglycerides, and low-density lipoprotein, while the high-density lipoprotein concentration increased. Moreover, superoxide dismutase and catalase activity increased (Zou et al., 2005). A similar protective effect was also found when tested on hyperlipidemic rats (Moghaddam et al., 2016). When ovariectomized rats were treated with *H. perforatum* ethanol extract, it was found that the extract prevented hypercholesterolemia and reduced obesity (You et al., 2014). Similar results were found on testing *H. perforatum* in hypercholesterolemic rabbits (Asgary et al., 2012).

Diabetic and diabetic-induced pain have also been found to be effectively tackled using SJW extract, which significantly reduced blood sugar level (Can et al., 2011). Moreover, when *H. perforatum* ethyl acetate extract was examined against streptozotocin-induced diabetic conditions, it was found to effectively treat such conditions and at the same time reduce serum cholesterol triglycerides (Arokiyaraj et al., 2011). Similar positive results have been seen when *H. perforatum* extract was administered in high fat diet-induced obese rats and fructose-fed rats (Husain et al., 2011).

H. perforatum's protective properties have also been tested against hepatic ischemia reperfusion injury in rats. The treatment significantly decreased alanine aminotransferase, aspartate aminotransferase, and alkaline dehydrogenase activity and reduced the MDA level too. However, catalase and GSHPx activity increased demonstrating *H. perforatum*'s protective properties against hepatic injury (Bayramoglu et al., 2014).

INTERACTIONS AND TOXICITY

Herbal preparations of SJW have been bought over the counter to treat a variety of illnesses in Europe and the United States for decades. Toxicity and interaction studies have been carried out on SJW alone or in combination with other drugs. Some of these studies are now discussed.

Interaction With Warfarin or Phenoprocumon

SJW has been reported to interact with warfarin and decrease its effect—see the first report in the *Bulletin of the Swedish Medical Products Agency* published in 1998. Thereafter, Maurer et al. (1999) reported an unexpected interaction between

SJW and warfarin or phenoprocumon. Ernst (1999) and Yue et al. (2000) witnessed unstable international normalized ratio (INR) values as a result of such drug interactions with SJW. These unstable INR values suggest that CYP2C9 has been induced. CYP2C9 breaks down the pharmacologically active S-enantiomer of warfarin (Kaminsky and Zhang 1997; Miners et al., 1998). Nevertheless, in vitro or in vivo studies still need to confirm this.

Interaction With Cyclosporin

A potential interactive effect between SJW and cyclosporin has been recorded in many studies (Barone et al., 2000; Mai et al., 2000; Ruschitzka et al., 2000). These mainly involved transplant rejection (e.g., two cases in which heart transplants were rejected and one case each in which a pancreas and a kidney were rejected). Transplant graft rejection was attributed in all these cases to patients being treated with SJW who had decreased levels of cyclosporin—between 25% (Rey and Walter, 1997) and 62% (Ruschitzka et al., 2000)—within 3–4 weeks of starting SJW. When SJW was stopped, some patients recovered suddenly while others required additional immunosuppressive therapy.

Breidenbach et al. (2000) threw some light on cases in which a decrease in mean cyclosporin concentration (47%) was recorded in patients with kidney grafts treated with SJW. To overcome this, SJW was stopped and the cyclosporin concentration was increased by a mean value of 187%, which further required the SJW dose to be decreased. Watkins (1990) and Lown et al. (1997) stated that cyclosporin, a substrate of *P*-glycoprotein, was being metabolized by CYP3A4. The plasma level of cyclosporin reduced both CYP3A4 and *P*-glycoprotein to subtherapeutic levels as a result of interaction with SJW (Ernst, 1999). This leads to rejection of transplanted organs.

Interaction With Oral Contraceptives

Alhough all oral contraceptives are metabolized by cytochrome P450 enzymes, their metabolism can vary between products (Ball et al., 1990; Guengerich, 1990; Schmider et al., 1997; Shader and Oesterheld, 2000). Bleeding has been reported in women who took both SJW and the oral contraceptive pill. In December 2001, seven unplanned pregnancies in the United Kingdom were reported as possibly being due to interactions with SJW; two cases were also reported in Sweden. It was believed that CYP3A4 induction caused drug levels to lower (Ernst, 1999; Yue et al., 2000), although no such reports have been documented. Moreover, no change in the concentration of estrogen in the blood has been observed after administration of *H. perforatum* extract (Kaufeler et al., 2001).

Interaction With Theophylline, Digoxin, and Human Immunodeficiency Virus Protease Inhibitors

The CYP1A2 hepatic enzyme is responsible for clearance of theophylline, which is upregulated by SJW. Thus the effect of theophylline is reduced (Ha et al., 1995; Nebel et al., 1999). Multiple-dose treatment with *H. perforatum* extract brought about a *P*-glycoprotein level that influenced the pharmacokinetics of digoxin. Hence, the absorption and distribution of digoxin is influenced and manifests itself as a reduction in maximum concentration and area under the curve (Johne et al., 1999). A specific pharmacokinetic study done by Piscitelli et al. (2000) showed a decrease in indinavir concentration that ranged, 8 h after dosing, from 49% to 99%. By administering SJW combined with indinavir the concentration of indinavir in blood decreased by a mean value of 57%, which led to complete failure and drug resistance.

CONCLUSIONS

SJW is an herbal plant whose beneficial effects in various oriental medicinal systems have been proven. It is an effective dietary supplement in treating depression and anxiety, thereby promoting mental health and positive mood. Moreover, SJW has been proven to have effective antiinflammatory, anticarcinogenic, antimicrobial, and antioxidant properties. Many medicines containing SJW as an active ingredient are commercially available. There have been reports that SJW interacts with other drugs and that caution should be taken in such cases. SJW is the only effective and safe natural medicine for the treatment of depression. Various chemical classes of compounds such as phloroglucinols, naphthodianthrones, flavonoids, tannins, polyphenols, phenylpropanoids, and volatile compounds have been detected and identified in SJW. More clinical trials on using SJW as a dietary supplement need to be conducted to check its long-term effectiveness. In addition, more studies on the mechanisms underlying SJW's capacity to treat various diseases need to be carried out to understand the molecular basis behind its effectiveness.

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Chapter 3.41

Tea Extracts

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TEA

Tea, native to China, is today the most widely consumed beverage in the world, aside from water. Tea is an infusion of *Camellia sinensis* leaves. The dried leaves of *C. sinensis* are used to produce several types of tea. Depending on the different manufacturing processes the leaves are subjected to, you can get three kinds of tea, green tea (nonfermented), oolong tea (partially fermented), and black tea (fermented) (Graham, 1991).

Steaming and drying freshly harvested leaves produces green tea, therefore, its composition is not very different from the composition of the leaves themselves.

During fermentation important molecules are lost, like polyphenols, which seem to be responsible for many of the benefits of tea. Around 20%–30% of the flavonoids can be oxidized during this process, originating similar polymers to those are found in black tea (Wiseman et al., 1997).

Thus, green tea is the tea that contains larger amounts of bioactive molecules and, therefore, is the tea most associated with beneficial human health effects.

CHEMICAL COMPOSITION OF TEA

The chemical composition of tea is complex: polyphenols, fluoride, vitamin K, caffeine, minerals (sodium, potassium, and calcium) alkaloids, amino acids, glycerides, volatile compounds, and trace elements like, aluminium, chromium, selenium, manganese, and iron (Reto et al., 2007a,b, 2008).

The polyphenols are the most interesting group of components found in the leaves of *C. sinensis* because many of the biological activities of tea are associated with them. The catechins, that is, catechin (C), epigallocatechin (EGC), epigallocatechin-gallate (EGCG), epicatechin (EC), and epicatechin gallate are the main flavonoids present in green tea. The beneficial effects of tea have been attributed to these compounds, especially to EGCG, which represents 40% of the total catechin content in the leaves of green tea, because they are the most biologically active group of tea compounds (MacKay and Blumberg, 2000). Caffeine is also present naturally in tea. Additionally, tea contains other compounds with physiological actions such as quercetin, phenolic acids, and vitamin K, among others.

HEALTH EFFECTS

The beneficial health effects of green tea have been demonstrated in a large number of studies (Sharangi, 2009).

Because of their potent antioxidant activity, tea catechins can play a very important role in preventing diseases in which oxidative damage is important, such as cardiovascular diseases, inflammatory diseases, and cancer.

Several studies have shown that tea catechins and other polyphenols are antioxidants through various mechanisms (Guo et al., 1999; Nakagawa and Yokozawa, 2002; Ostrowska et al., 2004; Paquay et al., 2000; Wiseman et al., 1997), however, this antioxidant action is dose dependent. For example, a human study revealed that doses lower than 3 cups of tea per day did not decrease the formation of compounds resulting from lipid oxidation (Cherubini et al., 1999) observed by Freese et al. (1999) in humans who drank 10 cups of tea per day.

Other works have related the effect of tea, or tea extract, consumption with the antioxidant capacity of human plasma. An increase in antioxidant capacity was observed for both green and black tea by Leenen et al. (2000), Sung et al. (2000), and Serafini et al. (1996), however, McAnlis et al. (1998) did not observe the same effect with black tea.

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Antiinflammatory capacities are also attributed to tea flavonoids. Both green and black tea extracts demonstrated this activity in animals (Das et al., 2002; Varilek et al., 2001).

Through cellular, animal, and human experiments, green tea and its major component, epigallocatechin-3-gallate (EGCG), have been demonstrated to have antiinflammatory effects (Nash and Ward, 2016; Ohishi et al., 2016; Reto et al., 2014).

Evidence from animal studies suggests that catechins inhibit cholesterol absorption and lower plasma cholesterol (Bursill et al., 2007; Zheng et al., 2011). Epidemiological studies indicated a significant inverse relationship between drinking green or black tea and plasma levels of cholesterol and low-density lipoprotein (LDL) (Davies et al., 2003; Peters et al., 2001; Zheng et al., 2011).

The association of drinking tea with reduction of serum cholesterol and LDL, prevention of LDL oxidation, as well as the antiinflammatory effect, contributes to control some of the risk factors linked to the development atherogenesis and, therefore, cardiovascular disease (MacKay and Blumberg, 2000; Suzuki-Sugihara et al., 2016).

The inhibitory activities of tea catechins against carcinogenesis and cancer cell growth have been demonstrated in a large number of laboratory studies, but EGCG, the principal catechin in green tea, has received the most attention (Chung et al., 2003, 2016).

Several human and animal studies suggest that green tea has an antioxidant effect, antibacterial and antiviral activity, cancer chemopreventive properties, contributes to a reduction in the risk of cardiovascular disease, and enhances weight loss, among other effects (MacKay and Blumberg, 2000).

The increased popularity of green tea is clearly related to the health benefits attributed to its consumption. In fact, the antiobesity effect associated with tea, especially green tea, is the main reason responsible for its increased consumption in recent years.

One of the most important reasons for this increased interest is that obesity has become a global epidemic and public health problem (Turk et al., 2009).

The effects of green tea on energy metabolism and body weight are related to its bioactive components—catechins and also caffeine.

Some studies reported that green tea affects the sympathetic nervous system and causes an increase in thermogenesis and substrate, together with fat oxidation. On the other hand, green tea can control body weight because of its inhibition of some enzymes, which work as part of the mechanism driving appetite, lipid metabolism, and nutritional element absorption (Rains et al., 2011). However, there are studies that suggest that the polyphenols in black tea have excellent antiobesity activity too, without having the apparent side effects associated with green tea, and that black tea polyphenols are more effective than green tea polyphenols (Haibo Pan et al., 2016).

The antiobesity mechanisms of black tea polyphenols include inhibiting lipid digestion, absorption, and intake, thus reducing calorie intake, attenuating lipogenesis, enhancing lipolysis, decreasing the differentiation and proliferation of preadipocytes, and suppressing oxidative stress (Haibo Pan et al., 2016).

Significant effects in humans are noted only at high doses, such as 400–500 mg of EGCG equivalent per day. One cup of *C. sinensis* green tea may contains approximately 50 mg of EGCG.

Fat-burning effects are highly synergistic, in fact almost dependent, on not consuming caffeine habitually.

TEA EXTRACTS—FOOD SUPPLEMENTS

As already noted, all biological effects/benefits are dose dependent, both in green tea itself (as a drink) as well as in its supplemental forms (tea extracts). Low concentrations of bioactive compounds do not exert effects and very high doses can be toxic. The amount of phenols and other compounds varies greatly in the leaves of *C. sinensis* and depends on the specific crop, the soil it is grown in, the weather conditions, and its harvest time. In addition, phenol concentration in tea beverages also depends on how the tea is obtained, its infusion time, and ratio of tea to water volume and temperature. Usually, the conditions for making tea at home involve the infusion of a tea bag (1.5 g of tea leaves) in a cup of 250 mL of boiling water for a period of 10 min. However, these conditions can vary greatly from consumer to consumer. Of course, some people do not like tea, specifically green tea, finding it a little astringent.

Although polyphenols and catechins are commonly found in tea, the highest concentrations can be found in forms of tea extract (Duygi and Nilufer, 2017).

Additionally, it is green tea, which has the greatest amount of active compounds associated with beneficial health effects. Therefore, green tea is the most common tea used to produce extracts used as food supplements. Green tea dietary supplements are the fourth most popular dietary supplement on the market in the United States (Sarma et al., 2008).

The catechin EGCG is one of the most beneficial elements in green tea, therefore, it is this material that is extracted and found in green tea tablets.

Thus, the use of tea extracts as a food supplement can have great benefits because they are standardized—the amounts of active compounds can be controlled to ensure that they are ingested in effective doses.

There are different processes used to make green tea supplements. One method is to dry green tea leaves and put them in capsules. Other types of supplements are industrially processed to make green tea extracts. In this production method tea leaves are brewed and catechins extracted and used in tablets, capsules, and pills.

Types of Green Tea Extracts

Strong infusions obtained when green tea leaves are processed by soaking them in aqueous solutions of alcohol (the catechin content is about 2% w/w); *soft extracts* obtained by concentrating strong infusions to 20%–25% (the catechin content is about 20% w/w); *dry extracts* obtained by concentrating strong infusions to 40%–50% solid (the catechin content is above 25% w/w) followed by subsequent spraying until the solid becomes a dehydrated extract and powder.

The extracts are usually processed as a powder containing inert processing aids suitable for a variety of uses (tablets, capsules, dry mixes, etc.) (Johnson and Williamson, 2003).

To increase the concentration of catechins it is possible to use solvent extraction and/or purification using membranes.

There are some green tea extracts, which are incredibly pure, providing 98% polyphenols of which about 45% is EGCG.

Green tea extracts also contain a small amount of natural caffeine, which can be a problem because it acts as a stimulant of the central nervous system and may cause side effects. However, caffeine-free tablets and capsules are already available on the market.

Some green tea capsules contain excessive amounts of polyphenols, namely EGCG.

Most green tea tablets or pills are highly concentrated, containing many more catechins than are found in five or more cups of green tea. It is very important to carefully choose the most suitable green tea extract supplement—very high doses of catechins can act as prooxidants and the use of some green tea extracts have been linked to occasional cases of acute liver failure (Molinari et al., 2006; Pezeshki et al., 2016).

It is equally important note if the supplement (capsules, tablets, and pills) has been subjected to adequate quality controls and if it is free of steroids, alcohol, and other contaminants.

CONCLUSIONS

Although consumption of dietary phytochemicals such as tea flavonoids has been suggested to have beneficial biological effects, it is not yet possible to state that such compounds are free of side effects. The risk of adverse effects is increased with the use of pharmacological doses used for prevention/treatment purposes as well as supplement situations or drug–drug interactions that increase the bioavailability of certain compounds (Lambert et al., 2007).

It is well known that food supplements have higher concentrations of active compounds than food, so it is easy to exceed recommended maximum doses. In contrast to medicines, food supplements are not usually supervised.

To conclude, there are many green tea extract supplements on the market, including those available online. However, there is still not enough scientific evidence to guarantee their beneficial effects and the absence of toxicity.

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Chapter 3.42

White Tea

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INTRODUCTION

The leaves of *Camellia sinensis* (L.) O. Kuntze, which belongs to the Theaceae family, have been used since ancient times to prepare tea infusions (Moderno et al., 2009). This plant is native to southwest China, but it is actually cultivated in tropical regions around the world (Hasimoto and Simura, 1978). Technically, only infusions made with the dried leaves (or buds) of this plant should be called tea. Different types of tea can be obtained from *C. sinensis*: white, green, oolong, and black (Alcazar et al., 2007). The distinguishing factor that defines the different types of tea is the "level of fermentation" of the leaves (or buds) during the manufacturing process (de Mejia et al., 2009). This process is more accurately called "oxidation," as it represents the degree of enzymatic oxidation that is allowed to occur to the freshly picked leaves until they are dried (Alcazar et al., 2007). White tea (WT) is considered the least processed tea type, followed by green tea (GT). These nonfermented or very lightly fermented tea types are very rich in polyphenolic, flavonoid-derived compounds known as catechins (flavan-3-ols) (Dias et al., 2014, 2017). On the other hand, during oolong and black tea processing, the catechins in the leaves are oxidized and polymerized, by polyphenol oxidase, forming yellow–orange pigments known as theaflavins (dimers) and thearubigins (oligomers) (Dias et al., 2013; Koech et al., 2013). These compounds contribute not only to the color of tea, but also the bitterness and astringency of oolong and black teas (Muthumani and Kumar, 2007). Although theaflavins have demonstrated a similar free radical–scavenging activity as catechins in certain biological processes (Leung et al., 2001), catechins are usually more potent antioxidants than theaflavins and thearubigins (Stewart et al., 2005). Hereupon, the amount of catechins is positively correlated with antioxidant potential. Therefore, in recent years, WT has become more attractive due to its high antioxidant activity. Evidence of this scenario can be found in many publications from the last two decades, reporting total amount of tea produced and consumed in the world. In these publications WT did not feature at all (Wang et al., 2011; Yang et al., 2000). However, statistical data about tea consumption in 2015 in the United States, indicated consumption of 85% black tea, 14% GT, and the remaining 1% oolong and WT (Tea Association of the USA Inc., 2015). Thus, WT does not seem very popular among tea consumers but is gaining ground. Unfamiliarity, controversial results, its subtle taste, and higher cost may be some of the reasons for low consumption of WT. Nevertheless, the potent antioxidant potential of WT has been demonstrated by many studies (Carloni et al., 2013; Dias et al., 2014), and might be an encouraging factor leading to its increased consumption. This type of tea has revealed interesting effects against oxidative stress–related diseases, including cancer, diabetes mellitus, obesity, and neurodegenerative and cardiovascular diseases (Dias et al., 2013), which will be further discussed in this chapter. However, most of the studies were conducted with cell cultures (Martins et al., 2014) and animal models (Dias et al., 2016), and some of these studies used very high doses of WT components which are not achievable in the human body based on daily ingestion. Moreover, it is known that after tea ingestion, catechins are readily absorbed but low plasma concentrations are attained (Manach et al., 2005). Thus, the development of WT-based food supplements has been proposed as the best way to achieve similar results to those reported in the studies. In modern societies, the use of supplements is becoming a trend. In 2015, the global annual sales of dietary supplements exceeded USD 120 billion and is expected to increase to USD 278 billion by 2024 (Market Research Report, 2016). There is a vast range of over-the-counter supplements in the market, including vitamins, minerals, and botanicals (Abourashed et al., 2016). GT preparations are among the top selling botanical dietary supplements, especially for anticarcinogenic (Bettuzzi et al., 2006), weight loss (Westerterp-Plantenga et al., 2005), and skin antiaging purposes

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(Hsu, 2005). Moreover, as no undesired effects have been found with tea ingestion, people are eager to try tea-based products to potentiate their health-promoting effects. In this chapter, we will discuss the potential use of WT as food supplement.

WHITE TEA PECULIARITIES

In contrast to the other tea types, there is no general accepted definition of WT. There is still great controversy concerning its manufacture and its content in caffeine and catechins. WT is best known in Asia and less known in western communities that prefer black tea. Nevertheless, in Europe, the flavor of WT seems to be more accepted than that of GT (Almajano et al., 2008). In fact, the availability of WT in European supermarkets has been increasing, which is certainly due to a higher demand from consumers. WT is characterized by its very pale yellow color and mild delicate and sweet taste. Though, there are also some versions with added flavors. Annually, very small quantities of WT are produced, once it is prepared from the new, unopened buds of the tea plant and/or immature leaves which are covered with tiny silvery-white hairs (Pettigrew, 2004)—the reason it is called "white" tea. In addition, WT harvesting should only occur once a year, more specifically in the early spring, in order to attain higher quality tea. Traditionally, after picking, the buds/leaves are gently spread out to dry under the sun for quite a lengthy period of time to ensure maintenance of the leaf structure and avoiding any breaks by curling or twisting of the leaves (Damiani et al., 2014). During drying, the tea becomes slightly "oxidized," as it contains very small amounts of theaflavins and thearubigins (Koech et al., 2013). Thus, it is wrongly called a nonfermented tea, it should be considered a very lightly fermented tea instead (Gopal et al., 2016). Nevertheless, there are some variations among the processing techniques that lead to different WT types and these should be taken into consideration. One of the best known and expensive WT types is the Bai Hao Yin Zhen, also known as Silver Needle. It is produced in the Fujian province in China using only new leaf shoots, which are preferably harvested by hand (Ugochukwu et al., 2003). The processing method for Silver Needle includes a one-day drying of the buds under the sun, on sieves or drying mats, followed by baking over a slow fire until the moisture content is fully removed (Damiani et al., 2014). Bai Mu Dan or White Peony is another type of WT from the Chinese Fujian province, which uses the leaf shoot and two youngest leaves, thus attaining a light golden-brown color when brewed and a more intense taste relative to Silver Needle (Ugochukwu et al., 2003). The manufacture of White Peony includes a withering process under the sun (taking 1–3 days), which is followed by drying in a basket (Damiani et al., 2014).

Generally, the main bioactive constituents of WT include amino acids, polyphenols, and methylxanthines (Dias et al., 2014). The most prevalent amino acid in tea is L-theanine (representing about 4% of leaf dry weight), which contributes to the pleasant and relaxing effects of the tea (Sun et al., 2014). Among the polyphenols, the catechin content really stands out, representing more than 20%–30% of leaf dry weight (Dias et al., 2014; Koech et al., 2013). These are the compounds to which are attributed the antioxidant properties of WT. The main catechin derivatives present in WT include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The latter catechin accounts for about 50% of all the catechins and is considered the most bioactive component of tea (Dias et al., 2014; Koech et al., 2013). The antioxidant potential of catechins is measured by their free radical–scavenging ability and metal-chelating properties (Frei and Higdon, 2003). The free radical–scavenging ability of a certain compound is related to its potential to act as a hydrogen/electron donor under identical conditions (Gopal et al., 2016). The most effective radical scavengers in WT are EGCG and EGC due to their characteristic structure, as presented in Fig. 3.42.1 (Dias et al., 2017). While both EGCG and EGC present hydroxyl (OH) groups at positions 3′, 4′, and 5′ of the B-ring, EGCG has also a 3-gallate group at the C-ring (Dias et al., 2017). The catechin content is often mistakenly used as a differentiation parameter between WT and GT, but it is not a reasonable approach to take. There are some WT types that contain a higher catechin content than GT (Koech et al., 2013; Rusak et al., 2008), but the opposite also occurs (Dias et al., 2014). This is due to the fact that tea chemical composition is influenced not only by differences in the processing techniques, but also by the geographical origin of growth, climate, soil, botanical variety, harvest time, horticultural practices, and even brewing conditions (Damiani et al., 2014; de Mejia et al., 2009). Thus, there is a high variance not only in catechin content, but also in other components, such as methylxanthines. On the other hand, it does seem feasible to use catechin content as a differentiating parameter when comparing either WT or GT with oolong and black tea (Koech et al., 2013). Indeed, most of the components of black tea are thearubigins (60%–70%), with only 3%–10% catechins (Kuhnert et al., 2010).

Concerning methylxanthines, the most abundant in tea is caffeine (about 3.5%). However, tea also contains low amounts of theobromine (0.15%–0.2%) and theophylline (0.02%–0.04%) (Gopal et al., 2016). Caffeine popularity is attributed to its potent stimulating properties and is usually associated with coffee or caffeinated energy drinks. However, the amount of caffeine among tea types has also been questioned. There are many reports stating that WT has the least caffeine content among the several tea types (Khokhar and Magnusdottir, 2002; Lin et al., 2003). However, this affirmation cannot

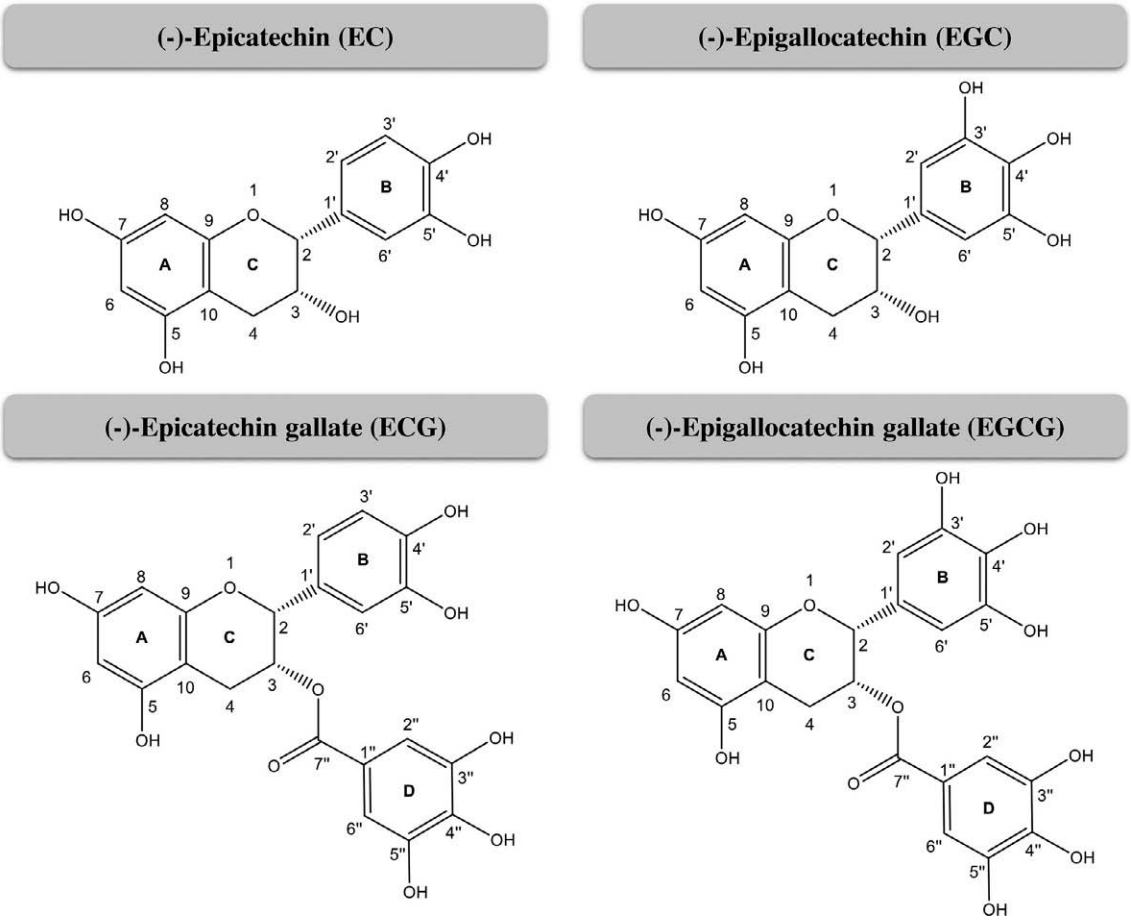

FIG. 3.42.1 Chemical structures of the main catechins present in white tea.

be so strictly made due to the abovementioned variables. In fact, there are some WT types with higher caffeine contents than GT (Dias et al., 2014). Hence, other parameters should be used to distinguish WT from GT, such as color, taste, and plucking and processing steps. Once there are already many GT-based dietary supplements on the market, in the form of concentrated extracts in tablets, capsules, and liquid formulations, WT's similar chemical composition and antioxidant activity to GT (Koech et al., 2013) makes it a potential product for inclusion in dietary supplements.

HEALTH BENEFITS FROM WHITE TEA

The ingestion of tea is usually associated with a pleasant sensation and a warm or refreshing feeling, when consumed hot or cold, respectively. Most people drink tea not only for its sensorial characteristics, but also for the wide range of health benefits associated with it. Similarly to GT, most of WT's health-promoting effects are ascribed to its high phenolics content and respective antioxidant activity (Dias et al., 2013; Koech et al., 2013). Although the human body possesses efficient defense systems to control naturally produced free radicals, unhealthy lifestyle habits (e.g., smoking, alcohol consumption, or poor diet), aging, and physiological disorders may reduce their efficiency. This may lead to the impairment of redox homeostasis, resulting in the overproduction of free radicals, such as reactive oxygen and nitrogen species (RONS) (Rietveld and Wiseman, 2003; Willet, 1994). Chronic exposure to RONS can lead to DNA damage, alterations in membrane lipids, and changes in functional and structural proteins (Halliwell et al., 1997; Sohal and Weindruch, 1996). Subsequently, this oxidative damage may trigger the development of numerous human dysfunctions, such as cardiovascular diseases (Dhalla et al., 2000), diabetes mellitus (Rochette et al., 2014), obesity (Marseglia et al., 2014), neurodegenerative disorders (Dasuri et al., 2013), subfertility/infertility (Agarwal et al., 2008), and certain types of cancer (Sosa et al., 2013). This highlights the importance of using exogenous antioxidants as a preventive measure or even to avoid the progression of oxidative stress–related diseases (Dias et al., 2017). Recently, natural compounds have been widely debated for their interesting antioxidant properties (Dias et al., 2016b). Polyphenols, especially catechins, demonstrated potent antioxidant potential

acting as quenchers of free radical species and metal chelators (Atoui et al., 2005). Thus, as WT is rich in catechins, it could be a cost-effective measure to maintain redox balance in certain human conditions. In fact, studies with animal models subjected to the ingestion of a WT extract demonstrated an increase in the antioxidant potential of different organs, such as the heart (Alves et al., 2015), lungs (Koutelidakis et al., 2009), brain (Nunes et al., 2015), and reproductive organs (Alves et al., 2015; Dias et al., 2016a). Although, there is a lack of human studies concerning the effects of WT, data from clinical trials showed that a single dose of black or GT (2 g tea solids in 300 mL water) administered to healthy adults, was able to improve their plasmatic antioxidant capacity 30–60 min after ingestion (Leenen et al., 2000). Interestingly, although the benefits of tea are mainly attributed to catechins, some studies argue that the synergism among all tea components is more therapeutically effective than some of the components consumed alone (Dias et al., 2016a; Horie et al., 2005). For instance, there are reports on the compensatory relaxing effect of L-theanine against the stimulatory effect of caffeine (Kakuda et al., 2000). Overall, WT may act as a protective agent against a wide variety of oxidative stress–induced health conditions, which are summarized in Fig. 3.42.2.

One of the major health concerns of modern society is diabetes mellitus, as its incidence has been increasing at an alarming rate (Geiss et al., 2014). This metabolic disorder, generally characterized by chronic hyperglycemia, is a treatable lifelong condition that affects the health of the whole body. Diabetes may be caused by or lead to a redox imbalance, which results in the impairment of insulin-producing pancreatic cells (Yan, 2014). The antidiabetic potential of tea components, and specifically of WT, has been reported. In vitro studies showed that WT extracts (0.5 g of dry leaves in 20 mL of hot water) have a stronger hypoglycemic and hypolipidemic potential relative to GT and black tea extracts (Tenore et al., 2013). Moreover, streptozotocin-induced diabetic rats fed with a 0.5% (w/v) aqueous WT extract during four consecutive weeks, demonstrated a significant decrease in blood glucose concentration, improvement in glucose tolerance, and decrease in total serum cholesterol (Islam, 2011). A more recent study using prediabetic rats (also injected with streptozotocin) also showed an improvement in glucose and insulin tolerance after replacing *ad libitum* water by *ad libitum* WT infusion (1 g of dry leaves in 100 mL of boiling water) for 2 months (Dias et al., 2016a). These results suggest that WT's antidiabetic potential is mediated by a decrease in insulin resistance and improvement in insulin sensitivity. Besides, diabetes mellitus is closely linked to the development of other human disorders, such as obesity (Johnson et al., 2007). Natural products also have a fair market share in antiobesity products and treatment (Newman and Cragg, 2012). Furthermore, WT may be a good complementary or alternative treatment against obesity and its associated complications. In fact, tea catechins (100 mg in 3 mL of fat emulsion), especially ECG and EGCG, have demonstrated an effective role in reducing cholesterol absorption in the intestines, thus decreasing its solubility and enhancing its excretion (Ikeda et al., 1992). They were also reported to

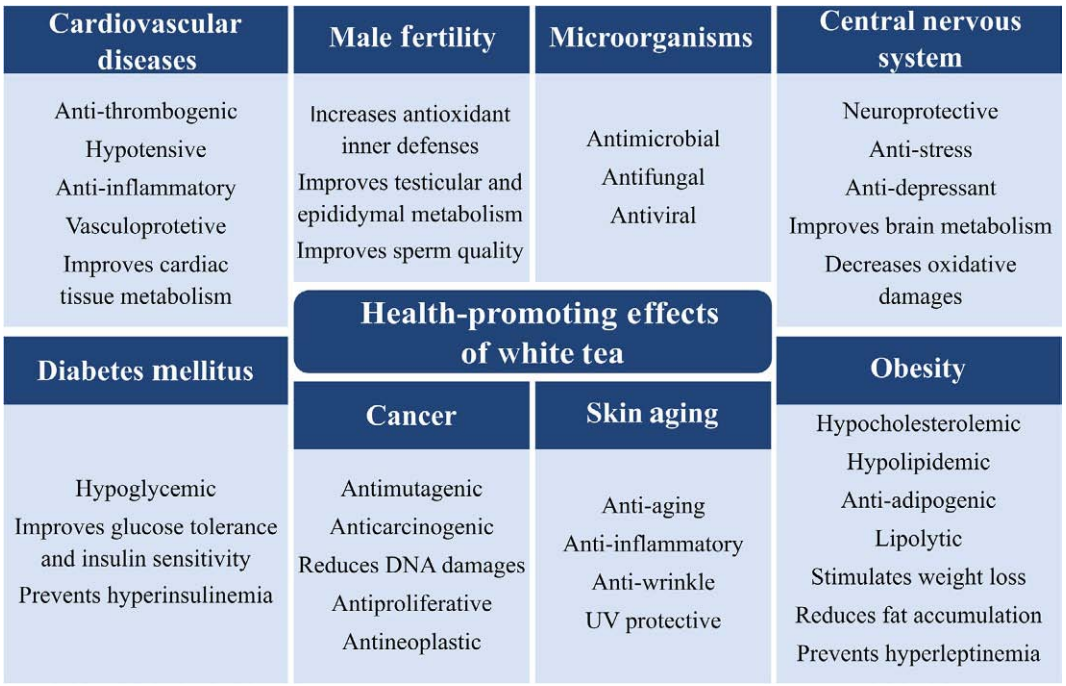

FIG. 3.42.2 The potential protective effects of white tea on human health.

reduce total serum cholesterol (Islam, 2011), fat accumulation in the abdominal cavity and subsequent body weight gain, as well as preventing hyperinsulinemia and hyperleptinemia (Murase et al., 2002). In addition to catechins, caffeine and L-theanine also demonstrated a suppressive effect on body weight increase and fat accumulation (Zheng et al., 2004). The antiobesity potential of tea components seems to be largely due to a synergistic effect between catechins and caffeine (Zheng et al., 2004). Moreover, an in vitro study using human preadipocytes indicated that WT has strong lipolytic and antiadipogenic activities (Söhle et al., 2009). Thus, WT consumption may reduce adipose tissue size and stimulate weight loss.

Both diabetes mellitus and obesity constitute a risk factor for the onset of hypertension and heart-related diseases (Johnson et al., 2007). So, it is of extreme relevance to find new therapeutic approaches to prevent the progression of human disorders to severe complications and WT looks quite promising. The antithrombogenic and antiinflammatory action of polyphenols has been reported (Stangl et al., 2006). Polyphenols may also inhibit lipid oxidation and have a vasculoprotective action (Stoclet et al., 2004). Since the oxidation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) is associated with atherosclerosis and even coronary heart disease infarction (Tijburg et al., 1997), WT can be an attenuating factor for the progress of such diseases. Additionally, the most prevalent amino acid present in WT, L-theanine, has been described as a blood pressure–reducing agent (Yokogoshi et al., 1995). The replacement of water intake by *ad libitum* WT infusion over 2 months in a prediabetic animal model demonstrated cardioprotective effects through the improvement of cardiac tissue metabolism (Alves et al., 2015).

In the central nervous system, oxidative stress may cause cell loss, triggering the development of many neurological disorders (Almajano et al., 2011). Oxidative stress is one of the main factors contributing to the aging processes and neurodegenerative diseases, such as Alzheimer's, Parkinson's, or Huntington's diseases (Halliwell, 2006; Wang and Michaelis, 2010). Polyphenols have demonstrated a neuroprotective role due to their potent antioxidant activity (Mandel and Youdim, 2004). In addition, L-theanine also induces a feeling of relaxation by lowering cortisol levels and reducing psychological and physiological stress (Kimura et al., 2007). Prediabetic rats drinking a WT infusion in *ad libitum* conditions over 2 months (as a replacement for water intake), demonstrated improvements in cerebral cortex metabolic and oxidative profiles (Nunes et al., 2015), which could be of great value to prevent the development of neurodegenerative disorders.

Oxidative stress may also contribute to the development of cancer. It may lead to DNA damage, which might culminate in an uncontrolled cell division. In fact, elevated levels of oxidative-induced DNA lesions have been described in many tumors (Valko et al., 2007). WT revealed important anticarcinogenic (Hajiaghaalipour et al., 2015) and antimutagenic (Santana-Rios et al., 2001) activities. In fact, it has been reported that WT has an antiproliferative effect against cancer cells, while protecting normal cells against DNA damage due to its strong antioxidant activity (Hajiaghaalipour et al., 2015). Additionally, nonsmall cell lung cancer cell apoptosis was induced after exposure to a WT extract through the modulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) and the 15-lipoxygenase (15-LOX) signaling pathways (Mao et al., 2010). Thus, WT consumption may have antineoplastic and chemopreventive effects that could be vital for cancer prevention.

Increased testicular oxidative stress, which may result from metabolic diseases, such as diabetes mellitus, has also been associated with impaired reproductive function (Rato et al., 2013). The effects of WT and its components in male reproductive health have been largely explored in recent years. The exposure of rat Sertoli cells to a WT extract (0.5 mg/mL) for 50 h led to alterations in cell glycolytic profiles, resulting in lactate stimulation (Martins et al., 2014). As lactate is the energy source and acts as an antiapoptotic factor for developing germ cells (Rato et al., 2012), the effects of WT were suggested to improve the reproductive health of males. Notably, the viability of rat spermatozoa was also highly improved during a 3-day, room temperature storage after exposure to a WT extract (0.5–1 mg/mL) (Dias et al., 2014), illustrating that it may have protective properties over sperm physiology. Prediabetic rats having free access to a WT infusion (1 g of dry leaves in 100 mL of water) for 2 months (as a replacement for water) demonstrated increased testicular antioxidant potential and improved sperm quality (Alves et al., 2015). Moreover, WT consumption was able to restore most of the prediabetes-induced metabolic dysfunctions in testicular and epididymal tissues (Dias et al., 2016a).

WT extracts also exhibited strong antimicrobial and antifungal activities, which were attributed to polyphenolic content (Gopal et al., 2016; Koech et al., 2013). For instance, catechins were reported to reduce *Escherichia coli* growth by about 50% (Nazer et al., 2005). EGCG seems to be the most effective antimicrobial catechin. However, it has been reported that the bactericidal effect of EGCG is less effective for gram-negative bacteria than for gram-positive bacteria, once this latter type can absorb higher amounts of EGCG (Taguri et al., 2006). Tea catechins also demonstrated the ability to inhibit human immunodeficiency virus (HIV) propagation by inhibiting the enzyme reverse transcriptase (Liu et al., 2005). Altogether, WT comprises antimicrobial, antifungal, and antiviral activity, which highlights its potential against infections. Besides, the synergistic effects between tea polyphenols and antibiotics have been explored (Koech et al., 2013). Interestingly, the antibacterial effect of WT has been maximized when in combination with ampicillin, possibly because they directly or indirectly attack the same binding site on the bacterial surface (Koech et al., 2013).

Recently, the beneficial effects of WT to prevent skin aging have been investigated (Hunt et al., 2010). WT extract exhibited a protective role on human dermal fibroblast cells, acting as an antioxidant and antiinflammatory agent (Thring et al., 2011). WT extract (2% in propylene glycol:ethanol:water (5:3:2)) was described as an effective antiwrinkle agent, as it was able to decrease epidermal thickness and increase collagen and elastic fiber content in mice suffering from ultraviolet-induced photoaging (Lee et al., 2014). Furthermore, clinical trials also demonstrated that the topical application of WT extract may provide protection from ultraviolet radiation–induced Langerhans cell and DNA damage, which could lead to suppression of the immune system and development of skin cancer (Camouse et al., 2009). Thus, WT oral or topical administration may have an antiaging effect and improve the health of skin (Saric and Sivamani, 2016).

Overall, WT and its interesting components may be of great value in the prevention and treatment of several human conditions. Many studies demonstrate the higher therapeutic potential of WT when compared to the other tea types. The development of WT-based food supplements could be a promising approach to enhance the beneficial effects of WT and improve human health.

FUTURE PERSPECTIVES

Recently, there has been a cultural resurgence of interest in alternative medicines by the general public, where botanical products have a great prominence. This has led to a widespread interest in nutritional supplements, which has contributed to the creation of a multibillion dollar industry. GT-based food supplements are currently quite popular. WT is poorly known among tea consumers and there are few supplements based on it. However, WT's interesting health-promoting properties are making it a "medical treasure." As WT is not very popular as a beverage, it could become an important industrial and pharmaceutical raw material in the development of food supplements. These products could increase the endogenous antioxidant capacity of the human body, thus preventing possible oxidative stress–induced diseases. Nevertheless, therapeutic-efficient doses of WT, as well as its possible side effects and interactions with other compounds should be investigated.

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Chapter 3.43

Tumeric or *Curcuma longa* Linn.

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THE *CURCUMA LONGA* PLANT AND ITS SOURCES

Among the plants known for their medicinal values, the plants of the *Curcuma* genus, which encompass 70 known species, have been traditionally used as a spices, food preservatives, and coloring materials, and are highly significant for their therapeutic potential (Krup et al., 2013). *Curcuma longa* L., or turmeric, is an everlasting herb and member of the Zingiberaceae (ginger) family which is cultured widely in South East Asia, mostly in India and China (Labban, 2014; Mehrotra et al., 2013). The height of the *C. longa* tree is approximately 91.44 cm and their leaves appear like lance structures with yellow flower prickles that ripen in its fleshy rhizome or in its underground stem. The source of turmeric medicinal powder is the orange pulp enclosed inside the rhizome (Kocaadam and Şanlier, 2015; Ulbricht et al., 2011). Dried *C. longa* is the main source of turmeric. The vigorous component of turmeric and the one responsible for its yellow color is known by different names in different countries, for example, it is named curcumin (Fig. 3.43.1) in Arab countries, in India it is called saffron or Haridra (Sanskrit, Ayurvedic), it is known as Jianghuang (yellow ginger) in China, and Kyoo or Ukon in Japan (Goel et al., 2008).

ACTIVE COMPONENTS

Curcumin is composed of several components, namely curcuminoids, which are comprised of three groups as shown in Fig. 3.43.1: 76.9% curcumin, 17.6% demethoxycurcumin, and 5.5% bis-demethoxycurcumin (Funk et al., 2006; Lawand and Gandhi, 2013). Additionally, it contains volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. Curcumin is poorly water soluble because it is a lipophilic polyphenol and it is fairly constant in the acidic pH of the stomach (Julie and Jurenka, 2009).

PHARMACOKINETICS AND SYSTEMIC BIOAVAILABILITY OF CURCUMIN IN HUMAN

Curcumin is harmless and effective which marks it as a potential composite for treatment and prevention of an extensive range of diseases according to pharmacological studies. Due to the poor bioavailability of curcumin its clinical uses have been discontinued (Bar-Sela et al., 2010) Curcumin poorly dissolves in water (Kakran et al., 2012). To increase its bioavailability, lengthen its circulation, provide it with better permeability, and resistance to metabolic processes, several formulations have been prepared which include nanoparticles, liposomes, micelles, and phospholipid complexes (Mohanty and Sahoo, 2010; Yadav et al., 2012). According to dose-intensifying studies, curcumin at doses of 12 g/day for 3 months are documented as safe (Gupta et al., 2015). Furthermore, pharmacologically when curcumin is administered at doses of 8 g/day for 12 weeks no toxicity is noticed (Pescosolido et al., 2014).

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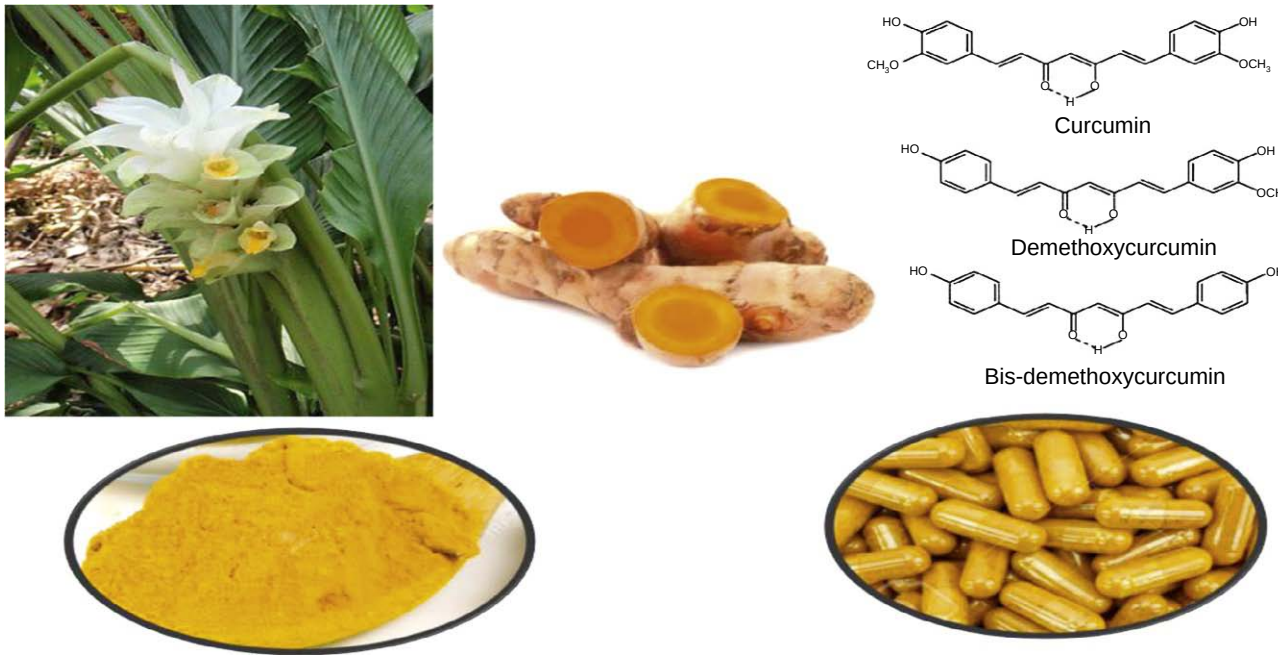

FIG. 3.43.1 The main components of *Curcuma longa.*

MEDICINAL AND PHARMACOLOGICAL PROPERTIES OF TURMERIC

Natural products from turmeric (*C. longa*) have been revealed to show antioxidant, analgesic, antibacterial, antifungal, antiviral, antiparasidic, antiinflammatory, and digestive-protecting properties (Anand et al., 2010; Hayakawa et al., 2011) and are under study as possible treatments for Alzheimer's disease (Venigalla et al., 2015), arthritis, diabetes, liver and kidney problems (Bar-Sela et al., 2010), cardiovascular disease (Wongcharoen et al., 2012), and several types of cancer (Huminiecki et al., 2017). The crushed rhizome of the plant is used externally as an antiseptic (Mehra et al., 2014).

ANTIINFLAMMATORY PROPERTIES OF *CURCUMA*

The antiinflammatory effects of *C. longa* may be related to its capacity to inhibit diverse molecule biosynthesis and pathways that are involved in inflammation, including phospholipase, lipooxygenase, cyclooxygenase 2 (COX-2), leukotrienes, thromboxane, prostaglandins, nitric oxide (NO), elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis factor, and interleukin-12 (Aggarwal and Sung, 2009; Labban, 2014). In one nonplacebo-controlled study for 106 patients the efficacy of Meriva® (a curcumin supplement) was assessed against recurring anterior uveitis. All patients were given NorFlo® which contains 600 mg of Meriva ®, twice daily for about 12–18 months. The results indicated that NorfFo® was well tolerated and could reduce eye discomfort symptoms and signs after a few weeks of treatment in 90 of the patients (Allegri et al., 2010).

GASTROINTESTINAL DISORDERS AND CURCUMIN'S PROPERTIES

The antiinflammatory properties and therapeutic benefits of *C. longa* have been established for variable gastrointestinal conditions, including indigestion, infection with *Helicobacter pylori*, gastric ulcers, irritable bowel syndrome (IBS), Crohn's disease, and ulcerative colitis (Julie and Jurenka, 2009). The tablet form of *C. longa* displays a major effect in declining IBS prevalence and abdominal pain/discomfort scores. After 8 weeks of treatment highly significant enhancements in IBS quality-of-life scales were noted (Rahimi and Abdollahi, 2012). In addition, a clinical randomized trial study established that an adjunctive therapy (clarithromycin, amoxicillin, and pantoprazole) with 500 mg/day of curcumin for 1 month showed improvement in dyspepsia symptoms in patients with peptic ulcers (Khonche et al., 2016).

RESPIRATORY DISORDERS AND EFFECTS OF *CURCUMA*

The bioactive components of *C. longa* that have potential antiasthmatic action include: turmerones, curcuminoids, curcumin, and tetrahydro curcumin (Mali and Dhake, 2011). It has been revealed that curcumin can act as a scavenger of NO and can prevent bronchitis in asthmatic patients (Nilani et al., 2009). In a study conducted by Abidi et al. (2014) 77 patients who had mild to moderate bronchial asthma were piloted with a predictable positive bronchodilator reversibility test with ≥15% improvement in forced expiratory volume one second (FEV1). The outcomes showed that curcumin capsules (500 mg) taken daily for 30 days assisted in improving bronchial obstructions in the mean FEV1 value (Abidi et al., 2014).

CARDIOVASCULAR DISEASE AND CURCUMIN'S EFFECT

In a randomized, double-blind, placebo-controlled clinical trial study conducted for 6 months in type 2 diabetes patients, contributors were trained to take three capsules, either 250 mg of curcuminoid or a placebo, twice day, to assess curcumin's properties on risk influences for atherosclerosis. The outcomes revealed that curcumin reduced the risk of atherosclerotic disease in patients by reduced pulse wave velocity, increased intensity of serum adiponectin, and decreased intensity of leptin (Chuengsamarn et al., 2014). In a study by Akazawa et al., conducted on 32 postmenopausal women for 8 weeks, 3 groups were allocated: a control group, a group taking exercise (moderate aerobic), and a group taking curcumin (administrated orally). The results indicated that curcumin ingestion and aerobic exercise can raise flow-mediated dilation in postmenopausal women, suggesting that both can possibly slow the age-related drop in endothelial function (Akazawa et al., 2012).

DIABETES MELLITUS

The consumption of 6 g of *C. longa* increased postprandial serum insulin levels but did not seem to affect plasma glucose level in healthy subjects. *C. longa* effectively decreases lipid levels in patients with type 2 diabetes mellitus and metabolic syndrome (Neerati et al., 2014; Yang et al., 2014). The administration of Meriva® (curcumin and phosphatidylcholine), at a dose of two tablets per day (corresponding to 100 mg of curcumin) for 1 month in patients with diabetic microangiopathy and retinopathy, was evaluated and provided evidence of an influence on reducing blood glucose levels (Steigerwalt et al., 2012). In a randomized control trial conducted on 35 patients with type 2 diabetes mellitus for 14 weeks, turmeric exhibited an effect analogous to glibenclamide in terms of declining the blood glucose level—the curcuma was well-accepted and no drug interferences were described throughout (Sukandar et al., 2014).

DERMATOPROTECTIVE ACTION

There is evidence that turmeric/curcumin products and supplements, both orally consumed and topically applied, may provide therapeutic benefits for acne, atopic dermatitis, eczema, pruritus, psoriasis, and vitiligo (Vaughn et al., 2016). In one study on 10 patients with focal or generalized vitiligo, one group was treated with narrow band of ultraviolet light type B (NB-UVB) plus topical application of tetrahydro curcuminoid cream and the other with UVB alone, twice weekly for 3 months. Results indicated a better repigmentation in the group receiving combination treatment at 8 and 12 weeks—the tetrahydro curcuminoid cream was well tolerated (Asawanonda and Klahan, 2010). A decrease in radiodermatitis symptoms in a group receiving Vicco® turmeric ointment was statistically noteworthy compared with a group receiving baby oil in a 7-week, random-sampling study on 50 subjects (37 men and 13 women) with radiodermatitis (Palatty et al., 2014).

ANTIMICROBIAL ACTIVITY

An antibacterial study of an aqueous extract of *C. longa* rhizome validated a minimum inhibitory concentration (MIC) value of 4–16 g/L and a minimum bactericidal concentration (MBC) value of 16–32 g/L against *Staphylococcus epidermis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumonia* ATCC 10031, and *Escherichia coli* ATCC 25922 (Lawhavinit et al., 2010). In another study the capability of *C. longa* rhizome extracts were found to prevent the growth of *S. aureus* ATCC 6571—a sign of the broad spectrum of antimicrobial properties which may be present (Gupta et al., 2015). A one percent turmeric supplement as part of the diet, results in an improved weight gain and a reduction in intestinal lesion in chicks infected with *Eimeria maxima* (Nasri et al., 2014). The topical use of turmeric oil caused inhibition of dermatophytes and pathogenic fungi in guinea pigs after 7 days of turmeric application (Dujic et al., 2009).

ANTICANCER ACTIVITY

The anticancer activities and effects of curcumin have been established as part of various biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, carcinogenesis, and metastasis (Kwon and Magnuson, 2009). Curcumin is well tolerated in humans and could communicate with novel drug targets and show synergism to chemotherapy (Ye et al., 2012). In a study by Carroll et al., curcumin (4 g/day) expressively reduced aberrant crypt foci (ACF) formation, something which might further reinforce curcumin's usefulness as a cancer chemopreventive agent that done on 44 smokers were given Curcumin orally at two different doses (2 or 4 g/day) for 30 days (Carroll et al., 2011). In a study by He et al. it was established that curcumin capsule (360 mg) administration 3 times daily for 10–30 days can advance the overall wellbeing of colorectal cancer patients via the mechanism of increased p53 expression in tumor cells (He et al., 2011). In an open-label, phase II trial the influence of curcumin in a mixture with gemcitabine, against pancreatic cancer, estimated in 21 patients registered in the study administrated 8 gm of curcumin orally per day plus gemcitabine. The result showed the safety and potential of this mixture (Kanai et al., 2011). Additionally, curcumin showed a potential effect in suppressing carcinogenesis in the forestomach and skin (Nautiyal et al., 2011; Ramawat et al., 2009), as well as suppressing B cell and T cell leukemia (Angelo and Kurzrock, 2009) and several types of breast carcinoma cells (Liu et al., 2009).

USING CURCUMIN AGAINST SEVERAL DISEASES

In a randomized, placebo-controlled study on 24 patients with relapsing lupus nephritis, administration of one capsule containing 500 mg of turmeric taken with each meal for 3 months showed a significant decrease in proteinuria, hematuria, and systolic blood pressure in all patients. Therefore, turmeric is a good candidate for consideration as a treatment against these diseases and can be used as a safe adjuvant therapy (Khajehdehi et al., 2012). In a randomized, double-blind, placebo-controlled study conducted on 60 healthy older adults (mean age 68.5 years), Longvida^{TM} curcumin (400 mg) has been revealed to improve working memory and mood after 4 weeks of administration (Cox et al., 2014). It was concluded that curcumin plus apigenin are unique candidates for an antiinflammatory treatment for Alzheimer's disease and other related degenerative syndromes according to the results reported from cell cultures, and animal and human studies (Venigalla et al., 2015). Curcumin plays the main role in slowing down obesity and minimizing the impact of its accompanied sequelae via regulation of lipid metabolism (Alappat and Awad, 2010).

PRECAUTIONS

The supplementation of turmeric and curcumin are harmless when consumes at the doses mentioned earlier in the text. Conversely, overdosing for long periods of time may possibly cause gastroesophageal reflux disease. Individuals who have gallstones or blockages of the bile passages should carefully consider their options before taking turmeric. Similarly, diabetic patients should be cautious as turmeric may lead to hypoglycemia. Taking high amounts of turmeric may also lower testosterone levels and decrease sperm movement, furthermore it prevents the absorption of iron, therefore, should be used cautiously by people with iron deficiency (http://www.turmericforhealth.com).

POSSIBLE INTERACTIONS

- Medications that slow blood clotting (anticoagulant/antiplatelet drugs).Administration of turmeric with these medications will increase the possibility of bleeding and may reinforce the properties of these drugs including warfarin (Coumadin), Clopidogrel (Plavix), and Aspirin, among others.
- Drugs that reduce stomach acid. Turmeric may increase the production of stomach acid by interfere with the action of Cimetidine (Tagamet), Famotidine (Pepcid), Ranitidine (Zantac), Esomeprazole (Nexium), Omeprazole (Prilosec), and Lansoprazole (Prevacid).
- Diabetes medications. Turmeric may increase the possibility of hypoglycemia, reinforcing the effects of these drugs, http://www.turmericforhealth.com.

INTERACTION WITH HERBS

Herbs that slow blood clotting, when taken with turmeric, can increase the risk of bleeding and bruising. Such herbs include cloves, angelica, garlic, danshen, ginger, *Panax ginseng*, ginkgo, willow, and red clover (Mayanglambam et al., 2010).

CONCLUSIONS

On the basis of results of several experimental studies and clinical data, mentioned in this chapter, it can be established that the use of curcumin can play an important role in improving human health. Therefore, its use as food supplements should be considered at the recommended doses. However, attention should be given to its use in combination with other drugs. More research studies and clinical data are required to use curcumin in the future for the chemoprevention of all kinds of neoplasia.

LIST OF ABBREVIATIONS

IL Interleukin-1
NF-κB Nuclear factor-κB
TNF-α Tumor necrosis factor-α
MCP-1 Monocyte chemo attractant protein-1
COX-2 Cyclo-oxygenase 2
FEV1 Forced expiratory volume 1
MIC Minimum inhibitory concentration
MBC Minimum bactericidal concentration
ACF Aberrant crypt foci

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Chapter 3.44

White Dead-Nettle (*Lamium album*)

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SOURCES AND AVAILABILITY OF *LAMIUM ALBUM*

L. album belongs to the blossoming family of plants known as Lamiaceae, which are populates of Europe and Asia. White dead-nettle (*L. album*) can be grown in partially shady areas and can be found near rock debris sites, roadsides, fences, midforest clearings, and in brushwood. White dead-nettle is also grown as a low-maintenance ground cover in gardens (Ani Ko, 2008; Rudy, 2004). *White dead-nettles are* native to Europe, Asia, North Africa, most abundantly in Mongolia, Iraq, Iran, Russia, eastern Turkey, central Asia, China, India, and Japan. This species can be grown in North America, New Zealand, and Iceland. This species also grows and is available in central and eastern Canada, as well as in the United States (NRCSU, 2011). White dead-nettle has been identified as present in Juneau and the Glacier Bay National Park in the Pacific Maritime ecogeographic region of Alaska and Anchorage in the Interior-Boreal ecogeographic region (AKEPIC, 2011). Extracts of *L. album* at different concentrations are used to treat diseases like hepatitis C and cancer among others. The combination of different compounds with *L. album* and its effects could be investigated to find more miraculous properties of this species. Yet, various mechanisms involved in the effects of active compounds present in *the white dead-nettle* has not been fully explored, which need to be expressed via cellular and molecular targets.

CHEMICAL COMPOSITION OF *LAMIUM ALBUM*

L. album shows an extensive variety of restorative mediations, which are allocated to different organically dynamic substances, for example, essential oil phytoecdysteroids, terpenes, flavonoids, isoscutellarein derivatives, phenolic acids and iridoids. Isoscutellarein derivatives mostly consist of 30% purified ethanolic extract phenolics. These derivatives include isoscutellarein-7-allosyl(1→2)glucoside, isoscutellarein-O-allosyl glucoside, and O-methylisoscutellarein-7-O-allosyl(1→2)glucoside. These compounds have possible associations with health benefits (Pereira et al., 2012). *White dead-nettle* hasiridoids have been approved clinically in vitro and in vivo as having biological activity as a glycoside, thus, proving that it has the ability to be used as an antiarthritic, antiinflammatory, antibacterial, anticancer, antioxidant, antiviral, and an immunomodulatory, as well as a wound-healing agent, and a neuroprotective (Ghule et al., 2012).

Flavonoids were found abundantly in the form of aglycone (quercetin) and glycosides (isoquercitrin, tiliroside, and rutin) from the aerial parts of *white dead-nettle* but as extracts of methanol and ethyl acetate. Quercetin, as a member of the flavonoid family has various pharmacological effects, including functioning as an antioxidant, scavenging highly reactive oxygen species. It also plays a prominent role in osteoporosis, pulmonary and cardiovascular diseases, and malignant cancer (Khan et al., 2016). Normal human skin fibroblasts can be protected against oxidative stress-induced cell injury with the help of flavonoids (Spencer et al., 2004). In *white dead-nettle*, phenolic compounds trigger physiological activities and these compounds are verbascoside (acteoside), isoverbascoside, and lamalboside. The methanol and ethyl acetate extracts from flowers of *white dead-nettle* also contained phenolic acids such as protocatechuic, chlorogenic, vanillic, caffeic, coumaric, and ferulic acids (Mattila and Kumpulainen, 2002; Pereira et al., 2013).

Ecdysteroids extracted from the aerial part of *L. album* including abutasterone, inokosterone, polypodine B, and pterosterone, are widely used as a dietary supplement and anabolic agent. Ecdysteroids play an important role in the stimulation of protein synthesis, immune response, reducing plasma cholesterol and glucose concentrations, antimutagenic activities, antioxidant function, and wound healing (Lafont and Dinan, 2003; Savchenko et al., 2001). The hemiterpene

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glucosidehemialboside has also been isolated from *white dead-nettle* (Damtoft and Jensen, 1995). The ethyl acetate, methanol, and heptane extracts of *white dead-nettle* flowers contains β-amyrin and triterpenesursolic acid. These metabolites appeared in excess in the heptane extract.

RELATION OF *LAMIUM ALBUM* TO HEALTH

Generally, *L. album* was first cultivated in North America. It was consumed both cooked and in its raw state. Its flower has the potential to attract honey bees and is an excellent source of nectar, so sometimes it is known as bee nettle (Grieve, 2013). The major pharmacological properties of *white dead-nettle* such as antiviral, antimicrobial, antioxidant, cytoprotective, anticancer, and antiinflammatory with relation to the specific extracts from *white dead-nettle* are described below.

As an antiviral, *white dead-nettle* extract used against hepatitis C virus (HCV) has proven to be effective. The aqueous *white dead-nettle* extract showed an anti-HCV inhibitory activity of 50% compared to a negative control against HCV pseudoparticle infection at a concentration of 100 μg/mL (Zhang et al., 2009). In addition, the antiviral activity of the chloroform extracts *in situ* and in vitro propagated plants of *L. album* showed significant inhibition of herpes simplex virus (HSV) type-1 and type-2 replication in Madin-Darby bovine kidney (MDBK) cells at inhibitory concentration-50 (IC_{50}) values of 668 and 552 μg/mL, respectively, with no apparent cytotoxicity. In the in vitro model, IC_{50} of chloroform was 552 and 487 μg/mL, respectively, while 50% of IC_{50} of chloroform in vivo study showed 668 and 780 μg/mL, respectively (Shishkov, 2013). When chloroform extracts were used at the highest accepted concentration (1.2 and 1 μg/mL, respectively), virus replication was inhibited by more than 90%. These results indicate the possible application of *L. album* in medical use due to its efficacy as an antiviral substance.

White dead-nettles have great potential as an antimicrobial agent. It has been proven that crude ethanol extract from the rhizome of *white dead-nettle* contrary to *Staphylococcus aureus* and *Bacillus cereus* (Kokoska et al., 2002), and 18 different extracts of *white dead-nettles* from 4 different solvents such as water, ethanol, chloroform, and methanol were experimented for diverse antimicrobial activities and having potential health benefits (Chipeva et al., 2013). All of the extracts exhibited antimicrobial activity against bacterial strains such as *Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Proteus hauseri, Pseudomonas aeruginosa, Salmonella enterica, S. aureus,* and *Staphylococcus epidermidis*. Gram-positive bacteria were found to be more sensitive than gram-negative bacteria. The lowest minimum inhibitory concentration (MIC) against *S. aureus, P. aeruginosa, P. hauseri,* and *E. faecalis* was attained by chloroform extracts from *in situ* plants (Chipeva et al., 2013).

Oxygen free radicals play a major role in the pathophysiology of several illnesses, including oxidative cancer, stress, and inflammation. The purified ethanol extract of *white dead nettle plants* not only has the capability of radical scavenging, it can also reduce the production of reactive oxygen species. The harmful effect of abundant production of reactive oxygen species is oxidative stress, which is connected to the process of aging and also plays an important role in diseases such as cancer, cardiovascular, neurodegenerative, and inflammatory disorders. Each of the identified phenolic compounds in *L. album* extracts have been shown to reduce reactive oxygen species production in stressed human hepatoblastoma Hep G2 cells and therefore these biologically active molecules may find potential therapeutic applications in the prevention of degenerative and neoplastic diseases (Pereira et al., 2013). Epidemiological experiments propose that the alimental intakes of flavonoids are related with a reduced risk of developing cancer, and these flavonoids are found in *white dead-nettle* (O'prey et al., 2003).

Consumption of the flowering part of *L. album* after boiling in water can enhance insulin secretion in the islets of Langerhans and show antidiabetic effects (Farzami et al., 2003). *L. album* is the second strongest antioxidant of the Lamiaceae species. *L. album* also has antispasmodic, diuretic, and hemostatic properties and can be used to lessen bladder, kidney, and menstrual problems (Farzami et al., 2003).

Aerial parts of *L. album* reveal phytoecdysteroids, which show an extensive range of beneficial pharmacological properties in vertebrates. These phytoecdysteroids are most abundant when the plant is young and at an immature stage. The mentioned compounds trigger immune response and protein synthesis, exhibit wound healing activities, reduce concentration of cholesterol and glucose in the blood, and have antimutagenic and antioxidant effects (Lafont and Dinan, 2003; Savchenko et al., 2001).

An experiment on the A549 cancer lung cell line was performed to observe the anticancer effects of *L. album* plant extracts at 3×10^4 cell/mL doses (Moskova-Doumanova et al., 2012). Different doses (0.25, 0.50, 1.0, 2.5, and 5.0 mg/mL) of methanol and chloroform extracts from in vivo and in vitro cultivated plants were used. Almost every extract exhibited anticancer effects, excluding chloroform and methanol extracts at concentrations of 0.5 and 2.5 mg/mL, respectively. The

strongest effect was observed from methanol extract from in vivo plants at the concentration of 4.5 mg/mL where around 50% of cells remained unattached after 6 h, which indicates the inhibition of further division of tumor cells. After 48 h of incubating alone with methanol and chloroform extracts in different absorptions, the G2-phase cessation of cell division advancement was observed, while a lesser quantity of apoptotic cells viability was seen in combined methanol/chloroform extracts. The experiment showed that specific treatments with chloroform or methanol extracts caused weaker action, while conducting with combined methanol/chloroform extracts revealed the most influential anticancer outcome (Moskova-Doumanova et al., 2012). It should be noted that *L. album* extracts did not harm the normal cells but was very helpful in inhibiting the replication of tumor cells.

POSSIBLE INTERACTIONS OF *LAMIUM ALBUM* WITH OTHER SUPPLEMENTS/DRUGS/FOODS

By controlling the environmental parameters and the components of the culture medium, it is possible to modify the metabolite content to obtain high-producing secondary metabolite genotypes (Arencibia et al., 2008; Kirakosyan et al., 2004). Aqueous extract of *L. album* at a concentration of 100 µg/mL and lamiridosins A/B at a concentration of IC_{50} 2.31 µg are effective against HCV (Zhang et al., 2009). An aqueous fraction of hydroalcoholic extract at a concentration of IC_{50} 280 µg/mL is used as a 1-diphenyl-2-picrylhydrazyl (DPPH) antiradical activator. The aqueous fraction of the hydroalcoholic extract acts as an antiinflammatory agent at an IC_{50} level of 1500 µg/mL (Trouillas et al., 2003). Chloroform extract at a concentration of IC_{50} 668 µg/mL and chloroform extract from in vitro plants at a concentration of IC_{50} 552 µg/mL is effective at inhibiting the replication of HSV type 1 and type 2 (Shishkov, 2013).

Methanol extract at a concentration of IC_{50} 1 µg/mL, methanol extract from in vitro cultivated plants at a concentration of IC_{50} 194 µg/mL, and butanol fractions of methanol extract at concentrations of IC_{50} 9.9 µg/mL are used as DPPH antiradical activators (Budzianowski and Budzianowska, 2006; Trouillas et al., 2003). Methanol extract is also effective as a cytotoxic agent and reduces cell adhesion at IC_{50} 800 µg/mL (Moskova-Doumanova et al., 2012). Ethanol extract and ethanol extract from in vitro cultivated plants at a concentration of IC_{50} 11.2 and IC_{50} 274 µg/mL, respectively, can be used as DPPH antiradical activators. Ethanol extract at a concentration of the 50 µg/mL is helpful in the decrease in production of reactive oxygen species (Table 3.44.1). Ethanol extract also acts as an antimicrobial agent, specifically against *B. cereus* and *S. aureus* at a MIC of 250 mg/mL. Ethanol extract at a concentration of 50 µg/mL performs cytoprotective activity (Kokoska et al., 2002; Pereira et al., 2013).

Chloroform extract has antimicrobial activity against *E. faecalis, S. aureus, P. hauseri,* and *P. aeruginosa* at a MIC of 313 µg/mL (Chipeva et al., 2013). Chloroform extract is also effective as a cytotoxic agent at an IC_{50} level of 500 µg/mL (Moskova-Doumanova et al., 2012). Ethyl-acetate extract performs antiproliferative activity at a concentration of IC_{25} of 188 µg/mL (Paduch et al., 2008). Heptane extract performs antiinflammatory activity at a concentration of 20 µg/mL (Paduch and Woźniak, 2012). The chloroform extracts derived from *L. album* propagated in vivo and in vitro showed antiviral capacity. The chloroform in vitro extract (CS) expressed strong inhibitory effects against the replication of HSV type 1 and type 2 in MDBK cells. The in vivo extract also inactivated extracellular HSV type 1 (Table 3.44.1). The results showed that *L. album* could be an interesting source of natural antiviral substances with potential use in medicine (Shishkov, 2013).

Thus, applying topical formulations of flavonoids may shield skin from oxidative injury and cancer. It has been revealed that the incubation of methanol extract of *L. album* with normal human skin fibroblasts showed antioxidative activity and was nontoxic. Free radical–scavenging activity was also a property of the extract. Hence, the *L. album* extracts could be used to aid wound healing and in skin protective formulations, but very careful selection of exposure time and sample concentration should be taken into account (Paduch et al., 2008).

CONCLUSIONS

The synergistic effects of the different compounds with *L. album* could be investigated to find more miraculous properties of this plant. Furthermore, the mode of action and molecular targets of the biologically active compounds isolated from *white dead-nettle* are still largely unknown. In certain diseases, *L. album* when given in its precursor form increases the effectiveness of a drug due to its antioxidant, antiinflammatory, antimicrobial, and antiviral activities. More research is needed to find out the diverse and protective effects of *L. album* in critical diseases like neurodegenerative disorders, cancer, and HIV/AIDS.

TABLE 3.44.1 Biological Activities of the Pure and/or Extracted Form of *Lamium album*

Extract or pure compounds	Concentration	Model system	Effect	References
Chloroform extract	MIC 313 μg/mL	Diffusion analysis	Antimicrobial activity	Chipeva et al. (2013)
Ethanol extract	MIC 250 μg/mL	Liquid dilution analysis	Antimicrobial activity	Kokoska et al. (2002)
Methanol extract	IC_{50} 1 μg/mL	DPPH free radical–scavenging test	DPPH antiradical activity	Matkowski and Piotrowska (2006)
Chloroform extract	IC_{50} 500 μg/mL	A549	Cytotoxic activity	Moskova-Doumanova et al. (2012)
Methanol extract	IC_{50} 800 μg/mL	A549	Lessens cell adhesion and cytotoxic activity	Moskova-Doumanova et al. (2012)
Ethyl acetate extract	IC_{25} 188 μL/mL	HSF	Antiproliferative activity	Paduch et al. (2008)
Ethanol extract	50 μg/mL	Hep G2 (HB-8065)	Cytoprotective activity	Pereira et al. (2013)
Ethanol extract	50 μg/mL	Hep G2 (HB-8065)	Lessens reactive oxygen species	Pereira et al. (2013)
Ethanol extract	IC_{50} 11.2 μg/mL	DPPH free radical scavenging test	DPPH antiradical activity	Pereira et al. (2013)
Chloroform extract	IC_{50} 668 μg/mL	MDBK	HSV type 2 and type 2 inhibit replication	Shishkov (2013)
Chloroform extract from cultivated plants (in vitro)	IC_{50} 552 μg/mL	MDBK	HSV type 2 and type 2 inhibit replication	Shishkov (2013)
Aqueous fraction of hydroalcoholic extract	500 μg/mL	B16 cell line	Antiproliferative activity	Trouillas et al. (2003)
Ethanol extract from cultivated plants (*in vivo*)	IC_{50} 274 μg/mL	DPPH free radical–scavenging test	DPPH antiradical activity	Valyova et al. (2011)
Methanol extract from cultivated plants (*in vitro*)	IC_{50} 194 μg/mL	DPPH free radical–scavenging test	DPPH antiradical activity	Valyova et al. (2011)
Aqueous extract	100 μg/mL	Huh-7	Anti-hepatitis C virus entry activity	Zhang et al. (2009)
Lamiridosins A/B	IC_{50} 2.31 μM	Hep G2 2.2.15	Anti-hepatitis C virus entry activity	Zhang et al. (2009)

HSF, human skin fibroblasts.

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All authors have directly participated in the planning or drafting of the manuscript and read and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 3.45

Wine Grapes (*Vitis vinifera*) and Wine-Based Food Supplements

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INTRODUCTION

One of the greatest concerns regarding dietary supplements is their quality. Nowadays grape products number in their several thousands. Due to the lack of assurance of product quality and regulatory elements many problems occur across Europe, especially regarding nonconforming grape products.

Neither manufacturers nor traders are obliged to carry out high-quality product analysis generating literary references. Food supplements are not required to be examined in clinical studies before being placed in the market, therefore, it is down to consumers to pay attention to their composition. It is also important to know that often translations from Chinese and Ayurvedic, in terms of dietary supplement components, are incorrect.

In this chapter we consider a few important examples of grape, grape seed, grape husk, grape leaf, and wine applications in terms of their medicinal applications.

GRAPES

The Museum of Leipzig University preserves the longest surviving and most comprehensive papyrus dealing with healing—the Ebers Papyrus. Georg Ebers was the first owner and scientific researcher of this medical papyrus, which consists of more than 33 stand-alone "books," or papyrus scrolls, that were copied together into one volume. This 20.23-m-long papyrus was written in the era of Pharaoh Amenhotep, 1515 BC by the Oxford History of Ancient Egypt (Győry, 2003).

This papyrus includes a few recipes, in which grapes (in fruit form, raisin form, or wine form) are important constituents. For example, in part of Eb 477 (67, 7–9):

The start of medicines, which are given to the liver: fig, 1/8, desert plum fruit 1/8, grapes 1/16, slashed sykomor fig 1/8, ground pumpkin crop 1/16, acacia gum 1/32, incense 1/64, cress 1/64, water 15 ro, exposed to the night dew, break, drink 4 days.

These medicaments were used to cure liver diseases. Liver diseases were common in ancient Egypt because of the everyday consumption of wine and fermented drinks, such as beer and fruit juices, instead of water. Lack of fresh water and floods of the Nile as well as Schistosomiasis (*Schistosoma haematobium*, *Schistosoma mansoni*) contributed to frequent intoxication cases, beside drunkenness feasts dedicated to Hathor (Katona et al., 2015, 2016).

Grape and wine as medicament components can be found in Avicenna's Canon. Avicenna (980–1037) was the magnificent Persian polyhistor and doctor. The five-volume-long Kanun fi Tib (Canon of Medicine, written between 1013 and 1021), is the most outstanding in the Islamic Golden Age (the 11th to 13th centuries). The second and fifth volumes contain those herbs (drugs) that Avicenna used with success to cure different illnesses.

This scientific work was effected by both Greeks and the Romans (Hippocrates, Galenos), the Persian physician Rhazes, and Far-Eastern, Ayurvedic, and Chinese medicines. Avicenna represented the "contraria contrariis" kind of medicine, originated from Galenos. Many drugs, including grapes, listed in the Canon are still widely applied in traditional medicine all over the world.

In traditional medicine grapes can be applied as remedies for several diseases, because of their laxative and diuretic properties as well as stomahicum. Grapes are currently used against biliary dyspepsia, haemorrhaging, chronic bronchitis, and dysuria, as well as a treatment for heart failure and gout. Ripe fruits or raisins are effective in treating subacute spleen and liver enlargement.

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Avicenna applied wine as a vehicle to prepare medicaments and narcotic and analgesic drinks during amputations and wound care. He recommended wine as a wound cleaner as well. These medicaments described in the Canon were used across Europe (Petrov, 1982).

The Canon was such a meaningful medical encyclopedia that even after Avicenna's death it reached Western Europe—by 17th century it had more than 30 editions. Schola Medica Salernitana, a late medieval medical school, also applied several medicaments of Avicenna's Canon. A complete version of the Canon was published in Russian between 1954 and 1960. The Salerno school was the institutionalized education center of medicine in Europe and the first secular medical school, founded around AD 850. It flourished between 1150 and 1180 (Blázovics, 2016; Buranova, 2015; Shamsi-Baghbanan et al., 2014).

The ancient Ayurveda and traditional Chinese medicines are also very important not only in regions of the Far East (India, China, Vietnam Cambodia, Korea, Ocean Archipelago) but also in the West which uses their traditional therapies integrated into complementary or alternative medicines (European and American regions)—their natural products can be found in nutritional supplements in today's markets.

The Sanskrit name for *Vitis vinifera* is draksha. Grape has general roborating effect on patients and increases their body weight. Ayurvedic medicine applied this fruit to cure nervous system problems in vata-type patients as a brain tonic for memory enhancement. Draksha is widely used in digestive disorders, in constipation, jaundice, and thirst helping vata and pitta regulatory functions. Grape acts as a cardiac tonic, because it strengthens the heart's functions. Grapes are very useful against diseases which arise due to vitiation of rakta (blood) and pitta.

Additionally, grapes are recommended in cases of gout and allergies. Grapes help to strengthen the respiratory system. In cases of dosha kapha–type people, expectoration can be achieved to improve conditions and tuberculosis, coughing, and bronchitis can be cured with Ayurvedic grape preparations.

Grapes have beneficial effects on the urinary system, because they acts as a diuretic and increases both frequency of urination and urine volume, therefore grapes can be recommended in cases of burning micturition and cystitis. Grapes can be applied to increase fertility in both sexes, increasing the quantity of semen, sperm count, and sperm motility, as well as helping improve erectile dysfunction and premature ejaculation. They also strengthen the female reproductive system. This fruit can moderate skin burning sensations and allergies and prevent several skin disorders (http://www.himalayawellness.com/herbfinder/vitis-vinifera.htm).

Traditional Chinese medicine used and still uses grapes (putao) to improve important life functions, namely, invigorating QI and blood, strengthening the bones and muscles, and benefiting diuresis. Chinese doctors treat patients with coughs, due to lung problems, heart palpitations, night sweats, rheumatic arthralgia, stranguria, and general edemas with grapes (Xinrong, 2003). The beneficial therapeutic effects of grapes can be explained due to their bioactive compounds, which were identified over the last 2–3 decades.

Two main types of *V. vinifera* L. are white and red. Both types of this fruit are very rich in bioactive agents. Fresh red grapes contain approximately 70%–80% water, sugars, nitrogenous and aroma-producing compounds, vitamin B6, thiamine, riboflavin, vitamin C, phenolic compounds, flavonoids, anthocyanins, proanthocyanidins, phenolic and fruit acids, tannins, pectine substances, and mineral elements. White grapes contain similar compounds but differ in that they contain no colorful polyphenolic compounds and smaller quantities of resveratrol. These compounds can be found in wine as well. More, than 500 compounds have been identified in wine.

The effects attributable to resveratrol are its free radical–scavenging function, inhibition of lipid peroxidation, d-field element-chelating abilities, several metabolic activities (such as influencing eicosanoid synthesis, lipoprotein metabolism, and the inhibition of activity of proteins against ageing), physiological effects (such as thrombocyte aggregation inhibition, protection of endothelium function, inhibition of inflammation), as well as having vasodilation and antihypertensive properties (Tyihák et al., 2003).

Polyphenols including the flavonoids in grapes also have similar beneficial effects on several life functions. These molecules, and vitamin C, can act as signal transducer molecules in the cells (Blázovics, 2007; Wang et al., 2011).

Wine is a very ancient beverage which was first produced at a similar time to the first occurrence of people growing grapes. Almost all the valuable compounds which occur in grapes can be found in the wine, depending on the grape species, method of cultivation, and wine-processing conditions (Janisch et al., 2006; Sárdi et al., 2000).

In the early 2000s, efforts were made to utilize agricultural by-products, like grape cores and husks, after pressing for juice. This was because tests supported that these parts of the fruit were valuable. The dried and pulverized seeds and husks, as well as leaves, contain polyphenols, flavonoids, and stilbenes which are released in the acid medium of the stomach, as well as the neutral medium of the small intestine, and can act as antioxidants. Recent studies also have shown that grape seeds contains significant quantities of fatty acids, linolenic, oleic, palmitic, and stearic acids, as well as tocoferol isoforms.

These seed oils can be used not only as a food but also as massage oils and for the treatment of cellulite.

It must be mentioned that *V. vinifera* leaves, mainly red ones, are a very important food. Stuffed grape leaves are well known not only in Arabia, Greece, and Turkey, but in several southern and central European regions as well as in Vietnamese cuisine. Red grape leaf extracts are also used in traditional herbal medicine in Mediterranean areas. Grape leaves were used (mainly anthocyanines) in folk medicine to stop bleeding, inflammation, and pain. Leaf products can also be found as food supplements in European markets. These are used to combat venous insufficiency and cutaneous capillary fragility. They can be used to combat medical conditions like heavy legs, capillary fragility, and varicose veins.

Experimental studies have showed that the bioactive agents of grape leaves can improve endurance, leading to increased athletic performance and active lifestyle (Janisch et al., 2006). As a result of these benefits, grape, grape leaves, and seed flour and oil all appear as ingredients in the food supplement market (Minegishi et al., 2014; Sabir et al., 2012).

So, the positive role wine plays, as well as other parts of the vine and grapevine products, in human health promotion has long been investigated. In these studies no, or very little, attention has been paid to examining endogenous "bound" formaldehyde and precursor molecules of methyl-donor compounds of HCHO (e.g., quaternary ammonium compounds). The role of quaternary ammonium compounds in connection with nutrition appeared primarily in medical science papers, but the majority of these publications did not deal with plants or plant parts, which are known to contain these important transmethylation molecules. These methyl-donor compounds have not been emphasized when considering grape and viticultural products, however, their beneficial effects can contribute to positive nutritional effects (Kovács Nagy et al., 2009) (Fig. 3.45.1).

A lot of data supporting the role of endogenous methylation and demethylation has been confirmed in the epigenetic regulation, posttranslational modification of proteins in the forming of membrane molecules as well as globin methylation, although the methyl balance and transmethylation fluxes are not known in detail in many cellular pathways. The most important pathways for *S*-adenosylmethionine–dependent transmethylation in mammals are the syntheses of creatine by guanidinoacetate-methyltransferase, of phosphatidylcholine by phosphatidylethanolamine-methyltransferase, and of sarcosine by glycine-*N*-methyltransferase (Mudd et al., 2007).

DNA methylation typically occurs at the cytosine–phosphate–guanine site. Methylation results in the conversion of cytosine to 5-methylcytosine, catalyzed by DNA methyltransferase. Therefore, adequate dietary intake of methyl-donor groups, e.g., methionine, one-carbon units, choline, and choline metabolite betaine, are very important in different biological systems (Blázovics et al., 2008, 2012; Sárdi et al., 2004).

WINE

The term "French Paradox" which is known now to everyone, was first used in 1986 in the newsletter of the International Vine and Wine Organisation. In 1991, Serge Renaud, professor at the Université Bordeaux, presented his results of a scientific discovery, namely that the risk of cardiovascular disease in French people is 3.5 times lower than the risk to Americans, thanks to their moderate consumption of red wine every day, despite an equivalent consumption of saturated fats. The article "Wine, alcohol, platelets, and the French Paradox for coronary heart disease (CHD)," by Renaud and de Lorgeril,

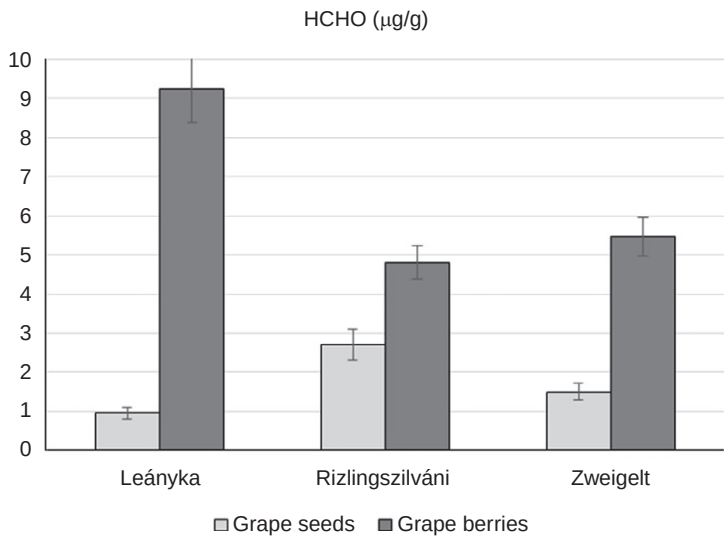

FIG. 3.45.1 HCHO concentrations in grape seeds, berries, and leaves.

published in *The Lancet* in 1992, was the next scientific publication about the beneficial effects of red wine on CHD. The researchers claim that red wine moderates high mortality from CHD despite an associated high intake of saturated fat. In the same article researchers reexamined their previous suggestion that, in the main, moderate red wine consumption does not prevent CHD through an effect on atherosclerosis, but rather through a hemostatic mechanism (Renaud and de Lorgeril, 1992; Simini, 2000).

Meanwhile, in 1999, Malcolm Law and Nicholas Wald wrote a paper in the *British Medical Journal* (BMJ) with the headline "Why heart disease mortality is low in France: the time lag explanation," which claimed that the "French Paradox," not because of statistical distortions, was an "illusion."

Since that time, numerous epidemiological and clinical studies have dealt with bioactive polyphenolic compounds in red wine. Resveratrol has been found to have particularly beneficial physiological effects of a variety of cardiovascular diseases (Bertelli and Das, 2009).

In France the Ministry of Health published guidelines which advised, in 2009: "The consumption of alcohol, and especially wine, is discouraged." INCA (Institut National du Cancer) says the consumption of only a small amount of alcohol can increase the risk of mouth and throat cancer by 168%. INCA's president Dominique Maraninchi said: "Small daily doses of alcohol are the most harmful. There is no amount, however small, which is good for you" (http://www.decanter.com/wine-news/french-government-new-advice-dont-drink-wine-73540/).

Considering these scientific battles, the question is whether to drink or not to drink? Known statistical data indicate that 30,000 people die every year because of alcohol-related diseases, and the number of so-called heavy drinkers is 2.5 million, so almost all families involved in Hungary. Within the next few years, the number of alcoholics may increase to 1 million from its current estimate of 800,000 (http://napidoktor.hu/korkep/eveken-belul-egymilliora-nohet-az-alkoholistak-szama-magyarorszagon/).

In our research group, clinical studies were carried out with red and white wines from different grapes grown in Hungary, to investigate the "French Paradox" and other recently raised questions.

Although one month's worth of moderate red wine (Egri cuvée) consumption did not cause hepatotoxicity, and was found to improve antioxidant parameters in both sexes of young healthy volunteers, different sex-dependent alterations were observed in metal homeostasis. Erythrocyte Ca, Mg, Pb, Sr, and Zn levels as well as the Zn/Cu ratio were found to have lower endpoint values than baseline values in women. In men, however, the endpoint values of Al, Ca, Li, Pb, and Sr were lower than their baseline values. The most remarkable alterations were the decreases in the Zn levels and the Zn/Cu ratio in women. These significant decreases can be considered problematic in terms of the susceptibility of women to alcoholic-induced hepatotoxicity (Bekő et al., 2010).

When the effects of moderate white wine consumption was examined in a 1-month study in patients with metabolic syndrome in both sexes, wine consumption was found to increase insulin sensitivity in the cases of both wine types: Müller-Turgau and Pintes. However, epidermal growth factor levels were also increased significantly in sick volunteers—an effect which is questionable (Ábel et al., 2013).

Through the formation of very low-density lipoprotein, triglyceride levels increase and this process causes alteration to lipid parameters. Alcohol metabolism causes centrilobular hypoxia and increases formation of toxic agents, acetaldehyde and superoxide radicals, which stimulate the autoimmune reactions in the liver. Therefore, alcohol hastens the necrosis of hepatocytes. Inflammatory responses, reticuloendothelial system (RES) activation, can be observed against neoantigens. Intestinal endotoxines, lipo-polysaccharides (LPS) aggravate the necroinflammatory processes. Proinflammatory cytokine production, on a large scale, amplifies tissue damage and fibrogenesis increases production of extracellular matrix and collagen. This may lead to cirrhosis (Lieber, 1997).

FOOD SUPPLEMENT

In an earlier study we reported the negative output of the commercially derived dietary supplement—among others containing grape—in colectomysed patients in relative high dose (2 × 3 g/die for 3 months). This food supplement was found to have diet-related bioactive compounds such as vitamins, polyphenols, flavonoids, anthocyanins, and stilbenes. Twenty anthocyanins and flavonoid glycosides were identified respectively by HPLC-DAD-ESI-MS/MS.

It was found that treatment with this food supplement influenced the plasma, erythrocyte free radical levels, and HbA1c concentration negatively, diminished the reducing power similarly reduced the glutathione peroxidase (GSHPx) level in the asymptomatic patients. The HbA1c levels correlated well with the free radical levels of erythrocytes and lowered antioxidant status. These results support the idea, that the body, due to a significant compensating effect, induces essential free radicals, because of nutritional antioxidant overflow. In this context, AFP, CEA, and CA-19-9 tumor marker levels in colectomysed patients were higher in most cases, and only in few cases did the normal values exceed baseline levels (Blázovics et al., 2016).

CONCLUSIONS

More than 1000 years of experience suggest that vine or grapevine nonalcoholic products exert their beneficial effects on human organs safely. However, wine and wine-containing beverages cause problems because of their alcohol content, especially when alcohol consumption is uncontrolled. Consuming large quantities of alcohol is harmful. Food supplements with grape seed oil and powder, as well as berry extracts, can be used safely in moderate amounts by keeping to the Recommended Dietary Allowance for vitamin and metal element content, and avoiding excessive consumption. Therefore, people must be prudent when choosing food supplements. Dietary supplements should not be used to replace a balanced diet and healthy lifestyle (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx).

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Chapter 3.46

Pomegranate (*Punica granatum* L.)

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INTRODUCTION

Punica granatum L. (pomegranate) comprises about 500 cultivars with worldwide distribution (Stover and Mercure, 2007). It differs in having pink flowers and smaller, less sweet fruit. Pomegranate is used in baking, beverages, and cooking. Moreover, *P. granatum* is largely used for the treatment of several diseases across different cultures and civilizations (Bhowmik et al., 2013). Many studies reported the functional properties of pomegranate extracts and juice. This chapter is focused on pomegranate antioxidant, antiinflammatory, antitumor, neuroprotective, cardiovascular, and antidiabetic effects.

BIOACTIVITY

Antioxidant Activity

The antioxidant activity of pomegranate juice and identified compounds has been largely investigated. Pomegranate's antioxidant activities depend on several factors, such as cultivar, part of the fruit used, climatic conditions, and ripening stage (Borochov-Neori et al., 2009). In this context, Li et al. (2015) demonstrated the influence of cultivars. Generally, Shandong and Xinjiang pomegranate cultivars had higher antioxidant activities than Shaanxi and Yunnan, and the activity is correlated to their total polyphenol content. In order to verify the antioxidant effect of pomegranate juice in humans, Matthaiou et al. (2014) investigated the effect of juice consumption in 14 healthy volunteers for a period of 15 days. The malondialdehyde (MDA) was reduced by 24.4% after one week of treatment, while protein carbonyls were reduced by 19.6% after 15 days of juice consumption. Moreover, glutathione (GSH) levels were significantly increased. The most consumed pomegranate variety, Gabsi, was investigated for its antioxidant potential by different in vitro methods. Generally, the antioxidant potency followed the trend: peel > flower > leaf > seed. Correlation analysis revealed that phenols are mainly responsible for this bioactivity (Elfalleh et al., 2012). Recently, pomegranate seed oil, rich in punicic acid, exhibited radical scavenging potential and iron chelation ability (Lucci et al., 2015).

The antioxidant properties exhibited by pomegranate may be related to different phytochemicals, including anthocyanins such as glucosides of delphinidin, cyanidin, pelargonidin, ellagic acid, punicalin, punicalagin, pedunculagin, and different flavanols. Tzulker et al. (2007) indicated that punicalagin is the main substance responsible for the total antioxidant capacity of juice, whilst anthocyanins play only a minor role. Numerous speculations were raised to explain the antioxidant mechanism of action of the pomegranate constituents. Madrigal-Carballo et al. (2009) hypothesized that pomegranate can act as a reducing agent since its phenols readily donate hydrogen to reducing agents. Several pomegranate constituents can act as reductones. Reductones break the free radical chain by the donation of a hydrogen atom and preventing peroxide formation (Naveena et al., 2008).

Antiinflammatory Properties

Several studies have demonstrated the antiinflammatory effects of pomegranate in different models.

Pomegranate downregulates the overexpressed cyclooxygenase 2 (COX-2) and prostaglandin E synthase levels in the colon mucosa and consequently decreases the levels of prostaglandin E2 (PGE2) (Larrosa et al., 2010). In a recent work, Aharoni et al. (2015) demonstrated that pomegranate juice and its main phenols decreased interleukin (IL)-6 and tumor necrosis factor (TNF)-α release in response to stimulation by IFN-γ and lipopolysaccharide (LPS) in J774.A1 macrophage-like cell lines. In in vivo studies, pomegranate juice inhibited macrophage inflammatory responses.

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00062-X

Recently, Mansouri et al. (2015) evaluated the interaction between ellagic acid and venlafaxine on pain. Ellagic acid and venlafaxine determined a dose-dependent inhibition of the writhing response induced by acetic acid. The coadministration of both compounds at increasing doses led to a synergistic interaction against writhing behavior. Xu et al. (2014) demonstrated the ability of punicalagin to reduce the LPS-induced production of PGE2, IL-1β, IL-6, nitric oxide, and TNF-α in RAW264.7 cells. The treatment with punicalagin reduced LPS-induced lung edema, increased IL-1β, IL-6, and TNF-α levels, inhibited NF-κB activation induced by LPS, myeloperoxidase activity, and increased macrophage and neutrophil infiltration of lung tissues in mice (Peng et al., 2015).

Ellagic acid–enriched pomegranate extract significantly reduced the overexpression of both COX-2 and iNOS, the phosporylation of MAPKs, and prevented the nuclear NF-κB translocation in a model of Crohn's disease (Rosillo et al., 2012).

Antitumor Properties

Different pomegranate secondary metabolites were investigated for their potential antitumor activity. Ellagic acid, punicic acid, luteolin, and anthocyanins were the most studied.

Ellagic acid was able to inhibit cell motility and invasion in androgen-independent human (PC-3) and rat (PLS10) prostate cancer cell lines (Pitchakarn et al., 2013). Moreover, this compound considerably reduced the proteolytic activity of collagenase/gelatinase.

In a recent study, a mixture of ellagic acid, luteolin, and punicic acid was able to inhibit PC-3M-luc primary tumor growth and the CXCL12/CXCR4 axis for metastasis (Wang et al., 2014). The mixture inhibited angiogenesis, prevented human endothelial cell tube formation in culture, and disrupted preformed endothelial cell tubes. Moreover, the mixture inhibited IL-8, vascular endothelial growth factor (VEGF), and their induced signaling pathways in endothelial cell tubes.

Galactomannan polysaccharide isolated from the pomegranate fruit rind was studied in the murine cancer cell lines (DLA and EAC), and in the human cancer cell lines (A375, HCT116, and Hep G2) (Joseph et al., 2013). A significant cytotoxicity through the induction of apoptosis was found. The polysaccharide alone, and in combination with doxorubicin, showed an important decline in the tumor burden and increased life span in both models in comparison with the controls.

Neuroprotection

Alzheimer's disease (AD) is one of the most common forms of progressive dementia. Subash et al. (2014) have suggested that pomegranate juice is able to slow the progression of cognitive and behavioral deficits. This evidence was confirmed by their analysis in APPsw/Tg2576 mice, treated at 4 years of age with a diet containing 4% pomegranate. A significant improvement in memory, learning, and motor function was detected. Velagapudi et al. (2016) defined the pomegranate as a strategy for slowing the progression of neurodegenerative diseases as it is capable of inhibiting inflammation and amyloidogenesis inhibiting the expression of Aß and β-amyloid precursor protein cleavage enzymes (BACE-1) in SK-N-SH cells treated with IL-1β. Pomegranate (25–200 mg/mL) is able to reduce the production of COX-2 dependent PGE2 and inhibits transactivation of NF-kB in HEK293 cells that were TNF-α stimulated. The integration of diet with 4% of the pomegranate for 15 months in APPsw/Tg2576 mice could lead to a reduction of acetylcholinesterase in the brain cortex and hippocampus (Subash et al., 2014). Moreover, pomegranate inhibited the accumulation of oxidant substances involved in the AD pathophysiology (Pirinccioglu et al., 2014).

Cardiovascular Health Properties

Several studies have investigated the effect of pomegranate on low-density lipoprotein (LDL) oxidation and atherosclerosis. Aviram et al. (2004) demonstrated a significant carotid intima-media thickness reduction by up to 30%, after 1 year of juice consumption. The same treatment induced an increase of 83% of serum paraoxonase 1 (PON 1) activity. A reduction of 90% and 59% was evidenced for serum LDL basal oxidative state and LDL susceptibility to copper ion–induced oxidation, respectively.

After 1 year of juice consumption, a reduction (12%) of systolic blood pressure was observed. In another study, Aviram et al. (2008) investigated the in vivo effect of arils (POMa), flowers (POMf), peels (POMxl and POMxp), and seeds (POMo) in comparison to juice in atherosclerotic mice. POMf determined a significant decrease in atherosclerotic lesion area by 70% and a reduction of serum lipids and glucose levels (18%–25%). Both POMf and POMa were able to reduce the MPM total peroxides content, and increase cellular paraoxonase 2 (PON2) activity. After consumption of juice, POMxl, or POMxp, the uptake rates of oxidized LDL by E (0)-MPM were reduced by about 15%. Ellagic acid, gallic acid, punicalagin, and punicalin are identified as bioactive constituents. Pomegranate juice and its main components punicalagin or

gallic acid are able to activate the PON1 gene promoter via the intracellular signaling cascade PPARgamma-PKA-cAMP, while ellagic acid elicited only a modest stimulatory effect. The secreted PON1 showed biological activity by protecting LDL and high-density lipoprotein (HDL) from oxidation (Khateeb et al., 2010). Moreover, the consumption of juice for 3 months reduced stress-induced ischemia. No changes in blood pressure, blood sugar, cardiac medications, hemoglobin A1c, or weight were observed (Sumner et al., 2005).

Antidiabetic Activity

Nekooeian et al. (2014) studied the effect of pomegranate seed oil in streptozotocin-induced diabetic mice. Pomegranate seed oil administered to animals determined higher levels of GSH peroxidase and serum insulin. Several investigations evaluated the effect of pomegranate juice administration in diabetic patients. The effect of pomegranate juice ingestion after a 12 hour fast, and then 1 and 3 h after consumption of 1.5 mL of pomegranate juice, per kg body weight, were investigated in 85 participants with type 2 diabetes. After 3 h of juice ingestion, fasting serum glucose, increase of β-cell function, and decrease of insulin resistance was observed. These effects depended on initial fasting serum glucose levels, since participants with lower fasting serum glucose levels demonstrated a greater hypoglycemic response compared with those who had higher fasting serum glucose levels (Banihani et al., 2014). Sohrab et al. (2015) demonstrated the antidiabetic effect of pomegranate juice in a randomized, double-blind clinical trial. In another study, 44 patients affected by type 2 diabetes were enrolled and invited to consume 250 mL/day of pomegranate juice, or a control beverage for 12 weeks. At the end of the study, plasma total antioxidant capacity increased, but MDA decreased in the treated group. No significant differences were detected in plasma concentration for pentosidine and carboxy methyl lysine between the groups.

CONCLUSIONS

The last five ten have seen a great increase in the trading of pomegranate fruits considered to be a "superfood" rich in healthy phytochemicals. The consumption of pomegranate juices and other derivatives have increased dramatically worldwide. Several research articles evidenced that pomegranate derived products, (juice, seed, flower, and peel extracts) are rich sources of bioactive phytochemicals including, ellagic acid, punicalin, punicalagin, pedunculagin, flavanols, punicic acid, etc.

Supplementation with pomegranate juice or standardized pomegranate extract capsules may prevent several diseases, including cardiovascular disease, diabetes, and cancers. Despite the great demand for pomegranate products by consumers, there are no studies that allow excluding its possible toxicity. For this reason, it is necessary to consider that the safety of this "superfood" is not fully understood. Table 3.46.1, Table 3.46.2, Table 3.46.3, Table 3.46.4.

TABLE 3.46.1 In Vitro and In Vivo Studies on *Punica granatum* Antioxidant Activity

Pomegranate parts	Methods	References
Flowers	DPPH	Elfalleh et al. (2012)
Peel	ABTS	Elfalleh et al. (2012)
	DPPH	Elfalleh et al. (2012)
	ABTS	Elfalleh et al. (2012)
Rind	DPPH	Naveena et al. (2008)
	Ferric reducing test	Naveena et al. (2008)
	Chicken patties oxidation model	Naveena et al. (2008)
Seed	DPPH	Lucci et al. (2015)
	ABTS	Lucci et al. (2015)
	Iron chelating assay	Lucci et al. (2015)
	β-carotene bleaching test	Lucci et al. (2015)
	ABTS	Elfalleh et al. (2012)
Juice	Administration of juice in 14 healthy volunteers for a period of 15 days	Matthaiou et al. (2014)

TABLE 3.46.2 In Vitro and In Vivo Studies on *Punica granatum* Antiinflammatory Activity

Pomegranate parts	Methods	Effects	References
Whole fruit	In vitro	Down-regulates the over-expressed COX-2 and prostaglandin E synthase (PTGES) levels in the colon mucosa	Larrosa et al. (2010)
Juice	In vitro and in vivo	Decrease of IL-6 and TNFα secretion, suppress macrophage inflammatory responses	Aharoni et al. (2015)
Ellagic acid-enriched Pomegranate extract	In vivo	MPO activity and TNFα levels were considerably reduced in fed rats; reduction of over-expression of iNOS and COX-2, phosporylation of MAPKs and prevention of nuclear NF-κB translocation	Rosillo et al. (2012)
Ellagic acid, urolithin A and urolithin B	In vivo	Antinociceptive and anti-inflammatory effect	Mansouri et al. (2015)
Ellagic acid			
	In vivo	Inhibition of the writhing response induced by acetic acid	Mansouri et al. (2015)
Punicalagin, punicalin, strictinin A, and granatin B	In vitro	Inhibitory effect on nitric oxide production	Lee et al. (2010)
Punicalagin	In vivo	Reduction of LPS-induced lung edema, increase of IL-1β, IL-6, and TNFα levels, and inhibition of NF-κB, myeloperoxidase activity and the increases in the macrophage and neutrophil infiltration of lung tissues	Peng et al. (2015)

TABLE 3.46.3 In Vitro and In Vivo Studies on the Effect of *Punica granatum* and Derived Products on Metabolic Syndrome

Pomegranate parts	Methods	Effect	References
Flowers	In vivo	Inhibiting or activating nuclear factor κB and peroxisome proliferator-activated receptor γ	Banihani et al. (2013)
	In vivo	Lowered plasma glucose levels	Li et al. (2005)
	In vitro	Inhibitory effect on alpha-glucosidase activity with IC50 value of 1.8 μg/mL	Li et al. (2005)
Seed	In vivo	Reduce fasting blood glucose levels	Banihani et al. (2013)
	In vivo	Animals treated with seed oil (200–600 mg/kg/day) exhibited higher levels of serum insulin and glutathione peroxidase activity	Nekooeian et al. (2014)
Peel	In vivo	Reduce fasting blood glucose levels	Banihani et al. (2013)
Juice	In vivo	Inhibiting or activating nuclear factor κB and peroxisome proliferator-activated receptor γ	Banihani et al. (2013)
	Humans	Decreased fasting serum glucose, increased β-cell function, and decreased insulin resistance, 3 h after juice ingestion	Banihani et al. (2014)

TABLE 3.46.4 In Vitro and In Vivo Studies on *Punica granatum* Effect on Cardiovascular Disease

Pomegranate parts	Methods	Effect	References
Flowers	In vivo	Decrease in atherosclerotic lesion area by 70%. Reduced serum lipids, and glucose levels by 18%–25%. Reduce the MPM total peroxides content, and increased PON2	Aviram et al. (2008)
Arils	In vivo	Reduce the MPM total peroxides content, and increased PON2	Aviram et al. (2008)
Peel	In vivo	Reduced by approximately 15% after consumption	Aviram et al. (2008)
Juice	Humans	After 3-years of supplementation a significant carotid intima-media thickness reduction was observed. PON 1 activity was increased by 83%, whereas serum LDL basal oxidative state and LDL susceptibility to copper ion-induced oxidation were reduced (90% and 59%, respectively). Additionally, serum against oxidized LDL were decreased by 19%, and serum total antioxidant status was increased by 130% after 1 year. Systolic blood pressure was reduced after 1 year of juice consumption by 12%	Aviram et al. (2004)
		Decrease the extent of stress-induced ischemia	Sumner et al. (2005)
	In vivo	Reduced by approximately 15% after consumption	Aviram et al. (2008)

ABBREVIATIONS

BACE1 β-amyloid precursor protein cleavage enzymes
COX-2 Cyclooxygenase 2
ED_{50} Fifty percent effective dose
GSH Glutathione
LDL Low-density lipoproteins
LPS Lipopolysaccharide
MDA Malondialdehyde
MMPs Matrix metallo-proteinases
PGE2 Prostaglandin E
VEGF Vascular endothelial growth factor

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Part IV

Animal Extracts

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Chapter 4.1

Bee Products: Royal Jelly and Propolis

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INTRODUCTION

Over the last few years, the concept of nutrition has changed significantly. In the past, adequate nutrition was considered to be a diet that provided sufficient nutrients to maintain the body's needs. With the modern advancement of the food industry, there is growing interest in natural products used as foods to promote health and reduce illness. New bee product discoveries continue to emerge every day. These nonvitamin, nonmineral, and nutritional supplements have been used by humans since ancient times. The primary bee products, including beeswax, venom, and royal jelly, are chemically synthesized by the bees. Secondary bee products such as honey, propolis, and pollen are obtained from plants and are altered and arranged by the bees for their particular utilization (Schmidt, 1997). Bee products' chemical composition depends on various factors, including plant species used by the bees, season, collector type, biogeographic localization, environmental factors, bee species' activity during royal jelly production (Sabatini et al., 2009), and propolis exploitation (Castaldo and Capasso, 2002). This chapter focuses on the biomedical importance of royal jelly and propolis, summarizing their physiological and protective functions.

PRIMARY BEE PRODUCTS: ROYAL JELLY

Royal jelly is produced by the hypopharyngeal and mandibular glands of worker bees. Although all bee larvae are fed with royal jelly for the first three days, only queen larvae continue to be fed extensively with this special food throughout their development (Scarselli et al., 2005).

Chemical Composition of Royal Jelly

Royal jelly is a complex compound, consisting of water (50%–60%), proteins (18%), carbohydrates (15%), lipids (3%–6%), trace minerals, water-soluble vitamins, free amino acids, and many other less well-characterized compounds (Nagai and Inoue, 2004). Proteins represent the most important portion of royal jelly. Royal jelly contains a family of proteins called major royal jelly proteins (MRJPs), which play an essential nutritional role in the diet of the queen bee (Tamura et al., 2009). Nine MRJPs (MRJP1-9) have been characterized and MRJP1 is the major protein in royal jelly (Albert and Klaudiny, 2004, 2007; Schmitzová et al., 1998; Tamura et al., 2009). MRJP1 can form an oligomer called apisin by polymerization with apisimin (Kimura et al., 2003). Recently, Furusawa et al. (2016) suggested that apisin could be used to evaluate the quality of royal jelly. Apart from proteins, royal jelly is also one of the richest natural products in free amino acids containing at least eight essential amino acids (Bărnuţiu Lavinia Ioana et al., 2011). Another important portion of royal jelly is its lipid fraction, which is composed of 80%–85% fatty acids (Sabatini et al., 2009). Organic acids of royal jelly usually have 8–12 carbon atoms, which are either hydroxyl fatty acids or dicarboxylic acids. Among them, the main acid is the 10-hydroxy-2-decenoic acid (10-HDA), which is a unique active substance present in royal jelly (Isidorov et al., 2009). 10-HDA is chemically stable and, therefore, has been adopted as an international standard for the quality and freshness of royal jelly (Sabatini et al., 2009).

Biomedical Importance of Royal Jelly: Modern Research Evidence

Royal jelly has a wide range of pharmacological and health-promoting functions in humans. Here, we will only focus on the antibacterial, epigenetic, anticancer, and antioxidant activities.

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Antibacterial Activity of Royal Jelly

Royal jelly shows considerable microbial stability even though it is very susceptible to colonization by microorganisms. In recent years, chemical components, including royalisin and jelleine, have been isolated from royal jelly, which show antibacterial activities (Bilikova et al., 2001; Fontana et al., 2004; Fujiwara et al., 1990). Although the detailed mechanism remains poorly understood, the primary antibacterial effect could be due to the cell membrane–peptides interaction (Brogden, 2005). Antibacterial peptides are positively charged due to the presence of arginine, histidine, and lysine residues, which allows them to interact with anionic phospholipids of the cell membrane causing disruption to it (Splith and Neundorf, 2011). Royalisin is one of the most potent antibacterial peptides found in royal jelly. It was found to have potent antibacterial activity against gram-positive bacteria at low concentrations, but not against gram-negative bacteria (Bilikova et al., 2001; Bílikova et al., 2015; Fujiwara et al., 1990; Shen et al., 2012). Royalisin contains three intramolecular disulfide bonds between six cysteine residues, which are essential for its antibacterial action (Bílikova et al., 2015). Another antibacterial protein identified in royal jelly is jelleine. So far, four jelleines, jelleine I–IV, have been identified by using reverse phase, high-performance liquid chromatography (HPLC). The differences between jelleine I–IV are limited to one residue in the sequence, and this difference has a significant impact on their antimicrobial efficacy. Jelleine I–III, but not jelleine IV, show antimicrobial activities against both gram-positive and gram-negative bacteria (Fontana et al., 2004). Table 4.1.1 summarizes the antibacterial activities of both royalisin and jelleine.

TABLE 4.1.1 Antibacterial Activity of Royalisin and Jelleines I–III Against Common Bacteria

	Activity (MIC)				
Microorganisms	Royal Jelly	Royalisin	Jelleine I	Jelleine II	Jelleine III
Gram-positive bacteria					
Bacillus cereus	+[a]	NR	R[b]	R[b]	R[b]
Bacillus subtilis	+[c]	+[d]	+[b]	+[b]	R[b]
Clostridium perfringens	NR	+[e]	NR	NR	NR
Clostridium tetani	NR	+[d]	NR	NR	NR
Lactobacillus acidophilus	+[e]	+[e]	NR	NR	NR
Listeria monocytogenes	+[f]	NR	+[g]	+[g]	NR
Staphylococcus aureus	+[a,c]	+[d,e]	+[b,g]	+[b,g]	+[b]
Staphylococcus saprophyticus	NR	NR	+[b]	+[b]	+[b]
Gram-negative bacteria					
Escherichia coli	+[a,e]	R[h,i]	+[b]	+[b]	+[b]
Klebsiella pneumoniae	+[j]	R[e]	+[b]	+[b]	R[b]
Pseudomonas aeruginosa	+[j]	+[h]	+[b,g]	+[b,g]	+[b]

R, Resistant; *NR*, not reported.

[a] *Ratanavalachai and Wongchai (2002).*
[b] *Fontana et al. (2004).*
[c] *Moselhy et al. (2013).*
[d] *Shen et al. (2012).*
[e] *Fujiwara et al. (1990).*
[f] *Attalla et al. (2007).*
[g] *Romanelli et al. (2011).*
[h] *Bílikova et al. (2015).*
[i] *Bilikova et al. (2001).*
[j] *García et al. (2010).*

Epigenetic Effect of Royal Jelly

Although both queen and worker bees share the same genotype, the queen bees have larger bodies, a longer lifespan, and well-developed gonads compared with the worker bees. In 2011, a highly publicized *Nature* paper by Kamakura M. showed that royalactin could induce differentiation of honeybee larvae into queen bees through differential DNA methylation in the genomic region encoding epidermal growth factor receptor (EGFR) (Kamakura, 2011). However, a study conducted by Kucharski and Maleszka argued that the EGFR gene in *Apis mellifera* contains a high guanine–cytosine content and is not methylated (Kucharski et al., 2015). Maleszka's laboratory also discovered that royal jelly induced the queen bee phenotype through epigenomic modification by DNA methyltransferases (DNMT) (Foret et al., 2012). They successfully developed queen bees from newly hatched honeybee larvae by silencing DNMT3 expression (Kucharski et al., 2008). Apart from DNA methylation, royal jelly could also affect histone modification. Spannhoff et al. (2011) reported that 10-HAD exhibits histone deacetylase (HDAC) activity in vitro and activates gene expression through epigenetic reprogramming. In addition, phenyl butyrate, another chemical component of royal jelly, is also a well-known HDAC inhibitor. All these data suggest that the development of queen/worker phenotype is driven by the combined action of epigenetic events such as DNA methylation and histone modification.

Other Biological Activities of Royal Jelly

Antioxidant and anticancer activities of royal jelly are summarized in Table 4.1.2.

SECONDARY BEE PRODUCTS: PROPOLIS

In contrast with the past, the interest in animal modified plant products like propolis is increasing rapidly. Propolis, also called bee glue or bee putty, is a natural lipophilic resinous material that honey bees collect from several plants and mix with beeswax and salivary enzymes (β-glucosidase) (Bankova et al., 2000; Marcucci, 1995; Pietta et al., 2002). Used in the construction and protection of hives, propolis possesses a characteristic aromatic smell, varies in color from yellowish-green, to red, to dark brown depending on a multitude of factors such as plant source, biogeographic localization, and environmental factors (Bankova et al., 2000; Marcucci, 1995), and shows adhesive properties because it strongly interacts with oils and proteins of the skin.

TABLE 4.1.2 In Vivo Antioxidant and Anticancer Activities of Royal Jelly

Experiment model	Royal jelly dose	Effect
Antioxidant activity		
C3H/HeJ mice[a]	60 mg/kg/day	↓ 8-OHdG; ↑ survival time
Fe-NTA-induced renal carcinogenesis in rats[b]	2 g/kg/day	↓LPO and total cholesterol
Wister albino rats with γ-radiation exposure[c]	1 g/kg daily for 14 days before and after γ-irradiation	↓ LPO, cholesterol, triglycerides, and LDL
STZ-induced diabetic rats[d]	100 mg/kg/day	↑CAT and FRAP activities
Anticancer activity		
Erhlich ascites tumor in mice[e]	0.5g/kg/day	↓PGE2; ↑survival
4T1 Murine breast cancer model[f]	1.5 g/kg/day	↓IL-6, TNF-α, IgG and tumor size; ↑IL-10

8-OHdG, 8-Hydroxy-2′-deoxyguanosine; *CAT*, catalase; *Fe-NTA*, ferric nitrilotriacetate; *FRAP*, ferric reducing antioxidant power; *IgG*, immunoglobulin G; *IL*, interleukin; *LPO*, lipid peroxidation; *LDL*, low-density lipoprotien; *PGE2*, Prostaglandin E2; *STZ*, Steptozotocin; *TNF-α*, tumor necrosis factor alpha.

[a] *Inoue et al. (2003).*

[b] *Guo et al. (2008).*

[c] *Azab et al. (2011).*

[d] *Ghanbari et al. (2016).*

[e] *Bincoletto et al. (2005).*

[f] *Zhang et al. (2017).*

Chemical Composition of Propolis

In recent decades, the chemical composition of propolis has been analyzed in several reviews (Bankova, 2005; Huang et al., 2014; Silva-Carvalho et al., 2015; Toreti et al., 2013). As with all bee products, the exact composition will vary with the plants sampled, and so also shows a biogeographic, seasonal, and bee species specificity. In general, despite the considerable diversity of factors that could affect the chemical composition of propolis, we can find a significant likeness between them. Usually, propolis is composed of resin and vegetable balsam (50%), a mix of bee and vegetable waxes (30%), essential and aromatic oils (10%), pollen (5%), and minor components including organic debris. Propolis is a rich mixture of polyphenols and their esters, phenolic aldehydes, ketones, terpenes, beta-steroids, vitamins, minerals, proteins, amino acids, and sugars (Bankova, 2005; Bankova et al., 2000; Silva-Carvalho et al., 2015; Walker and Crane, 1987). Polyphenolic compounds are one of the most extensive groups of chemicals in the plant kingdom, they form the major part of propolis' biologically active substances, and can be subdivided into three main subclasses: the flavonoids, phenolic acids, and the stilbenoids. Flavonoids are a water-soluble, broad polyphenol family found in plants and synthesized by plants via photosynthesis (Pereira et al., 2009; Yao et al., 2004). Flavonoid aglycones and especially flavanones are typical components of poplar propolis (Bankova et al., 2000). The β-glucosidase secreted by bees hydrolyzes glycosides of flavonoids to the corresponding aglycones and sugars (Sahinler and Kaftanoglu, 2005). Caffeic acid and its by-products are principally derived from phenolic acids. The product of the reaction of caffeic acid with phenethyl alcohols is caffeic acid phenethyl ester (CAPE). CAPE is a natural bioactive compound bearing a polyphenolic ring that occurs in many plants, and is acquired from propolis obtained through extraction from honeybee hives (Bankova, 2005, 2009; Bankova et al., 2000). Stilbenoids are derived from polyphenols and play the role of phytoalexins in plants (Xiao et al., 2008). One of the most important subclasses of stilbenoids is polyhydroxylated stilbenes, the main representative being resveratrol (Pereira et al., 2009). Some stilbenoids have been found in the propolis of different geographic areas; schweinfurthin A and schweinfurthin B in Africa (Zhang et al., 2014), 5-farnesyl-3'-hydroxyresveratrol in the Solomon Islands (Inui et al., 2012), while other stilbenes were identified on Australia's Kangaroo Island (Abu-Mellal et al., 2012).

Biomedical Importance of Propolis: Modern Research Evidence

The biological and pharmacological actions of propolis have been extensively studied, highlighting its importance in cell biology and the development of potential drugs. In general, despite the considerable diversity of chemical components of propolis, some common effects for most propolis types have been reported.

Antioxidant Activity of Propolis

Cell injury caused by oxidative damage is the main consequence of an imbalance between the prooxidant and antioxidant levels in favor of prooxidants. High levels of oxidative stress can inflict damage to biomolecules such as DNA, proteins, and lipids. Antioxidant activity is one of the most important activities of propolis, and has been confirmed in several studies. The propolis extracts showed the highest antioxidant activity of all bee products (Nakajima et al., 2009). Because a considerable diversity of factors could affect the chemical composition of propolis (see the previous section "Chemical composition of propolis"), its antioxidant activity correlates with the type and total concentration of its polyphenols (Huang et al., 2014; Kumazawa et al., 2004). Table 4.1.3 shows a brief extract of studies presented in the literature in which the antioxidant activity of propolis was tested in vivo.

Antiinflammatory Activity of Propolis

Inflammation is a process that normally occurs in response to exogenous or endogenous stimulus such as toxic chemicals, stress, or infection. Flavonoids and phenolic acids such as caffeic acid and their derivatives could be responsible for the antiinflammatory activity of propolis. Table 4.1.4 shows a brief extract of studies presented in the literature in which the antiinflammatory activity of propolis was tested in vivo.

Anticancer Activity of Propolis

Despite advances in cancer biomarkers and therapy, cancer remains one of the most common causes of mortality worldwide. Propolis' anticancer activity has been confirmed in several studies. Sawicka et al. (2012) and Chan et al. (2013) in reviews, summarized different mechanisms for antitumor properties of propolis, such as suppressing cancer cell proliferation via its antiinflammatory effects, decreasing cancer stem cell populations, blocking specific oncogene signaling pathways, exerting antiangiogenic effects, and modulating the tumor microenvironment. Table 4.1.5 shows a brief extract of studies presented in the literature in which the anticancer activity of propolis was tested in vivo.

TABLE 4.1.3 In Vivo Antioxidant Activity of Propolis

Experimental model	Propolis dose	Effect
Plasmodium chabaudi infection in mice[a]	25, 50, 100 mg PE	↓MDA, ↑CAT, ↑GSH
IsoP-induced stress in mice[b]	25 mg/kg/day PF	Activation of PI3K /AKT
Hcy-induced stress in mice[c]	0.05% or 0.25% propolis diet	↓ROS in U-251MG cell
DTZ-induced KD in rats[d]	100 mg/kg bodyweight	↓MDA, ↑CAT, ↑GSH, ↑SOD
MeHg-induced stress in rats[e]	0.10 or 1.0, and 10 mg/kg	↑GSH, ↑DNA damage
DXR-induced stress in rats[f]	250 mg/kg bodyweight	↓MDA, ↑CAT, ↑SOD, ↑GST
CTX-induced stress in mice[g]	1, 0.5, or 0.25 mg/ml PFM	↓MDA, ↑SOD, ↑GSH-Px
Alloxan-induced LP in mice[h]	50 mg/kg (EEP and WSPP)	↓MDA
HSV2-induced stress in mice[i]	50 mg/kg HPE	↓RS, ↓TyrN, ↓MPO, ↑CAT

CAT, Catalase; *CTX*, cytoxan; *DTZ*, diatrizoate; *DXR*, doxorubicin; *GSH*, glutathione; *GSH-Px*, glutathione peroxidase; *GST*, glutathione-*S*-transferase; *HSV-2*, herpes simplex virus type 2; *Hcy*, homocysteine; *IsoP*, isoproterenol; *LP*, lipoperoxidation; *MDA*, malondialdehyde; *MeHg*, methylmercury; *MPO*, myeloperoxidase; *KD*, kidney disease; *OEP*, oil extract of Brazilian propolis; *PFM*, PF microemulsion; *PF*, propolis flavone; *PE*, propolis extract; *ROS*, reactive oxygen species; *RS*, reactive species; *SOD*, superoxide dismutase; *TyrN*, tyrosin nitration; WSDP, water-soluble derivative of propolis; *EEP*, ethanolic extract of propolis; *HPE*, hydroalcoholic propolis extract.

[a] *AlGabbani et al. (2017)*.

[b] *Sun et al. (2016)*.

[c] *Miyazaki et al. (2015)*.

[d] *Baykara et al. (2015)*.

[e] *Manzolli et al. (2015)*.

[f] *Singla et al. (2014)*.

[g] *Fan et al. (2014)*.

[h] *Oršolić et al. (2012)*.

[i] *Sartori et al. (2012)*.

Other Biological Activities of Propolis

Antibacterial, antifungal, antiviral, antiparasite, and stimulation of the immune system were also reported as biological activities of propolis (Silva-Carvalho et al., 2015).

SIDE EFFECTS AND INTERACTIONS OF ROYAL JELLY AND PROPOLIS

In humans, oral administration of both royal jelly and propolis is nontoxic. Royal jelly is considered relatively safe, and despite propolis treatment over 30 days not showing any side effects or significant alterations in antioxidant status, lipid profile, and routine red blood cell parameters (Jasprica et al., 2007), it has been reported that some side effects of propolis can include seizures, gastrointestinal symptoms, and acute renal failure (de Groot, 2013). An excellent review by de Groot summarized contact allergy and allergic contact dermatitis including coreactivity in patients allergic to propolis (de Groot, 2013). Both of these bee products could cause allergic reactions such as asthma, anaphylaxis (Callejo et al., 2001; Leung et al., 1995; Nyman and Hagvall, 2016), and contact dermatitis (Matos et al., 2015; Uter et al., 2016). These allergic reactions depend on several factors such as protein components, plant source, and biogeographic localization (Blank et al., 2012; Hegyi et al., 1990). In addition, both royal jelly and propolis have been reported to interact with anticoagulants such as warfarin and heparin (Akbay et al., 2017; Lee and Fermo, 2006).

CONCLUSIONS AND PERSPECTIVES

Royal jelly and propolis are natural products used as foods that may promote health and reduce illness. Based on modern research evidence, bee products have a wide range of pharmacological and health-promoting functions in humans. The main functions attributed to royal jelly include antibacterial, epigenetic, anticancer, and antioxidant activities, and propolis has antioxidant, antiinflammatory, and anticancer functions. The use of royal jelly and propolis is expected to increase as a consequence of almost daily research evidence emerging regarding bee products, an increasing interest in natural products,

TABLE 4.1.4 In Vivo Antiinflammatory Activity of Propolis

Experimental model	Propolis dose	Effect
Ovalbumin in mice[a]	5 mg/kg CAPE	↓MMP9, ↓MDA, ↓ROS, ↓IL4, ↓TNFα
Carrageenan in mice[b]	1, 3, or 10 mg/kg EEP	↓NM, ↓TNF-α, ↓IL1β, ↓CL1, ↓CL2
IR-induced injury in rats[c]	10 μg/kg CAPE	↓MPO, ↓ICAM-1
LPS-, collagen in mice[d]	3 or 10 mg/kg PE	↓NM, ↓LRA, ↓ICAM-1, ↓IL-6
TPA skin inflammation[e]	Topical CAPE (0.5%)	↓TNF-α, ↓COX2, ↓iNOS, ↓NF-κB
Mycobacterium butyricum in rats[f]	50 or 250 mg/kg PE	↓Arthritis scores, ↓cachexia
TNBS (colitis) in mice[g]	10 or 23.3 mg/g diet PE	↓Th1 cell differentiation
Eccentric EMI in mice[h]	5 or 10 mg/kg CAPE	↓Pn, ↓MDA,↓ IL1β, ↓COX1, ↓iNOS
High-fat diet in mice[i]	30 mg/kg CAPE	↓p-JNK-1, ↓IκB, ↓COX1

TPA, 12-*O*-tetradecanoylphorbol-13-acetate; *CAPE*, caffeic acid phenethyl ester; *CL*, chemokine ligand; *JNK*, c-jun N-terminal kinase; *COX*, cyclooxygenase; *EMI*, eccentric exercise muscle injury; *TNBS*, hapten 2,4,6-trinitrobenzene sulfonic acid; *iNOS*, inducible nitric oxide synthase; *IκB*, inhibitor of nuclear factor kappa B; *IL*, interleukin; *LRA*, leukocyte rolling and adhesión; *MDA*, malondialdehyde; *MMP-9*, metalloproteinase-9; *IR*, myocardial ischemia-reperfusion; *NM*, neutrophil migration; *NF-κB*, nuclear factor-κB; *Pn*, protein nitrotyrosylation; *TGF*, transforming growth factor; *Th1*, type 1 T helper cell; *ICAM*, intercellular adhesion molecule.

[a] *Ma et al. (2016)*.

[b] *Bueno-Silva et al. (2016)*.

[c] *Du et al. (2016)*.

[d] *Franchin et al. (2016)*.

[e] *Lim et al. (2015)*.

[f] *Mossalayi et al. (2014)*.

[g] *Okamoto et al. (2013)*.

[h] *Shen et al. (2013)*.

[i] *Bezerra et al. (2012)*.

TABLE 4.1.5 In Vivo Anticancer Activity of Propolis

Experimental model	Propolis dose	Effect
Oesophageal xenograft[a]	25 mg/kg Gal	↑PCD, ↓TV,↓Ki-67, ↓Wnt3a, ↓P53
Prostate cancer xenograft[b]	15 mg/kg CAPE	↓NF-κB, ↓MMP9, ↓Snail, ↓βcat
Lung cancer xenograft[c]	24 mg EEP	↓H&E, ↑PCD
OSC in rat[d]	50 or 100 mg/kg	↓TV, ↓TN
HNSCC xenograft models[e]	25 mg/kg Gal	↓TV, ↓TW, ↓cyclin D1, ↑PCD
Renal carcinogenesis in rats[f]	80 or 160* mg/kg P	↓TN, ↓PCNA, ↑P53, ↓COX-2
Ehrlich ascites tumor in mice[g]	50 mg/kg WSDP	↓TC, ↓CisTG, ↑Macrophages
Prostate cancer xenograft[h]	10 mg/kg CAPE	↓TV, ↓ p70S6K and Akt signaling
Colon carcinoma xenograft[i]	500 or 1000 mg/kg	↓TV, ↑P53, ↓Ki-67, ↓H&E

Gal, Galangin; *TV*, tumor volumen; *TM*, tumor number; *TW*, tumor weight; *TC*, tumor cells; *PCD*, programmed cell death; *P*, propolis; *OSC*, oral squamous carcinomas; *Ki-67*, marker for proliferation; *Wnt3a*, Wnt family member 3A; *NF-κB*, nuclear factor-κB p65; *MMP9*, metalloproteinase-9; *Snail*, zinc finger protein; *βcat*, β-catenin; *EEP*, ethanolic extracts of propolis; *H&E*, haematoxylin and eosin; *HNSCC*, head and neck squamous cell carcinoma; *PCNA*, proliferating cell nuclear antigen; *COX-2*, cyclooxygenase-2; *WSDP*, water-soluble derivative of propolis; *CisTG*, cisplatin toxic and genotoxic; *CAPE*, caffeic acid phenethyl ester.

[a] *Ren et al. (2016)*.

[b] *Tseng et al. (2016)*.

[c] *Khacha-Ananda et al. (2016)*.

[d] *Ribeiro et al. (2015)*.

[e] *Zhu et al. (2014)*.

[f] *Rashid et al. (2013)*.

[g] *Oršolić et al. (2013)*.

[h] *Chuu et al. (2012)*.

[i] *Sulaiman et al. (2012)*.

and advances in food industry. Despite administration of these bee products in humans not being toxic, a few side effects including allergic reaction have been reported. More clinical and epidemiologic studies of these bee products in relation to their side effects and interactions with other supplements/medicines are needed.

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Chapter 4.2

Chitosan

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INTRODUCTION

Marine organisms produce bioactive substances with important applications. Chitosan is a polysaccharide of marine origin produced from chitin of the exoskeletons of crustaceans (Santhosh et al., 2006). Chitosan is a linear polymer formed by D-glucosamine and *N*-acetyl-D-glucosamine. This biopolymer and its derivatives are considered promoters of diverse biological activities, including antioxidant, antihypertensive, antiinflammatory, anticoagulant, antitumoral, antimicrobial, hypocholesterolemic, and antidiabetic effects (Ngo and Kim, 2014).

Chitosan is listed as a safe material for use in food, cosmetics, and medical devices in countries such as the United States, Japan, Italy, and Finland (Baldrick, 2010). Additionally, Waibel et al. (2011) evaluated the safety of a chitosan-based bandage in patients allergic to seafood and found that all participants tolerated the bandage without any reaction. Similarly, Rizzo et al. (2014) reported no important secondary effects of the use of chitosan, although it could cause mild stomach ache, constipation, and gas.

Chitosan is marketed using a great variety of names and is available without prescription in health food and supplement stores, as well as in many online sites. It is known that chitosan supplements are very similar but not identical. Specifically, they differ in the degree of deacetylation (DD) and in molecular weight, which has a direct impact on the viscosity of the chitosan solution and its capacity to trap fats (Gades and Stern, 2005). Different authors have reported the beneficial effects of chitosan in lowering cholesterol and triglycerides (Rizzo et al., 2014). However, studies have shown inconclusive results with regard to weight loss. Chitosan also acts as an antioxidant in the circulatory system by lowering the levels of oxidized albumin, which represents an important indicator of oxidative stress (Anraku et al., 2009). Furthermore, the reports of Anraku et al. (2012) suggest favorable results for the antioxidant capacity of chitosan in studies related to the renal system.

This chapter is based on information published in clinical studies in relation to the potential benefits of chitosan in human health when used as a dietary supplement. Nevertheless, it is important to increase the number of clinical studies in the future to confirm the safety and efficacy associated with chitosan and each biological property attributed to this compound.

MOLECULAR STRUCTURE

Chitosan (PubChem CID: 71853) is a linear biopolymer constituted of units of D-glucosamine and *N*-acetyl-D-glucosamine, which are linked together by a glycosidic β (1–4) bond. The molecular weight of this natural polymer ranges from 10,000 to 1 million Daltons (Pillai et al., 2009). Its molecular structure has an amino group (C2) and 2 hydroxyl groups (C3 and C6), which form intermolecular hydrogen bonds that determine the stability of the polymer (Ríos-Donato et al., 2006). Chitosan can be chemically modified through amino and hydroxyl groups.

Source

Chitosan is found naturally in the cell walls of some fungi. However, this polymer is obtained industrially by deacetylation of chitin (PubChem CID: 6857375), which is extracted from the exoskeletons generated by crustacean-processing plants from animals such as shrimp, lobsters, crabs, and krill. Chitin is associated with proteins, minerals, lipids, and pigments (Xu et al., 2008).

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The chemical purification of chitin from crustaceans involves two processes. Initially, the wastes are demineralized to remove inorganic matter by applying dilute HCl and then deproteinized in a dilute aqueous solution of NaOH or KOH (Philiibert et al., 2017).

Currently, there is a preference for the extraction and purification of chitin by lactic acid fermentation (Arbia et al., 2013) with *Lactobacillus paracasei* strain A3, *L. paracasei* subsp *tolerans* KCTC-3074, *Lactobacillus pentosus* 4023, and *Lactobacillus* sp. B2, for deproteinization and demineralization (Bhaskar et al., 2007; Hamed et al., 2016).

Chitin can be converted to chitosan by chemical or enzymatic methods and both processes are based on the deacetylation of chitin. Deacetylation is the process of converting chitin to chitosan by removing acetyl groups from *N*-acetylglucosamine to form D-glucosamine units, which contain free amino groups and increase the solubility of the polymer in aqueous media (Philiibert et al., 2017). The chemical hydrolysis of the acetamide groups is achieved in a strongly alkaline medium and at high temperatures. Generally, the reaction is carried out in a heterogeneous phase using concentrated solutions of NaOH or KOH (40%–50%) at temperatures above 100°C, preferably under an inert atmosphere to avoid depolymerization of the polymer. The reaction-specific conditions depend on factors such as the source of material used, and the desired DD (Al Sagheer et al., 2009). Currently, enzymatic methods using chitinases or chitin deacetylases (EC 3.2.1.14) are a viable alternative to produce biological chitosan. These enzymes were identified and partially purified for the first time from extracts of the fungus *Mucor roxii* and *Absidia coerulea*. All enzymes have been reported as glycoproteins secreted intracellularly or extracellularly, have a remarkable thermostability, and act optimally at 50°C (Suresh et al., 2011).

Physicochemical Characteristics

The composition and size of the polymer chains of chitosan vary depending on their origin and the chitin deacetylation method. According to Rinaudo (2006), the solid chitosan is a semicrystalline polymer of white or slightly yellow appearance. Chitosan is insoluble in water, in alkaline solutions, and in organic solvents. Conversely, it is also soluble in dilute acid solutions below pH 6.3 (Zhang et al., 2011).

The most important parameter for the characterization of chitosan is the DD, which influences its properties and applications. The DD represents the proportion of D-glucosamine units relative to the total number of units (*N*-acetyl-D-glucosamine plus D-glucosamine) (Pillai et al., 2009). For some researchers, chitin with more than 50% DD is considered chitosan, while others define it as such when it is higher than 60%. Commonly, the DD of chitosan is in the 60%–98% range. The most common techniques for accurate DD determination are infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, as well as potentiometric titration (Dash et al., 2011; Dimzon and Knepper, 2015; Lin et al., 2017). Chitosan exhibits a wide range of viscosities in dilute acids depending on the molecular weight (Rinaudo, 2006). Li et al. (2005) have reported that commercially available chitosan has a high molecular weight; however, in the food and pharmaceutical industry, low molecular-weight chitosan is required.

Chitosan is bioadsorbable and biodegradable and it has been shown to be slowly degraded by chitosanases and lysozyme enzymes. Kean and Thanou (2010) have stated that the rate of degradation depends on the physicochemical characteristics of chitosan. Lysozyme reduces the viscosity of chitosan by up to 60%.

Jamnongkan and Kaewepirom (2010) point out that chitosan is a biopolymer that behaves like a hydrogel because its three-dimensional structure can absorb and retain high amounts of water, allowing it to swell without the need to completely dissolve. In this context Xie (2011) has discussed the development of a superabsorbent material based on chitosan, because its hydroxyl groups and other active sites are capable of strongly interacting with water and various organic molecules, allowing their application in areas such as agriculture, medicine, and the production of industrial materials.

BIOLOGICAL ACTIVITIES

Antioxidant Activity

Reactive oxygen species (ROS) can cause oxidative damage as a consequence of normal metabolic processes and external factors. An antioxidant acts by eliminating the ROS and repairing damage. Normal metabolism produces sufficient antioxidants to act against the normal number of free radicals generated by an organism (Harish Prashanth et al., 2007). However, the excessive production of ROS generates oxidative stress that can lead to illness and death (Essick and Sam, 2010). There is also a correlation between oxidative stress and cancer, diabetes, atherosclerosis, arthritis, and chronic inflammation (Halliwell, 2011).

Proteins constitute more than half of the dry mass of cells, and because of their immediate reaction with ROS, they are the main target of free radicals in vivo. Several findings support the theory that serum albumin is a passive protector

of extracellular fluids, given its capacity to eliminate ROS. However, oxidized proteins can propagate ROS and act as intermediates in oxidative stress. To avoid biological damage associated with oxidative stress, it is necessary to inhibit the formation and reactions of free radicals and protein hydroperoxides (Anraku et al., 2004). All this suggests that the level of oxidation of human serum albumin reflects the degree of oxidative stress in human plasma.

Clinical studies on the use of chitosan as a dietary supplement for its antioxidative activity are insufficient (Table 4.2.1). The effect of chitosan as an antioxidant has mainly been studied on laboratory animals such as rats (Anandan et al., 2013; Anraku et al., 2010; Yoon et al., 2008), prawns, dogs, and cats. There are also in vitro trials where the antioxidant activity has been measured by the reducing power and radical scavenging activity of chitosan (Hajji et al., 2015; Yen et al., 2007).

Anraku et al. (2008) studied the ability of 2800-Da chitosan to protect human serum albumin from oxidation by peroxyl radicals. The authors found that chitosan was equally efficient as vitamin C in preventing the formation of carbonyl and hydroperoxide groups when human serum albumin was exposed to peroxyl radicals. In addition, chitosan can act as a potent inhibitor of conformational changes of serum albumin. The results suggest that administration of low molecular-weight chitosan can inhibit neutrophil activation and oxidation of serum albumin, suggesting a reduction of oxidative stress.

Based on the work of Tomida et al. (2009), a low molecular–weight chitosan (<30 kDa) is proposed as a dietary supplement based on its ability to protect plasma proteins against oxidation by peroxyl radicals. For the in vitro assay, human plasma was obtained from healthy volunteers. The authors also suggested that low molecular-weight chitosan can be absorbed in the gastrointestinal tract and that it inhibited serum albumin oxidation, which is frequently observed in patients receiving hemodialysis.

The benefits of water-soluble chitosan as an antioxidant in the circulatory system have been studied (Anraku et al., 2009). The authors found that treatment significantly reduced the ratio of oxidized albumin to reduced albumin in plasma. Blood glucose (BG) and the atherogenic index (AI) also decreased. Specifically, the chitosan supplementation was proposed for young individuals to prevent oxidative stress associated with renal failure.

Anraku et al. (2011) studied the effect of a high molecular-weight chitosan as a dietary supplement on indices of oxidative stress monitored by the amount of oxidized albumin in plasma. The intake of high molecular-weight chitosan over a period of 8 weeks significantly reduced the levels of total cholesterol (TC) and AI, and increased high-density lipoprotein (HDL) cholesterol. The authors also suggested that the chitosan studied could reduce the levels of prooxidants like cholesterol and uremic toxins in the gastrointestinal tract, which inhibited oxidative stress in the human circulatory system.

Oxidative stress has been associated with patients with chronic renal failure. Anraku et al. (2014) studied the effects of the ingestion of chitosan supplements on oxidative stress in patients receiving hemodialysis. According to their hypothesis, chitosan can reduce the levels of lipids and uremic toxins that induce the production of ROS in the intestinal tract. They found that chitosan binds 38.5% of indoxyl sulfate (IS) and 17.8% of phosphate, and both of them induce the production of ROS. The study also reports a beneficial relationship between oxidized albumin and IS. These findings could prevent cardiovascular diseases in patients with chronic renal failure. The authors report that chitosan represents a new strategy in the treatment of many diseases that require antioxidants. With regards to its antioxidant effect, chitosan is unique and differs from all other natural antioxidants like vitamins and *N*-acetylcysteine.

TABLE 4.2.1 Clinical Trials with Chitosan as an Antioxidant Supplement

Dosage regimen	Participants	Patient parameters	References
Single dose of 540 mg	10 healthy subjects Aged 28 ± 2 years	BG, TC, LDL, HDL, AI, and OSA	Anraku et al. (2009)
180 mg three times per day	10 healthy subjects Aged 34 ± 4 years	TC, LDL, HDL, AI, and OSA	Anraku et al. (2011)
500 mg/day	11 patients with chronic renal failure Aged 52–78 years	HAS, ALP, TC, IS, Ca, LDL, and HDL	Anraku et al. (2014)

BG, Blood glucose; *TC*, total cholesterol; *LDL*, low-density lipoprotein; *HDL*, high-density lipoprotein cholesterol; *AI*, atherogenic index; *OSA*, oxidized serum albumin; *HAS*, human serum albumin; *ALP*, alkaline phosphatase; *IS*, indoxyl sulfate; *Ca*, calcium.

Antibacterial Activity

The antibacterial activity of chitosan has been observed in a wide range of microorganisms, including fungi, viruses, and bacteria. There are studies about the inhibition of growth of *Salmonella typhimurium* and *Staphylococcus aureus* (Rodríguez-Núñes et al., 2012; Liu et al., 2016), *Pseudomonas fluorescens* and *Escherichia coli* (Tang et al., 2010), and *Listeria monocytogenes* (Rodríguez-Nuñez et al., 2014). This property has favored the application of chitosan in food preservation and medicine. However, such a property is influenced by various factors such as molecular weight, DD, polymerization degree, host, and environmental conditions (Dutta et al., 2009). An important characteristic for the understanding of chitosan is the positive charge that the molecule acquires in slightly acidic media (pH 5.5); this is due to the protonation of the amino group present in the glucosamine monomers, facilitating its solubility in aqueous media, and according to different authors, leading to its biocidal properties (Guzman-Villanueva et al., 2014).

Body Weight Reduction

According to Rodríguez and Albertengo (2005) and Yun (2010), the incidence of obesity has increased in recent years becoming a global health issue, with incalculable social costs. In addition, obesity is associated with diabetes, hypertension, osteoarthritis, and cardiovascular diseases. At present, there are numerous natural products for the treatment of obesity, which need to be proved as safe and effective.

Over the past few decades, several authors have proposed the use of chitosan as a supplement to prevent the absorption of ingested fat, thus controlling body weight (Muzzarelli, 1996). However, some studies have reported that chitosan has no effect on fecal fat excretion (Guerciolini et al., 2001).

The interaction of chitosan with sunflower oil has been studied in an experimental chemical model of the human digestive tract (Rodríguez and Albertengo, 2005). In accordance with these results, the authors propose that chitosan dissolves in the acidic medium of the stomach forming an emulsion with oil. Subsequently, by increasing the duodenum's pH, chitosan precipitates, and the trapped oils cannot be absorbed through the intestinal walls.

Various studies involving animal experiments have demonstrated that chitosan interferes in the intestinal absorption of dietary lipids and leads to loss of body weight. In rats that were given chitosan, a loss of 15% body weight has been reported (Anraku et al., 2012), and according to Egan et al. (2016) chitosan has an antiobesogenic potential in pigs, due to its ability to alter eating behavior and control appetite.

Despite the many studies, the mechanism of action of chitosan has not been completely established. It is known that in the acidic environment of the stomach, chitosan acts as a soluble fiber and can increase its volume given its water absorption capacity. After this, in the nonacidic environment of the small intestine, chitosan becomes insoluble and forms a gel that encapsulates dietary fats. This insoluble complex travels through the large intestine and is naturally eliminated (Kaats et al., 2006). Additionally, it has been proposed that the positive charges of chitosan attract negatively charged molecules such as lipids and biliary salts (Elsabee et al., 2009).

The efficacy of chitosan as a dietary supplement to promote weight loss has been evaluated in 250 overweight and obese adults. During this prolonged clinical trial, Mhurchu et al. (2004) measured changes in body weight, body mass index, waist circumference, body fat percentage, blood pressure, serum lipids, plasma glucose, and fecal fat. This study demonstrated that chitosan had no clinical effect on weight loss or on the increase in fecal fat excretion when the participants received a dosage of 3 g/day. However, other researchers believe the type and chemical composition of chitosan can affect its ability to trap gastrointestinal lipids and affect weight loss. In this trial β-chitosan from squid pens was used with a level of DD of 75.5% and 130 kDa.

Mhurchu et al. (2005) also carried out a systematic review to determine if chitosan is an effective treatment for being overweight or obese. They searched specialized websites for all the controlled studies on chitosan as a dietary supplement. From a total of 36 studies, 14 were selected, those with a minimum treatment of 4 weeks in overweight or obese adult patients with hypercholesterolemia. In total, 1071 participants were involved. The results of the systematic review indicate that chitosan treatment has a slight effect on weight loss, 1.7 kg in a short period of time.

Gades and Stern (2005) studied the effect of diverse chitosan-based products on fecal fat excretion. Men and women participated in the study, with a total of 24 individuals, during a period of 12 days. The different formulations contained chitosan, psyllium husk, malic acid, and *Aloe vera*, the total consumption of chitosan was 2.5 g/day. These authors found that fecal fat excretion in men was 1.8 g/day and 0.0 g/day in women. The fat bound by chitosan lacks clinical significance. It would take men 7 months to lose 1 pound of body fat.

Kaats et al. (2006) determined the efficacy of chitosan on body composition. The total intake of chitosan was 3.0 g/day, and it was ingested with beta-glucan, snowhite oat, fiber, betaine HCL, and aloe saponins. The study was carried out over a

period of 60 days, with 150 subjects. At the beginning and the end of the study, every participant completed a dual-energy X-ray absoptiometry test for body composition and bone density, as well as a blood chemistry test. There was evidence that chitosan enables the depletion of excess body fat with a minimal loss of lean body mass.

Trivedi et al. (2016) investigated the efficacy of chitosan from *Aspergillus niger* in the treatment of excess body weight with an absence of any dietary restrictions. During the clinical trial, 5 commercial capsules of 500 mg were administered to 96 obese patients daily for 90 days. The researchers reported that chitosan could reduce body weight by up to 3 kg during 90 days of study, as well as improving body composition and anthropometric and lipid parameters. The authors suggested that chitosan supplements should be ingested at least 15 min before each meal to result in weight loss. This time allows the chitosan to dissolve in the acid of the stomach and then bind to dietary fats.

Antihyperlipidemic

Some studies have demonstrated that chitosan has the property of lowering cholesterol levels in animals and humans. In studies conducted in Sprague-Dawley rats, results have shown chitosan to reduce TC and low-density lipoprotein (LDL) cholesterol and increase the amount of HDL cholesterol (Park et al., 2010).

In an in vitro study with conditions that replicated the human gastrointestinal tract, Panith et al. (2016) has shown that chitosan's ability to bind fats can be related to its molecular weight and the morphology of the chitosan particles. Chitosan was also found to bind effectively to cholesterol and biliary salts. Therefore, chitosan has a potential use in the treatment of obesity in individuals with high fat consumption. However, there are insufficient clinical studies regarding the use of chitosan as a dietary supplement for body weight reduction (Table 4.2.2).

Gallaher et al. (2002) investigated the intake of chitosan and glucomannan in patients with obesity, observing a significant reduction in HDL and LDL cholesterol, with no changes in total triglyceride concentration. Another important finding was the minimum dose requirement and efficacy of chitosan. The authors observed a reduction in blood cholesterol and an increase in the fecal excretion of cholesterol; however, there was no increase of fecal fat excretion, which suggests that the action of chitosan involves two different mechanisms.

Liao et al. (2007) reported that both water soluble and insoluble chitosan decreased the blood lipid content and maintained normal Ca, Mg, and Fe levels in patients with advanced hyperlipidemia. TC decreased by 8.9% with water insoluble chitosan. This group of researchers confirmed that blood lipid levels can be reduced when the absorption of cholesterol from the diet is diminished and the hepatic bile acid reserves are depleted, because cholesterol is diverted to produce new bile acids.

Tapola et al. (2008) found that the consumption of chitosan tablets was safe, but did not find significant effects on the concentration of total plasma cholesterol. Two dosages of chitosan were studied in men and women with hypercholesterolemia over an 8-week period, in which patients consumed the typical Finnish diet.

Rizzo et al. (2014) reported significant beneficial effects of chitosan on plasma lipids and lipoproteins. After the treatment, TC was reduced by 8% and triglycerides by 19%. During this pilot study, 28 patients with hypercholesterolemia participated, and they had no other treatment with lipid regulators.

TABLE 4.2.2 Clinical Trials With Chitosan as an Antihyperlipidemic Supplement

Dosage regimen	Participants	Patient parameters	References
Two tablets, three times per day before meals One tablet (300 mg) contained 52% chitosan; taken for 8 weeks	3 groups of 20 hyperlipidemic patients Aged 61 ± 2 years, 62 ± 3 years, and 64 ± 3 years	TC, triacylglycerol, LDL cholesterol, HDL cholesterol, Ca, Mg, Fe, and vitamin C	Liao et al. (2007)
4.5 or 6.75 g/day for 8 weeks	65 Subjects Aged 18–55 years	Vitamin A, vitamin E, α-carotene, β-carotene, 25-hydroxyvitam D, TC, LDL cholesterol, HDL cholesterol, and total triglycerides	Tapola et al. (2008)
150 mg/day 4 months	28 patients with hypertriglyceridemia Aged 63 ± 12 years	TC, triglycerides, HDL cholesterol, LDL cholesterol	Rizzo et al. (2014)

TC, Total cholesterol; *LDL*, low-density lipoprotein; *HDL*, high-density lipoprotein; *Ca*, calcium; *Mg*, magnesium; *Fe*, iron.

Mineral Absorption

In the past few years, chitosan as a supplement has been reported to affect Ca metabolism in animals. These studies found that chitosan can increase urinary excretion and decrease serum Ca levels, without inhibiting the absorption of the mineral (Wada et al., 1997).

Similarly, an incorrect balance of minerals in an organism is directly related to bone deterioration. Deuchi et al. (1995) reported an abrasive effect in the intestinal mucosa, which affected the absorption of Fe and an imbalance in the bone mineral content. Yang et al. (2002) also found a reduction in bone density induced by the consumption of chitosan. Both studies were conducted with laboratory rats given chitosan supplements.

These studies suggest that a controlled intake of chitosan is needed to avoid mineral imbalance. However, recent studies conducted on older patients did not report differences in levels of serum Mg or Ca, although it has been suggested that a high molecular-weight chitosan can influence the absorption mechanism of Fe and its consumption may provoke changes in the level of mean corpuscular hemoglobin (Liao et al., 2007).

CHITOSAN OLIGOSACCHARIDE

Chitosan oligomers are obtained by enzymatic hydrolysis of commercial chitosan. Chitosan oligomers are of great interest, primarily in biomedical research, due to their characteristics such as water solubility, low molecular weight, low viscosity, and short chains. These characteristics increase their physiological functionality within in vivo systems (Hamed et al., 2016).

In a study conducted in healthy volunteers, Kang et al. (2016) investigated the effect of chitosan oligomers on postprandial BG. They confirmed that these supplements decreased BG levels by 25% after 30 min of bread ingestion in comparison to the control group. This was due to the inhibition by the oligomers of intestinal enzymes that hydrolyzed the carbohydrates present in the intestine, and this inhibition resulted in the reduced release of glucose.

Kim et al. (2014) evaluated the effect of chitosan oligomers in patients with prediabetes. During the study, a significant reduction in the levels of BG was observed 30–60 min after intake of the oligomers. Likewise, the results showed improvement of the levels of HbA1c (hemoglobin A1c), which is used as a diagnostic marker in patients with diabetes, reflecting their long-term glycemic levels.

Choi et al. (2012) demonstrated the hypocholesterolemic effect of chitosan oligomers. The study was conducted over a period of 6 weeks in male patients with normal cholesterol levels who had a smoking habit; these patients are more prone to cardiovascular diseases. The results showed a significant decrease in levels of TC and LDL cholesterol, for both smoking and nonsmoking groups. Oligomers can form a complex with biliary acids, removing them and making them unavailable for reabsorption and recirculation in an organism, resulting in an increase in the synthesis of bile acid, and reducing blood cholesterol.

Jo et al. (2014) evaluated the effect of oligomers in the level of postprandial glucose in nondiabetic adult patients. In this clinical trial, 13 patients with an average age of 28.9 $\pm$ 1.7 years, orally consumed 500 mg of oligomers before the administration of 75 g of sucrose in 200 ml of water. The authors found that ingestion of oligomers resulted in a reduction of blood postprandial glucose caused by the inhibition of the enzymes that hydrolyze carbohydrates. These results are in accordance with the observations from animal trials by the same research group.

Potential Interactions

Several studies have shown the low oral toxicity of chitosan when it is used as a nutritional supplement or as a food additive. However, Huang et al. (2007) reported that the anticoagulant effect of warfarin can be increased by chitosan because it interferes with the absorption of vitamin K and other vitamins such as A, E, and D, and bile acids. According to Baldrick (2010), more studies are needed to demonstrate the safe use of chitosan by parenteral route due to its haemostatic property.

CONCLUSION

There have been many diverse experimental assays with chitosan, its biocompatibility, and nontoxicity, and beneficial effects towards health have been acknowledged. In the last 20 years, the commercialization of chitosan as a dietary supplement has increased mainly because of its antioxidant activity, antilipidemic effect, and capacity to reduce body weight. All these biological activities have indicated the use and application of chitosan for the prevention and treatment of chronic diseases. Although there are few human clinical studies, there is enough evidence to indicate its beneficial value in human health.

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Chapter 4.3

Shark Cartilage

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INTRODUCTION

Almost 50 years ago a group from Harvard Medical School reported that tumor growth, as well as metastatic processes, were angiogenic-dependent (Folkman et al., 1971) and thereafter Brem and Folkman (1975) reported that cartilage appeared to inhibit tumor-induced capillary proliferation.

Cartilage is avascular tissue in which a lack of angiogenesis is observed due to the overexpression of angiogenesis inhibitors (Langer et al., 1976). This antiangiogenic characteristic has led researchers to isolate these inhibitors and suggest their use in applied studies aiming to create treatments for cancer and other malignant diseases in humans (Hammerness et al., 2002).

Several studies were performed to investigate the antiangiogenic effects of different sources of cartilage and shark cartilage (SC), and concluded that SC presented advantages such as its abundance (about 6% of its bodyweight) and higher antiangiogenic activity compared to other sources (Hamllet, 1999; Lee and Langer, 1983).

SC has been investigated for the treatment of degenerative diseases such as cancer, arthritis, osteoarthritis, systemic sclerosis, and neurovascular glaucoma in humans and animals (Habermann et al., 2009; Patra and Sandell, 2012; Venugopal, 2009).

The consumption of SC became popular in 1980, when its therapeutic biological activities became known (Sim et al., 2005). To date, the benefits of these activities remain under evaluation and controversial results have been identified. The efficacy of the treatments is unclear, and SC has not yet been approved by the food and drug administration (FDA) for use in the treatment of diseases, being available only as a dietary supplement (Ernst and Consortium, 2017).

This chapter focuses on the chemical constituents and biological activities of SC, in order to showcase the available knowledge concerning their properties.

CHEMICAL CONSTITUENTS

Cartilage is a connective tissue composed of a specialized cell type, the chondrocyte, as well as an extracellular matrix (ECM) which contains mostly water, and fibrillar and nonfibrillar constituents (Báez et al., 2001; Merly and Smith, 2013). An important by-product from shark processing is the cartilage that is harvested as a tough elastic tissue or endoskeleton from multiple species of shark (Escobar-Sanchez et al., 2010; Hamllet, 1999). Chemically, water is the major component of SC (66.84–70.29 g/100 g), while also showing significative protein content (14.01–14.85 g/100 g), ash (12.09–15.79 g/100 g), and trace amounts of fat (0.21–0.23 g/100 g) (Kittiphattanabawon et al., 2010).

In the ECM of SC, the principal fibrillar component is type II collagen (66%), followed by type I (33%), which represents 90%–95% of the total protein content (Báez et al., 2001; Jeevithan et al., 2015). Kittiphattanabawon et al. (2010) isolated and characterized collagen from the cartilage of brownbanded bamboo sharks (*Chiloscyllium punctatum*) and blacktip sharks (*Carcharhinus limbatus*), and observed that all collagen comprised of two types (I and II), containing glycine amino acid as the most abundant component, and high contents of alanine, proline, and hydroxyproline.

The nonfibrillar components were mainly comprised of complex carbohydrates (glycosaminoglycans) which are attached to a core protein, named proteoglycans (Khoshnoodi et al., 2006; Merly and Smith, 2013). Condroitin sulfate is an essential component of SC, which presents in the form of proteoglycans that are used to improve cartilage function, playing important roles in its elasticity and function of articular cartilage (Hardingham and Bayliss, 1990).

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BIOLOGICAL ACTIVITIES AND DRUG INTERACTIONS

It is well known that SC was initially claimed as having anticancer properties, and thereafter other bioactivities (Fig. 4.3.1) were also attributed to it such as having a preventive role in arthritis and other diseases (Lee and Langer, 1983; Patra and Sandell, 2012).

In this context, Hassan et al. (2005) isolated two polypeptides from SC with low molecular weight (14 and 15 kDa) that showed the ability to modulate immune response and inhibit angiogenesis. The authors demonstrated that this polypeptide fraction stimulated delayed-type hypersensitivity against sRBC, modulated CD4+, and CD8+ T cells infiltrated into the tumor, and inhibited endothelial cell growth.

Ratel et al. (2005) evidenced fibrinolytic activity in SC extract (SCE). They demonstrated nonplasmin fibrinolytic effects within SCE, which cleave all chains of fibrin and fibrinogen, and increase clot retraction. The authors suggested that SCE was a notable raw material for therapeutic treatment of vascular disorders.

Zheng et al. (2007) reported a new polypeptide angiogenesis inhibitor obtained from blue shark (*Prionace glauca*) cartilage with a molecular weight of approx. 15.5 kDa (PG155), and suggested that PG155 could be responsible for antiangiogenic activity.

Thereafter, studies using a mouse glioma model proposed a mechanism of angiogenesis inhibition by SC related to tissue plasminogen activator (t-PA) induction, which is a key enzyme in fibrinolysis, through intensification of plasminogen cleavage into plasmin (Simard et al., 2011). The authors demonstrated that the modulation process of plasminogen systems in experimental glioblastomas occurred in association with antitumor and antiangiogenic activities of SCE which were associated with stimulation of t-PA activity leading to the release of plasmin and endogenous angiostatin. Thus, this pathway can be suggested as a direct player in the therapeutic effects of SCE.

Although there is evidence of potent antiangiogenic and antitumoral activities of SC, in recent years its efficacy as a therapeutic compound in the treatment of angiogenesis-dependent diseases, especially in cancer, has been a controversial issue. This contrary viewpoint is derived mainly from two factors: first, ineffective results in cancer patients after clinical trials that have used SC, and second, the absence of pharmacokinetic studies correlating bioavailability with pharmacological effects using oral SC (González et al., 2001; Simard et al., 2013).

However, SC constituents were shown to have an effect on immune function as presented below.

Feyzi et al. (2003) showed the immune stimulatory effects of a fraction isolated from SC after the infiltration of CD4+ and CD8+ lymphocytes into a murine tumor model. However, although their results indicated a significant increase in the CD4+/CD8+ ratio in tumor infiltrating lymphocytes, changes were not found in the peripheral blood lymphocytes. Indeed, Kralovec et al. (2003) showed that SCE induced a cytokine response in human leukocytes, and this immune-stimulating activity was assigned to a complex mixture of molecules, particularly proteoglycans and collagen. Merly et al. (2007) revealed that collagen type II (alpha 1 protein) is the bioactive component of SC involved in the immune-stimulating activities, inducing preferentially a Th1 type inflammatory cytokine response in human leukocytes. More recently, Merly and Smith (2015) in a study with a commercial SC supplement, demonstrated strong evidence of the immunological

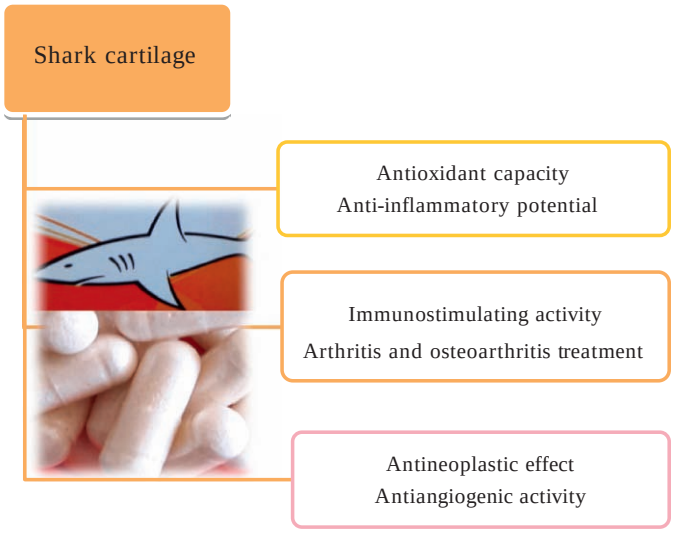

FIG. 4.3.1 Biological activities of shark cartilage.

activity of its components and suggested its consumption could be a potential health risk, particularly to those consumers with underlying inflammatory diseases such as irritable bowel syndrome and arthritis. Their results showed that it has bioactive component(s), which stimulate the production of proinflammatory cytokines, and activate specific signaling immune pathways.

The interest in SC has also turned to its beneficial effects on oxidative stress. Jeevithan et al. (2015) examined functional properties of type II collagens (CII) isolated from whale shark cartilage, and their results have shown an important antioxidant property related to the higher content of proline and hydrophobic amino acids (valine, leucine, and methionine), suggesting that the antioxidant capacity of CII is associated to certain specific amino acid residues and their sequences.

The biochemical and antioxidant properties of CII from *P. glauca* cartilage and its peptide (CII-P) were investigated (Bu et al., 2017). It was revealed that the difference between CII and CII-P related to their different amino acid composition. Moreover, this study tested the immunological tolerance of CII and CII-P, the function of apoptosis-regulating genes in human acute T-lymphocyte leukemia (6T-CEM) cells, and their mRNA expression levels. Their results showed that 10 μg/mL of CII and CII-P could efficiently and substantially induce apoptosis in 6T-CEM cells. Indeed, the authors suggested that the mechanisms of immunological tolerance and apoptotic regulatory mRNA expressions of human 6TCEM cells might be influenced by the percentage of glycosylation and molecular composition of CII. Bu et al. (2017) suggested that shark collagens could be used as a nutraceutical because of their higher antioxidant capacity, however, more studies with fish collagens may be required to study the effect of their immunological tolerance and biocompatibility for their commercial application.

Although several studies have been carried out with SC to date, only a small number investigated the side effects as well as drug interactions. However, it was reported that the consumption of SC supplements was associated with gastrointestinal side effects such as nausea, vomiting, and constipation (Ernst and Consortium, 2017). Indeed, SC can cause hepatotoxicity even though the mechanisms involved in drug-induced hepatotoxicity are not clear (Smith and Dillon, 2009). Cerda et al. (2013) reported elevation in aminotransferase levels in glucosamine patient consumers. It must be remembered that glucosamine is a constituent molecule of SC. Other cases were reported in the literature on hypersensitivity reactions such as pruritus and eosinophilia by glucosamine and chondroitin sulfate obtained from SC (Hammerness et al., 2002; Ossendza et al., 2007). Furthermore, it must be highlighted that SC has a high calcium level that could cause hypercalcemia as well as interactions with drugs such diuretics, tetracycline, doxycycline, and thyroxine absorption (Goodman and Trepanier, 2005; Ulbricht and Basch, 2005).

CONCLUSIONS

This chapter highlighted the rise in SC use as an anticancer drug, its efficacy in the treatment, and prevention of other diseases such as arthritis, neurovascular glaucoma, and vascular disorders. To date, several studies were performed with SC in order to investigate its chemical constituents, biological activities, and drug interaction. Although substances that exhibit antitumor activity have been identified that inhibit angiogenesis, their efficacy in curing cancer and other diseases is unclear due to controversial results.

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Part V

From Yeast and Fungi

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Chapter 5.1

Saccharomyces cerevisiae

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SOURCES AND AVAILABILITY OF *SACCHAROMYCES CEREVISIAE*

Single-celled fungi are termed as yeast. Hundreds of economically important varieties of yeast exist. Yeasts have a substantial role in the fermentation of numerous food products. *Saccharomyces cerevisiae* is frequently referred to as brewer's or baker's yeast, as some of its associated products are beer, wine, and baked goods. It has also been promoted by health food enthusiasts as a nutritional supplement in the form of brewer's yeast tablets or powder containing viable organisms. *S. cerevisiae* is widespread in nature and can be found on plants and fruit, and in the soil (Kwon-Chung and Bennett, 1992). *S. cerevisiae* can be found in abundance in the fermentations of artificially gathered fruit. It is scarcely distributed on fruit and oak tree bark (Goddard and Greig, 2015). It has been established that *S. cerevisiae* is not confined to a particular habitat, but is mobile. Yeast is able to grow in environments with changing concentrations of carbon and nitrogen (Goddard and Greig, 2015).

Some of the previous research suggests that *S. cerevisiae*, which is one of the types of yeast that widely grows in natural ecosystems, is mainly cultivated for use in the wine production industry. It grows abundantly in wine cultures, usually being found in the juice-filled fruit that falls to the ground that is used in wine production (Martini, 1993). Important habitats for this species are fruits, agriculture-related products, drinks, and vegetables. These are the important and most common habitats for *S. cerevisiae* (Wan Aida, 2012). According to some sources, *S. cerevisiae* can be found in the soil below white oak (*Quercus alba*), black oak (*Quercus velutina*), and chestnut oak (*Quercus prinus*) trees. Usually *S. cerevisiae* is domesticated, but sometimes synothropic natural populations are also obtained (Sniegowski et al., 2002).

HEALTH EFFECTS OF *SACCHAROMYCES CEREVISIAE*

Probiotic Effect

Living microorganisms showing health benefits to their hosts when administered in adequate quantities are defined as probiotics (WHO, 2001). According to the European Food Safety Authority (EFSA), probiotics show various technological properties in food fermentation, in which *S. cerevisiae* is the most common yeast used (Moslehi-Jenabian et al., 2010). Isolating *S. cerevisiae* from Litchi fruit has been used by pharmaceutical manufacturers as a dietary supplement (Moslehi-Jenabian et al., 2010).

Effects on Intestinal Bacterial Pathogens

One of the subspecies of *S. cerevisiae*, *S. cerevisiae* var. *boulardii*, binds to enteropathogens, thus preventing adherence and migration of bacteria in the intestinal epithelial cells. The cell wall of this subspecies has been shown to adhere to enterohaemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica* serovar Typhimurium (Gedek, 1999). *S. cerevisiae* var. *boulardii* produces a 54 kDa serine protease, which provides protection against *Clostridium difficile* infections by cleaving toxins A and B. In vivo studies have shown that due to the activity of this enzyme, oral administration of *S. cerevisiae* var. *boulardii* or its supernatant leads to the decrease in toxin A–induced intestinal secretions (Castagliuolo et al.,1996; Castagliuolo et al., 1999; Pothoulakis et al., 1993). A protein phosphatase extracted from *S. cerevisiae* has been shown to have a role in dephosphorylating endotoxins and inhibiting their cytotoxic effects such as the lipopolysaccharides

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of *E. coli* 055B5 (Buts et al., 2006). Research carried out on the ingestion of *S. cerevisiae* in rats showed a lower prevalence in bacterial translocations than would be seen with antibiotics (Moslehi-Jenabian et al., 2010).

Antiinflammatory Effects

The stimulation of peroxisome proliferative-activated receptor gamma (PPAR-γ) by *S. cerevisiae* causes the cells of the human colon to lower their response to proinflammatory cytokines. By interfering with the host cell–signaling pathways, it also reduces inflammation during bacterial infection (Lee et al., 2005). The inflammatory effect of inducible nitric oxide synthase (iNOS) is inhibited by *S. cerevisiae*. This has been investigated in rats with castor oil–induced diarrhea (Dijkstra et al., 1998; Girard et al., 2005).

Effects on Immune Response

As a response to pathogen infection, *S. cerevisiae* activated both the innate and adaptive immunity of the host. While analyzing the effect of the oral administration of *S. cerevisiae* in healthy volunteers, it was noticed that many cellular and humoral responses showed variations. *S. cerevisiae* affected the innate immune system, turning on the reticulo-endothelial and complement system (Caetano et al., 1986). The intravenous administration of *E. Coli* in germ-free mono-associated with *S. cerevisiae* demonstrated prominent clearance of pathogens from the bloodstream, in contrast to germ-free Swiss/NIH mice mono-associated with probiotic has higher TNF-alpha, IFN-gamma and IL-12 in serum, which revealed that yeast *S. boulardii* modulates immune response (Rodrigues et al., 2000).

Folate Production by *Saccharomyces cerevisiae*

High levels of folate per weight are produced by *S. cerevisiae*, which can also be used as a rich dietary source of folate (Patring et al., 2005).

Mycotoxin Absorption

Shetty and Jespersen (2006) explored that *S. cerevisiae* attaches to mycotoxins. *S cerevisiae* binds to ochratoxin A and zearalenone and inhibits their activity (Bejaoui et al., 2004; Yiannikouris et al., 2004).

Application of *Saccharomyces cerevisiae* in Clinical Trials

The use of *S. cerevisiae* in different clinical trials has shown promising results against different diarrheal diseases. Treatment with *S. cerevisiae* is effective in immune-compromised patients and those with serious or general intestinal diseases, of which most cases are affected by the presence of a central venous catheter (De Llanos et al., 2006; Hennequin et al., 2000; Lherm et al., 2002).

In the prevention of antibiotic-associated diarrhea (AAD), *S. cerevisiae* has been comprehensively evaluated and yeast has been potentially effective in adults and children in decreasing AAD (Kotowska et al., 2005; Surawicz et al., 2000). A reduction of 60% was seen in disease manifestation in patients with mild to moderate ulcerative colitis by using yeast in traditional therapies (Guslandi et al., 2003).

Ascorbic Acid Synthesis

Cultivating *S. cerevisiae* cells with sugars like D-glucose (D-Glc), D-galactose, or D-mannose (D-Man) produces products such as D-erythroascorbic acid (D-EAA) with the exception of L-ascorbic acid (L-AA) (Hancock et al., 2000). In *S. cerevisiae* metabolism, L-AA cannot be synthesized indigenously, however, by inducing the use of nonphysiological substrates like the enzymes of D-EAA biosynthesis, L-AA can be generated (Hancock et al., 2000).

Effect on Intestinal Mucosa

The trophic effects exerted by *S. cerevisiae* restore intestinal homeostasis (Moslehi-Jenabian et al., 2010). Many Enzymes like sucrase-isomalatase and lactase, which help in the breakdown of nutrients and their absorption, are functionally enhanced upon oral consumption of the yeast (Moslehi-Jenabian et al., 2010). *S. cerevisiae* is able to inhibit responses to food antigens (Moslehi-Jenabian et al., 2010).

Antioxidant Effect

Protective oxidative stress responses have been shown by *S. cerevisiae* against both hydrogen peroxide and superoxide anion generators, such as menadione (Jamieson, 1992; Collinson and Dawes, 1992; Flattery-O'brien et al., 1993; Jamieson et al., 1994; Stephen et al., 1995). In *S. cerevisiae*, one of the major antioxidant molecules is tripeptide γ-L-glutamyl-L-cystinylglycine (glutathione, GSH), which makes the cell a good buffer against reactive oxygen species and toxic electrophiles (Stephen and Jamieson, 1996).

S. cerevisiae contains two genes encoding for glutaredoxins, which are minor redox enzymes of about one hundred amino acid residues that utilize GSH as a cofactor. The product of these genes is required for protection against conditions of oxidative stress (Luikenhuis et al., 1998). The two genes encoding for thioredoxins in this organism are TRX1 and TRX2. Thioredoxins are required to maintain the redox balance of GSH (Luikenhuis et al., 1998).

Use in Biotechnology

In biotechnology, *S. cerevisiae* has been in widespread use as a cloning vector (single or multiple copies) (Murphy and Kavanagh, 1999). It has been approved in the food and pharmacological sectors (Schreuder et al., 1996). In the hepatitis B vaccine, yeast like *S. cerevisiae* is used to produced small hepatitis B surface proteins in transfected yeast cells which shows higher anti-hepatitis B titres after immunization (Shouval et al., 1994).

Effect on Immune System

Yeast supplements have been found to enhance cellular innate immune response in trout (Ortuño et al., 2002). An autopsy recorded on a HIV+ patient recorded the presence of this yeast in a number of organs. *S. cerevisiae* has been implicated in pneumonia which has led to the suggestion that yeast first colonized in the oropharynx of an immune-compromised host is then aspirated to the lungs, where it enters the bloodstream and hematogenously spreads to the spleen and intestines (Murphy and Kavanagh, 1999).

Role in Cancer Immunotherapy

S. cerevisiae can express one or more antigens in large quantities which are very stable after purification and propagation (Cohn et al., 2005). It has been shown as a potential component for cancer immunotherapy protocols (Wansley et al., 2008). For cancer immunotherapy, tumor-associated antigens (TAA) have been engineered and expressed from recombinant *S. cerevisiae* (Franzusoff et al., 2005; Stubbs et al., 2001).

Role in Diarrhea

S. cerevisiae has been administered for the treatment of severe diarrhea (Murphy and Kavanagh, 1999) and found to be effective in treating acute infectious diarrhea in children (Szajewska et al., 2007).

Disorders Caused by *Saccharomyces cerevisiae*

Among the disorders caused by *S. cerevisiae*, fungemia is most severe and verified disease (Muñoz et al., 2005). The only high threat factor for *S. cerevisiae* infection is to use probiotic containing *S. boulardii*. Its usage is high in Europe for the treatment and prevention of *Clostridium difficile*-associated diarrhoea. Use of *S. cerevisiae* probiotics should be wisely evaluated, reassessed with special focus on immunosuppressed or critically ill patients (Muñoz et al., 2005).

S. cerevisiae has been known on occasion to be the cause of fungal vaginitis, an infection of the female genital tract (Nyirjesy et al., 1995). Based upon the presence or absence of a 3kb fragment upon agarose gel electrophoresis, the isolated fragments of *S. cerevisiae* found to cause fungal vaginitis indicated that it could be divided into two subtypes (Murphy and Kavanagh, 1999).

INTERACTION OF *SACCHAROMYCES CEREVISIAE* WITH FOOD, DRUGS, AND SUPPLEMENTS

The interaction of food, and drug with body own cytokines and enzymes has good and adverse effects which increase in the physiological and biological activities of living organisms (Table 5.1.1). The interaction of *S. cerevisiae* also has various beneficial physiological activities and effects.

TABLE 5.1.1 Use of *Saccharomyces cerevisiae* Against Different Diseases

Yeast host	Use	Product
S. cerevisiae	Diabetes mellitus	Short-acting recombinant insulin
S. cerevisiae	Bone marrow transplantation, regulation of hematopoiesis, and acute myologenous leukemia	Granulocyte macrophage colony stimulating factor
S. cerevisiae	Anticoagulant	Hirudin/lepuridin
S. cerevisiae	Hyperuricemia	Urate oxidase
S. cerevisiae	Diabetic ulcer	Platelet-derived growth factor
S. cerevisiae	Excipient, shock, cirrhosis, and other uses	Human serum albumin
S. cerevisiae	Renal disease	Erthyropoietin
S. cerevisiae	Hypoglycemia	Glucagon
S. cerevisiae	Dwarfism and tissue repair	Human growth hormone
S. cerevisiae	Anemia	Platelet-derived growth factor
S. cerevisiae	Diabetes	Insulin
S. cerevisiae	Hepatitis A	Hepatitis A vaccine
S. cerevisiae	Hepatitis B	Hepatitis B vaccine
S. cerevisiae	Hepatitis B	Diphtheria, tetanus, pertussis
S. cerevisiae	Combination vaccines and polio	*Haemophilus influenzae* type B

Saccharomyces cerevisiae and Milk Production in Animals

Previous studies have shown that yeast cultures (YCs) of *S. cerevisiae* can increase the productivity of milk because dry matter (DM) intake is increased in dairy cows by the inclusion of YCs in their diet. Giving YCs to cows means that their ruminal pH increases due to a decrease in L-lactate concentration in the rumen. Incubating YCs for 12 h in the rumen increases the rate of DM breakdown of hay, which in turn increases the intake of forage (DM), and increases the total yield of the milk from the dairy cows (Williams et al., 1991). Similar results were obtained by treating dairy goats with live *S. cerevisiae*, which were newly lactating, resulting in an increase in the milk production in relation to an increase in DM intake (Stella et al., 2007). Lactating Zaraibi goats also increased their milk production when consuming a diet supplemented with *S. cerevisiae* (El-Ghani, 2004).

Interaction Between β-glucan From *Saccharomyces cerevisiae* and Inflammatory Cytokines

β-glucan, which is a cell wall component of *S. cerevisiae*, can be used for enhancing and regulating the humoral and cell-mediated immunity in pigs for better growth rate. This β-glucan reduces the inflammatory effects of cytokines by elevating the antiinflammatory cytokines after exposing the pigs to an immunological challenge. Studies also show that β-glucan increases the ability to regulate the immune system in animals exposed to an immunological challenge, stress, and inflammation (Li et al., 2005). β-glucan is responsible for reducing serum cholesterol and decreasing the absorption of triglycerides.

Interaction Between *Saccharomyces cerevisiae* and Meat

Use of yeast supplements in the diet of broiler chicks which were used as a source of meat has been shown to increase both meat production and quality. Incorporating of either whole yeast, *S. cerevisiae* extracts and/or *S. cerevisiae* cell walls in the broiler chick's diet increased the quality and production of meat. The quantity of 2-thiobarbituric acid–reactive substance (TBARS) was seen to be reduced in the breast meat of the chicks feed with whole yeast, *S. cerevisiae* extracts and *S. cerevisiae* cell walls, after 10 days of incubation. By using whole yeast and *S. cerevisiae* extracts, shear forces were

also reduced in the breast and leg meat of broiler chicks after cooking (Zhang et al., 2005). Yeast supplements improved growth performance and meat tenderness. It was found that *S. cerevisiae* also suppresses aflatoxicosis in broiler chicks, increases some enzymes like ceretine phosphokinase, lactate dehydrogenase, and alanine transaminase, and helps maintain the normal function of some internal organs (Stanley et al., 1993).

Interaction Between *Saccharomyces cerevisiae* and Carbohydrates

The growth performance in the Nile tilapia fish (*Oreochromis niloticus*) can be increased by formulating its diet with 40% protein with the addition of 0.1% *S. cerevisiae*. The supplementation of its diet with *S. cerevisiae* makes the utilization of nutrients more effective, resulting in increased growth and energy levels (Lara-Flores et al., 2003). *S. cerevisiae* was also used as a growth promoter in hybrid striped bass *(Morone chrysops)* by supplementing its diet with *S. cerevisiae*. This resulted in an increase in body weight and also maximized feeding efficiency compared to when it consumed its normal diet (Li and Gatlin, 2003).

Interaction Between *Saccharomyces cerevisiae* and Ca^{2+}

Benzylisoquinolinealkaloids (BIAs) are metabolites of plants which are derived from tyrosine and have a large structural diversity. Reticuline is its key intermediate which acts as a Ca^{2+} transport inhibitor, resulting in antispasmodic effects. Reticuline also acts as a depressant of the central nervous system (CNS). *S. cerevisiae* can be used to produce a family of products from reticuline and BIAs by engineering some of the synthetic pathways into *S. cerevisiae*—they have a large range of applications and uses in the field of pharmacology and medicine (Hawkins and Smolke, 2008).

Interaction Between *Saccharomyces cerevisiae* and Terpene

The purified extract (1-3 β-glucan) of *S. cerevisiae* can be used to promote the healing process of venous ulcers and to increase the production of plasmocytes and fibroblasts in humans (Medeiros et al., 2012). Sesquiterpene lactones, having sesquiterpenoids and lactone rings, play an important and beneficial role in human health and plant life from a pharmacological point of view (Chadwick et al., 2013). The previous study revealed that the *S. cerevisiae* could be effectively induced to produce sesquiterpene (Asadollahi et al., 2008).

Interaction Between *Saccharomyces cerevisiae* and Naringenin

Naringenin is one of the flavonoids which is produced from phenylalanine and has many beneficial effects on the human body such as aiding metabolism, acting as an antioxidant, antitumoral, and antiinflammatory, as well as preventing cardiovascular diseases (Yao et al., 2004). It also inhibits the osteoclastogenesis and resorption of osteoclastic bone in the human body (La et al., 2009). *S. cerevisiae* can be engineered to produce naringenin from glucose by introducing the biosynthetic genes of naringenin into yeast selected from *Arabidopsis thaliana* by comparative gene expression profiling (Koopman et al., 2012).

Interaction Between *Saccharomyces cerevisiae* and Plasminogen Activator Inhibitor

S. cerevisiae can be used as an effective source for the mass production of plasminogen activator inhibitor (PAI) type-2 (Steven et al., 1991). PAI type-2 or SerpinB2 which is mostly known as extracellular urokinase PAI, also have roles in the immune system where they regulate the adaptive immune responses (Schroder et al., 2011).

Interaction Between *Saccharomyces cerevisiae* and Artemisinin

S. cerevisiae can be used to produce toxin-free proteins and nonhost DNA virus-like particles which can act as useful tools for diagnosis, gene delivery systems, and the production of new antiviral vaccines (Sasnauskas et al., 2003). Artemisinin, which helps in the treatment of malaria by inhibiting the sarco/endoplasmic reticulum Ca^{2+}–ATPase action of *Plasmodium falciparum* (Eckstein-Ludwig et al., 2003), and the precursor of artemisinin which is artemisinic acid, can be produced in significant volumes by using engineered *S. cerevisiae* (Ro et al., 2006).

Saccharomyces cerevisiae and Engineering

The application of recombinant DNA technology to the restructuring of metabolic networks can enhance the production of metabolites and essential protein products by changing the pathway distribution and rates. The incorporation of the heterologous proteins prolongs the already exiting pathways in order to get a new product and hence changes posttranslational protein processing. Though, it is important that few experimental and mathematical tools are needed for the rational metabolic engineering (Bailey, 1991).

CONCLUSIONS

S. cerevisiae has long been utilized as a functional food and supplement. A number of biologically active secondary metabolites including naringenin, reticuline, artemisinin, PAI, and other pigments have been isolated from *S. cerevisiae*. *S. cerevisiae* produces pigments and other bioactive secondary metabolites that have been shown to exhibit neuroprotective, antioxidant, antidiabetic, antiinflammatory, antimalarial, and antitumoral properties. *S. cerevisiae* has potential safety concerns due to its levels of aflatoxin which has nephrotoxic, hepatoxic, and cytotoxic effects. Therefore, *S. cerevisiae* is a potential bioactive agent and food supplement that requires further clinical studies and development.

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Chapter 5.2

Red Yeast Rice (*Monascus purpureus*)

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SOURCE OF RYR

Fermented RYR is consumed throughout Asia as traditional food spice, food colorant and medicinal remedy. It has been used since 800 AD as food and medicinal agent and known as "red rice koji,""ang-kak," "akakoji," "red fermented rice," "red kojic rice," "red koji rice," "anka," or "RYR" (Hong et al., 2008). Basically several fungi species of *Monascus* (*Eurotiaceae*) have been used to make fermented food, red soybean cheese, red wine, medicines and preserving meat (Akihisa et al., 2005). This particular species is one among the several of Ascomycets and belongs to the family of Monascacea. There are four species under the genus *Monascus*, namely: *M. froridanus*, *M. purpureus*, *M. pilosus* and *M. ruberand*, which are frequent strains isolated from traditional oriental foods (Panda et al., 2010). In the western society, the fungus *Monascus* was recognized after Van Teighem, (1884) noted the utilization by local population in java to produce red powder (RYR). Currently there are more than thirty *Monascus* strains are deposited with the American Type Culture Collection (Ma et al., 2000). *Monascus* spp. can produce a mixture of bioactive metabolites such as polyketidemonacolins, dimerumic acid, isoflavones, γ-aminobutric acid (GABA) and pigments (Su et al., 2005). The pigments are a group of *Monascus* metabolites called azaphlones derived from polyketidechromophores and β-keto acids via esterification. Monascorubrin, monascorubramine, monascine, rubropunctatin, rubropunctamine and ankaflavin are the six identified structures applicable as food additives and pharmaceutical products (Silveira et al., 2008). The *M. purpureus* pigments are widely used in various Asian and European countries; however their usage as food additives is authorized only in Japan. In comparison to conventional food additives like E-120 (cochineal), E-249 (nitrite salts) and E-252 (potassium nitrate), the *Monascus* pigments are suitable for enhancing meat color (Sabater-Vilar et al., 1999). The RYR monacolin K is the most bioactive among the naturally occurring monacolin form of mevinolin (Abrams et al., 2011). It is the same substance isolated from *Aspergillus terreus* (*A. terreus)* and approved by FDA as lovastatin. Other related secondary metabolites such as monacolins-J,- L, -M, and -X, dihydromonacolin-L, and dihydromevinolin have also been isolated from *Monascus* spp. grown in liquid culture (Childress et al., 2013). *Monascus* secondary metabolites are liable to vary easily with the different growth conditions (Shi and Pan, 2011). Traditionally, making of RYR extract starts with preparing "seed Koji." Non-glutinous, long shaped rice is the best raw material. Initially, it is washed and soaked in water for 24 h or more and drained thoroughly. It is then cooked and while cooling the rice is mixed with diluted vinegar or alum solution (since the *Monascus* spp. is acidophilic). On the next stage, it is inoculated with "seed Koji" and mixed thoroughly and then inoculated at an ambient temperature between 32-42_^{o}C. In the next few days, it will be stirred and shaken to redistribute the moisture and water will be added when necessary to keep the mixture moist. In a period of about two weeks, the color of rice would be deep purple (Fig. 5.2.1) (Wang and Lin, 2007).

AVAILABILITY OF RYR/*MONASCUS PURPUREUS*

In USA, the demand of RYR has grown dramatically to nearly 80% between 2005 and 2008, with a reported sale of 20 million in 2008 (Childress et al., 2013). The FDA has not officially registered the RYR manufacturers and distributors thus it had no data to report on these companies regarding their number, compliance with CGMP regulations or product testing regulations. However, according to NMCD, currently there are more than 145 products containing RYR available in market (Childress et al., 2013). Despite the FDA's warning, the RYR products are available in market as dietary supplements in supermarkets, vitamin stores, health food stores, pharmacies and in the internet. In addition, the product labels of the existing brands do not contain any information about the levels of lovastatin or other monacolins (Gordon et al., 2010).

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00067-9

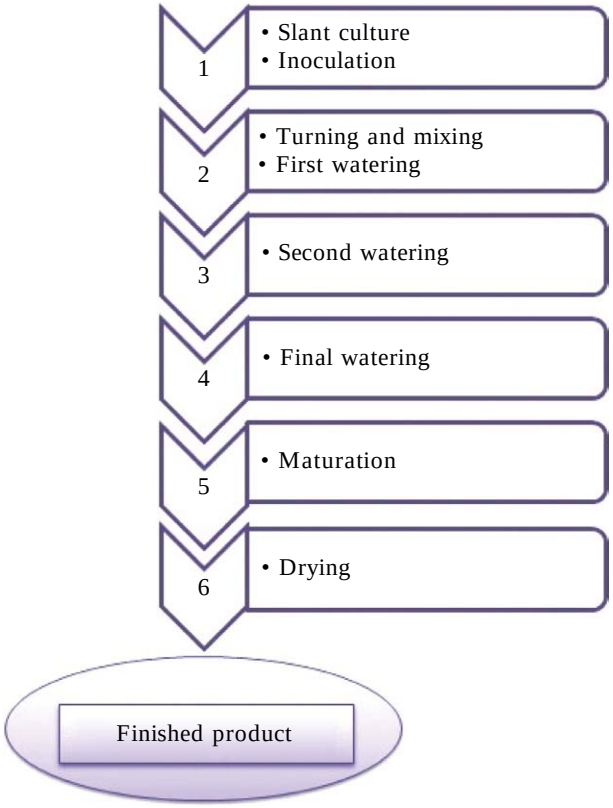

FIG. 5.2.1 The production scheme of RYR.

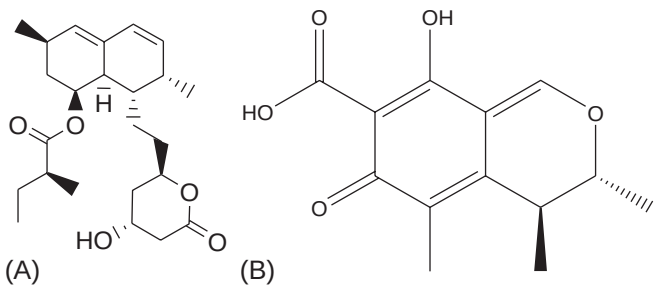

FIG. 5.2.2 The structure of bioactive metabolites of RYR extract a-Monacolin K and b- Citrinin

Although monacolin K production (Fig. 5.2.2a) is the marker for the significance of *Monascus* product, RYR manufacturers avoid disclosing the level of lovastatin and other monacolinsnot to be considered an unapproved drug by the FDA (Lee et al., 2007a; Gordon et al., 2010). Thus, there is no standardization of content levels across manufacturers. Studies indicated that the citrinin content of commercialized RYR product ranges from 0.28 to 2458.80 mg/kg, which is beyond the maximum permissible limit of citrinin (200 ng/g) in Japan, where as this limit is under consideration in China and Europe (Pattanagul et al., 2008).

RELATION OF RYR/*MONASCUS PURPUREUS* WITH HEALTH

The pharmacological activities of RYR were described by traditional healers of the Ming Dynasty (1368-1644). RYR has been used as food, preservative, food colorant in fish and meat and for medicinal purposes (Wei et al., 2003). The pigments that *Monascus* produces have been used to improve test in food. In addition, the bioactive secondary metabolites of *Monascus* were utilized as antifatigue, antidiabetic, antioxidant, antihypertensive, anti-inflammatory, neuroprotective, antihyperlipidemic, antitumor, antibiosis, etc. *Monascus* has been used widely without any adverse health effects in morethan twenty food items. However, currently scientists have indicated the unacceptable level of citrinin (Fig. 5.2.2b) in *Monascus*, which is a mycotoxin to that have detrimental effect on human liver and kidney

(Bogsrud et al., 2010; Zhang et al., 2017; Hsu et al., 2012). Furthermore, a plenty of *Monascus* metabolites are yet to be characterized chemically, and thus the pharmacological and toxicological profiles of most of its metabolites is fragmentary (Wild et al., 2002).

Nutritional supplements and functional foods are becoming the mainstay alternatives for people who are reluctant to take drugs (Verhoeven et al., 2015). In the Chinese official monographs, monacolin K (Fig. 5.2.2a) also known as mevinolin or lovastatin is the main ingredient and considered an indicator in quality control (Li et al., 2005). RYR containing lovastatin is reported to have effectively reduced the low-density lipoprotein (LDL) up to 20-30% and the triglyceride (TG) level by 10-20%. Typically, these products are considered helpful for children and patients in whom standard pharmacotherapy has failed (Hunter and Hegele, 2017). Various clinical trials demonstrated the effectiveness of RYR in hyperlipidemic patients intolerant to conventional statins (Abrams et al., 2011). A meta-analysis of randomized controlled trials on RYR and life style modification indicated the change in lipid profile of patients were more significant than placebo but similar to the effect of statins such as simvastatin, lovastatin, or pravastatin (Lin, 2010). RYR can decrease the level of LDL and increase the level of high-density lipoprotein (HDL) without affecting creatinine phosphokinase (CPK) level (Lin, 2010; Li et al., 2005). Monacolines are responsible for the hypocholesterolemic effect in RYR and acts via inhibiting HMG-CoA reductase and thus the catalytic conversion of HMG-CoA to mevalonate, the precursor of cholesterol (Setnikar et al., 2005). The enzyme HMG-CoA reductase plays a major role in the biosynthesis of ubiquinone and cholesterol; hence statin-containing health foods like RYR that functions through this pathway may also interfere with the production of ubiquinone, especially CoQ10, and cause myocardial dysfunction (Yang et al., 2005).

Atherosclerosis is the main cause of heart attack and stroke and it is characterized by elevation of accumulated cholesterol, oxysterols and lipid peroxides. The statin dihydromonacolin-MV, which is characterized from *M. purpureus* has antiatherosclerotic activity as a result of its radicals scavenging activity and ability to inhibit lipid peroxidation (Dhale et al., 2007). The treatment with food supplement such as RYR extract known for their lipid lowering effects reduce the serum total cholesterol (TC) and LDL, and increase serum HDL, hence improve the atheroprotection index. The atheroprotection index is the ratio of non- HDL cholesterol to HDL-cholesterol (HDL/TCx100), is considered another important risk factor for atherosclerosis (Wei et al., 2003). Various studies have indicated that RYR extract improves the ratio significantly, even at lower doses. Beside the statin drug, other secondary metabolites monacolins in the RYR extract are responsible for its antiatherosclerotic activity. Furthermore, experimental results showed that RYR extract effectively down regulates homocysteine induced endothelial adhesion property, which is related with dysfunction and risk of cardiovascular disease (CVD), reduces intracellular reactive oxygen species (ROS) formation and nuclear factor-κB (NF-κB) activation. This clearly shows dietary supplements with RYR extract will treat atherosclerosis effectively (Kalaivani et al., 2010).

Antihypertensive properties of RYR extract was demonstrated in*in vivo* to spontaneously hypertensive rats (Shi and Pan, 2011). Twenty four strains of the genus *Monascus* were tested for the angiotensin-converting enzyme (ACE) inhibitory activity of RYR extract. The most potent strain was *M. purpureus* IFO 4489 with IC_{50} value of 0.71 mg/mL. From this strain of *M.purpureus* mainly four peptides Ile-Tyr, Val-Val-Tyr, Val-Phe, and Val-Trp were isolated with IC_{50} values of 4.0, 22.0, 49.7 and 3.1 mmol/L respectively. These results from RYR extract by *M. purpureus* suggest that it could help alleviate hypertension (Xue-Mei et al., 2011). RYR contains GABA, which is inhibitory neurotransmitter in the sympathetic nervous system has been reported to play an important role in treating hypertension in experimental animals and humans after oral administration. Besides the yellow pigment monascin has anti-inflammatory activity. Thus, the anti-inflammatory action of monascin is proved to prevent the occurrence of blood pressure via monitoring the renin angiotensin system (RAS) (Wu et al., 2009). Another animal study done on fructose-induced hypertension demonstrated that intragastric administration of *M.purpereus* containing GABA (1mg/kg/day) reversed the elevated blood pressure into a normal level. In the similar experiment, administration of equal dose of pure GABA, however failed to show similar activity in fructose-induced hypertension (Wang and Lin, 2007).

A great number of evidence support the potential effect of statins, including lovastatin might inhibit colon-cancer cell growth and their activity in reducing the incidence of the disease. *In vitro* test results of RYR extract demonstrated monacolins and the red yeast pigments are involved in the stimulation of apoptosis and inhibition of proliferation in human colon cancer (Hong et al., 2008). In addition, other pigments such as monascin (Fig. 5.2.3a), ankaflavin (Fig. 5.2.3b), and rubropunctatin have been stated for their observed anti-tumor activities (Chang et al., 2016). Moreover, ankaflavin demonstrated tumoricidal effects on human lung carcinoma A549 and hepatoma cell Hep G2 (Su et al., 2005). On the other hand, the bioactive metabolite in RYR extract, monacolin K act synergistically with ankaflavin to increase the tumor cell apoptosis rate (Shi and Pan, 2011). Another study has revealed that monacolin K down regulates metastasis and tumor progression in Lewis lung carcinoma cells (Chang et al., 2016). Monacolin K acts through inhibiting tumor invasion induced by vascular endothelial growth factor (VEGF) and the formation of new vascular tissue, as a result inhibits tumor metastasis (Shi and Pan, 2011) (Xue-Mei et al., 2011). Therefore, chemotherapeutic approach using RYR extracts with or without lovastatin could be another option to inhibit cell proliferation and stimulate apoptosis (Xue-Mei et al., 2011).

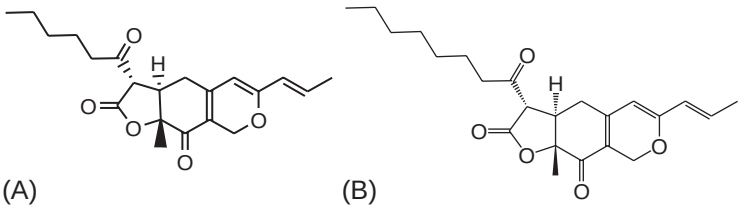

FIG. 5.2.3 Structures of bioactive metabolites of RYR extract a-Monascin and b-ankaflavin.

Other than their cholesterol lowering, anticancer, antiatherosclerotic and other biological activities, RYR extracts demonstrated antihyperglycemic activity in streptozotocine induced diabetes in rats. Diabetes is a group of metabolic disorders which results in high plasma glucose level (Issa and Hussen Bule, 2015). Oral administration of RYR in the range 50-350 mg/kg/day to a diabetic rat (streprtozotocine induced) for two weeks reduced the plasma glucose dramatically. This and other animal studies in streptozotocin-induced diabetic model showed reversed mRNA levels of phosphoenolpyruvatec arboxykinase (PEPCK) in liver by treating with RYR extract three times daily for two weeks (Kalaivani et al., 2010). RYR has the ability to delay the development of insulin resistance, enhance insulin sensitivity and promote insulin secretion. Stimulation of muscarinic M3 receptors of the pancreatic β-cells and boosting the release of acetylcholine (ACh) release by cholinergic nerve terminals are the mechanisms through which RYR extracts act to decrease the plasma glucose in diabetes (Xue-Mei et al., 2011). Furthermore, animal study revealed that RYR products fermented with *M. purpereus* and *M. pilosus* orally administered over 1.5 h to fasting rats decreased the blood glucose level in a dose-dependent fashion. Besides a parallel increase in plasma insulin and C-peptide level was observed (Wang and Lin, 2007). Moreover, oral administration of RYR could lower the serum glucose level by decreasing the hepatic gluconeogenesis in diabetic rats lacking insulin (Kalaivani et al., 2010). The anti-inflammatory properties of RYR are observed against diabetes associated inflammatory injuries by inhibiting inflammatory cytokines production and enhancing IL-2 expression. Thus, the RYR extracts might reduce the risk of diabetic associated complications (Shi et al., 2011).

In a state of obesity accumulation of excess fat in the body causes adversities on health, increased health problems and leads to reduced life expectancy. Several studies have showed that statins would regulate adipocyte differentiation. In addition, aqueous extracts of RYR suppress adipogenesis in 3T3-L1 preadipocytes via down regulation of adipogenic transcription factor along with other genes expression (Chen et al., 2008). The impacts of RYR on adipocyte have been studied by using differentiation of 3T3-L1 cells. These extracts decreased significantly glycerol-3-phosphate dehydrogenase (GPDH) activity and lipid accumulation in a dose-dependentmanner. In addition, it significantly reduced fatty acid binding protein αP2 gene expression along with leptin in adipocyte. This shows the RYR extract acts mainly via down regulating the expression of adipogenic transcription factors and related genes (Xue-Mei et al., 2011). Furthermore, *in vitro* experimental studies on aqueous and ethanolic extracts of RYR demonstrated inhibitory activity on preadipocyte proliferation. The *Monascus* secondary metabolites, monascin and ankaflavin are biologically active towards inhibiting the proliferation of 3T3-L1 cells and increase basal lipolysis of mature adipocytes (Shi and Pan, 2011). Monakolin K also reduced the 3T3-L1 preadipocyte proliferation and the appetite in HF rats. These effects of RYR extract are pleiotropic and would result from a number of bioactive metabolites (Chen et al., 2008).

Alzheimer's disease (AD) is caused by extracellular deposit of fibrils β-amyloid (Aβ) in the brain, inflammatory reaction mediated by fulminant microglial and neuronal death (Xue-Mei et al., 2011). Aβ is a product of proteolytic cleavage of the integral membrane amyloid precursor protein (APP) by β-and γ–secretase. Cholesterol is known to enhance the activity of β–secretase and increases the protein expression (Lee et al., 2010).Free radicals and *in vivo* oxidation would cause the formation of Aβ-fibrils and on the other hand the Aβ-fibril induces inflammatory responses and oxidative stress and ends in the formation of more Aβ-fibrils (Lee et al., 2008). An animal model experiment revealed that by inducing impairment of memory via intracerebral injection of Aβ40 for 28 days and gavaging the rats RYR of doses from 151-755mg/kg/day effectively improves memory and learning ability (Shi and Pan, 2011). Moreover, monacolin K can repress Aβ40 neurotoxicity and other metabolites of RYR have showed antioxidant property against oxidation due to Aβ (Xue-Mei et al., 2011). Inaddition, *M. purpereus* fermented rice ethanol extract could down-regulate the production of NO, PEG2 and proinflammatory cytokines in animal model (Hsu et al., 2013). Statins have been reported to prevent the formation of Aβ and hence ameliorate Aβ induced memory deficit in the progression of AD but their protection against Aβ induced neurotoxicity has scarcely studied (Lee et al., 2007b). In contrast, the RYR extract amelioration effect on memory deficit and Aβ-40 accumulation is due to the suppression of brain cholesterol and lipid formation which results in down regulation of β-secretase activity and ApoE expression (Lee et al., 2010). Furthermore, administration of RYR extract repressed the oxidative stress, acetylcholinesterase (AChE) activity and inflammatory response in cortex and hippocampus (Lee et al., 2007b).

Osteoporosis is a disorder of bones in which the risk of bone fracture is increased. *In vivo* animal model study on rat has demonstrated the anabolic effects of RYR extract. The bone mineral density of rats treated with RYR extracts at a dose of 3.6mg/kg/day were significantly increased (Xue-Mei et al., 2011). Since there appears has no detectable increase in total protein, the effect of RYR on new bone formation is a general biosynthetic activity in the cell (non-specific bone formation). RYR stimulates new bone formation in fractured bones in*in vivo*model and increases significantly bone formation in *in vitro*trial (Wong and Rabie, 2008). Currently, the RYR extract is reported to induce increases in osteogenic activity, cell viability and mitochondrial activity. Mechanistically bone cell formation effect of RYR extract is due to inhibition of HMG-CoA reductase in the mevalonate pathway (Kalaivani et al., 2010). RYR is a natural food supplement with potential pharmacologic activity in treating bone defects and probably also osteoporosis.

POSSIBLE INTERACTIONS OF RYR/*MONASCUS PURPUREUS* BASED FOOD SUPPLEMENTS WITH OTHER SUPPLEMENTS/DRUGS/FOOD

Although RYR extract is a natural product viewed as dietary supplement, it can't be devoid of adverse effects. Adverse drug reactions of statins including acute hepatitis and myopathies can possibly occur in patients taking RYR extracts. It should also be remembered that just like statins, pregnant or lactating women should avoid using RYR (Lin, 2010). The Cytochrome P450 (CYP450) enzymes metabolize the statins, and taking the RYR products with CYP450 enzyme inhibitors such as HIV protease inhibitors, ketoconazole, erythromycin, can lead to worsening of statin like adverse effects (Nguyen et al., 2017). On the other hand, RYR extract administration at high dose depletes the concentration of CoQ10 in the liver and heart. Muscle ache, fatigue, pain and other related adverse effects will occur when CoQ10 depletion occurs (Yang et al., 2005). Grapefruit reported to enhance the statins like effects of RYR and its concentration in blood, increases the risk of side effects and liver damage (Kalaivani et al., 2010). RYR products should not be taken with lovastatin and other drugs that have risk of muscle adverse effects, such as the antidepressant nefazodone and other antihyperlipidemicagents (Commissioner, N.D. 2007). RYR products increase the risk of bleeding thus anticoagulants, like warfarin, clopidogrel and daily aspirin should be avoided. Furthermore, combining RYR products with prescription medications including azathioprine, cimetidine, clarithromycin, cyclosporine, diclofenac, erythromycin, gemfibrozil, itraconazole, ketoconazole, methotrexate, rosiglitazone and valproicacid may pose some potential risk of liver damage (http://umm.edu/health/medical/altmed/supplement/red-yeast-rice (accessed 3.15.17). In regard to health risk related to RYR extracts citrinin is drawing much attention due to its mutagenic effects in animal models, genotoxicity to human lymphocytes and ability to cause kidney failure. The mycotoxinlike citrinin is mainly found in poorly produced RYR products (Nguyen et al., 2017). Citrinin shows remarkably variable cytotoxicity among cell cultures. Applied to hepatoma cell culture at a dose up to 25 μmol/L citrinin was cytostatic, while it was cytotoxic from 50 μmol/L to 200 μmol/L (Flajs and Peraica, 2010). Generation of oxidative stress, interference with electron transport system and alteration of Ca^{2+} homeostasis are the possible toxicological mechanisms of citrinin (Liu et al., 2005). Nonetheless, the clinical studies that showed the effectiveness of RYR in dyslipidemia also demonstrated that it is a relatively safe product (Nguyen et al., 2017).

CONCLUSION

The RYR extract has long been utilized as functional food and supplement. A number of biologically active secondary metabolites including monascin, monakolins, ankaflavin and other pigments have been isolated from the RYR extract. Besides, *M. purpureus* produces pigments and other bioactive secondary metabolites providing the RYR extract activities such as antihypertensive, antihyperlipidemic, antifatigue, neuroprotective, antioxidant, antidiabetic, anti-inflammatory, antibiosis, and anti-tumor. The safety of RYR is compromised with the level of the mycotoxin;citrininwhich has nephrotoxic, hepatoxic and cytotoxic effects. In addition since it is metabolized by the CYP450 enzymes it has various drug interactions with CYP450 inhibitors such as HIV protease inhibitors, ketoconazole and erythromycin leading to statin like side effects. Therefore,the RYR extract is a potential bioactive agent and food supplement that requires further clinical studies and development.

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AUTHOR CONTRIBUTIONS

All authors have directly participated in the planning or drafting of the manuscript and read and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 5.3

Mushrooms reishi (*Ganoderma lucidum*), shiitake (*Lentinela edodes*), maitake (*Grifola frondosa*)

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INTRODUCTION

Life longevity of the human species had significantly increased since the beginning of the twentieth century mainly because of an improvement in medicine, diet and hygiene conditions. However, this increased lifespan is also associated with increases in the prevalence of many diseases such as cancer and neurodegenerative disorders (Abdullah et al., 2015; Ostan et al., 2016). Fungus has been related to medicine and food since ancient times mostly in Chinese culture. The mushrooms are macrofungi belonging to the Fifth Kingdom and taxonomically are classified in two different groups: most of the known genera are Basidiomycetes, and the other group is Ascomycetes. Nowadays, there is an increasing consumption of mushrooms, which can be attributed not only to their pleasant taste and flavor but also to the current search for natural products containing healthy bioactive compounds. Regardless the fact that some mushrooms have been described to have a high nutritional value, little is known about its medical benefits since limited or no clinical trials have been performed. However, these species have been related to pharmaceutical properties because of their chemical and bioactivity diversity; some works have pointed out antioxidant (Thyagarajan et al., 2006), anti-inflammatory (Dudhgaonkar et al., 2009), antitumoral (Hetland et al., 2011; Hsieh and Wu, 2011), or antimicrobial activities, among others, so that indicating a possible health benefit due to their consumption. These actions have related to the constituents of mushrooms, which mainly comprise terpenes, polysaccharides, and proteins. These components have been described to participate and modulate signaling pathways (Chen et al., 2016; Lin et al., 2003; Sliva, 2004).

There are more than 140000 species of mushrooms that have been predicted but only 22000 are known and about a 5% has been investigated. Within this small percentage, Reishi (*Ganoderma lucidum*), Shiitake (*Lentinela edodes*), Maitake (*Grifola frondosa*) are three of the most consumed and studied mushrooms. Although these three species have been widely used in ancient medicine for many years, up to date many researchers have conducted scientific studies last years to determine their potential clinical uses.

GENERAL CHARACTERISTICS OF MUSHROOMS

Ganoderma lucidum (Curtis) P. Karst., commonly called reishi (Japan) and Ling Zhi (China) (Stamets and Yao, 2002) is a fungus species that belongs to the Ganodermataceae family (Polyporales). It occurs throughout the world in temperate and subtropical locations including North and South America, Europe, and Asia. This species can grow solitary or sometimes in small groups, and it is typically located at the base of living tree species or rarely on the roots of a wide range of deciduous species such as *Acer*, *Quercus*, *Fraxinus*, *Celtis*, *Salix* or *Ulmus* (Gerhardt et al., 2000). It is considered a parasitic (first grows on living hosts) and saprophytic (then grows on dead hosts) species. *G. lucidum* is commercialized under different nutraceutical brands, under the form of dietary supplements, health drinks and powders, as well as specific functional agents (Jong and Birmingham, 1992; Ying et al., 1989). For more than 2000 years, *Ganoderma* species is highly regarded as

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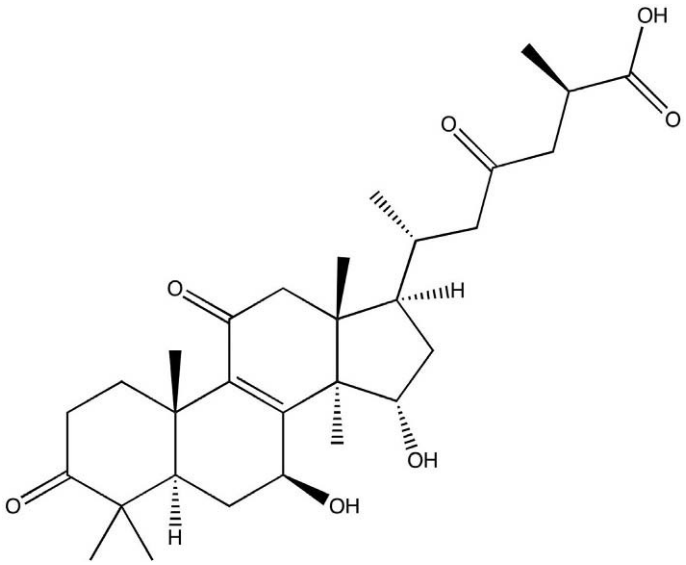

FIG. 5.3.1 Chemical structure of the polysaccharides ganoderic acid A found in *Ganoderma lucidum*.

herbal medicine, and its popularity in China has spread to the rest of the world (Stamets, 1993; Zhu et al., 1994). *G. lucidum* has been cultivated artificially by maintaining growth parameters such as temperature, water content, relative humidity, pH and light intensity together with the use of several different growing substrates (Chang and Miles, 2004). This method is used to produce a whole body of fruits and it is denominated traditional basidiocarp cultivation. Another method, called submerged fermentation, consisted on cultivation of the mycelium in liquid nutrient broth, to obtain natural products such as ganoderic acid (Figure 5.3.1) and polysaccharides that takes two or three weeks, opposite to the traditional that needs at least 3-5 months (Kapoor and Sharma, 2014; Wagner et al., 2003).

Lentinula edodes (Berk.) Pegler (syn. *Lentinus edodes*) is a saprophytic species, commonly known as shiitake, black oak mushroom, or shiang-gu. It is an omphalotaceae (Agaricales) species naturally occuring throughout Southeast Asia; where has been reported from China, Japan, Vietnam, Korea, Burma, Thailand, the Philippines, North Borneo, Papua New Guinea and Taiwan (Chiu et al., 1999; Stamets, 2000). It is a gregarious species that grows on fallen wood of a wide variety of genus of deciduous tree species such as *Acer, Alnus, Carpinus, Castanea, Castanopsis, Diospyros, Fagus, Liquidambar, Morus*, *Populus or Quercus,* in a warm and moist climate (Wasser, 2005). Traditionally, it has been cultivated in countries like China and Japan for many centuries (Chang and Miles, 1987). In fact, it was first cultivated in China for more than 800 years ago (Chang and Miles, 1987; Zhang and Lai, 1993). Nowadays, shiitake is produced on a commercial scale worldwide (Hibbett, 2001), becoming one of the most popularly cultivated mushrooms in the world (Royse, 1995), and producing more than two million tons, only surpassed by the button mushroom, *Agaricus bisporus* (Wasser, 2005). The cultivation process is based on the inoculation of the mycelia in a mixture of bark and wood of the selected hosted tree species, and to cover them with soil. This cultivation technique was probably introduced to Japan by the Chinese during the 16th century. (Suzuki et al., 1990).

Grifola frondosa (Dicks.) Gray (syn. *Polyporus frondosus*), commonly named maitake, is a saprophytic species from the family meripilaceae (Poryporales). Naturally, it occurs in Asia, North America and Europe (Breitenbach and Kränzlin, 1986; Gilbertson and Ryvarden, 1986; Zhao and Zhang, 1992). *G. frondosa* is a species that tends to grow on the rhizosphere of a variety of hardwoods, particularly fagaceous species such as *Castanopsis cuspidate, Fagus carenata*, *Quercus crispula* or *Q. serrata*, (Shen et al., 2002), and also in other species like *Prunus ume*, *P. salicina*, *P. armeniaca*, *P. persica* and *Diospyros kaki* (Mizuno and Zhuang, 1995). It tends to prefer the transition between open fields and wooded areas (Stamets, 2005). This species is generally classified as a saprophytic species, though it is sometimes found growing on dying trees. Nevertheless, this species is rarely the initial pathogen, and will consume previously weakened trees by other organisms (Stamets, 2000). It has been appreciated and consumed for 4000 years by the Chinese and Japanese culture due to its medicinal properties (Siquier and Constantino, 2008). Nowadays it is commercialized all over the world, although Asian and American countries are the main consumers (Chang, 1999; Royse, 1997).

Cultivation techniques were developed in 1979 prior to which maitake were only available from the wild (Sadler, 2003). Production of *G. frondosa* has been tested in three different forms: i) bottle culture in controlled conditions, ii) bag culture, consisted on a mixture of sawdust, rice and wheat brans packed in plastic under controlled conditions and iii) outdoor bed culture under natural climatic conditions (Mayuzumi and Mizuno, 1997).

CHEMICAL COMPONENTS WITH BIOLOGICAL ACTIVITIES: TRITERPENOIDS AND POLYSACCHARIDES

More than 150 lanostane-type triterpenoids have been identified to date from the genus *Ganoderma* (Boh et al., 2007). This kind of compounds are the typical chemical constituents in *G. lucidum* and they possess a huge diversity of functional groups, e.g. acids (Kikuchi et al., 1985; Kohda et al., 1985; Kubota et al., 1982), highly oxygenated (el-Mekkawy et al., 1998), lactones (Mizushina et al., 1999), aldehydes (Gao et al., 2002) or esters (Liu, Zhu, et al., 2014). Besides triterpenoids, a number of meroterpenoids have also been isolated. The chizhines A-F, lingzhifuran A and lingzhilactones D–F are phenolic meroterpenoids that display renoprotective effects (Ding et al., 2016; Luo et al., 2015). Recently, four polycyclic alkaloids, lucidimines A–D, have been isolated from *G. lucidum* (Zhao et al., 2015). In addition, a polysaccharide was isolated from a hot water extract of the fruiting bodies of *G. lucidum* exhibiting antitumor activity (Miyazaki and Nishijima, 1981). It was designated as GL-1 and it was concluded to be a branched arabinoxyloglucan. Since then, other polysaccharides with biological activities have been isolated, some of them are β-(1→3)-linked D-glucan with a (1→6)-β-D-glucopyranosyl branches (Bao, Wang, et al., 2002; Liu, Zhang, et al., 2014) but also others are heteroglycans, composed of glucose along with rhamnose, galactose or mannose (Bao, Zhen, et al., 2002), that display immunological activity. More recently, a hyperbranched glycoprotein, designated *FYGL-n*, was obtained from *G. lucidum* and investigated for anti-diabetic activity (Pan et al., 2015).

Shiitake (*L. edodes*) mushrooms produce a secondary metabolite named eritadenine [(2R,3R)-4-(6-amino-9H-purin-9-yl)-2,3-dihydroxybutanoic acid] that was previously named lentinacin (Chibata et al., 1969) and lentysine (Kamiya et al., 1969; Rokujo et al., 1970). Eritadenine is an alkylated adenosine analog with an acyclic sugar fragment which has been shown to possess hypocholesterolemic activity. Six polysaccharides from *L. edodes* were purified and especially one of them, lentinan (Figure 5.3.2), had strong antitumor activity (Chihara et al., 1970). Lentinan was extracted with hot water and the content was reported to be 0.0155% of fresh fruiting bodies of *L. edodes*. The structure is β-1,3-linked-D-glucan with β-1,6 branching, and since its discovery a number of biologically active polysaccharides have been isolated from *L. edodes*. So that, for instance, the chemical structure of a heteropolysaccharide (fucomannogalactan) extracted from shiitake was determined and found to possess antinociceptive and anti-inflammatory properties (Carbonero et al., 2008). Also, an antitumor and antiviral substance was isolated from the culture media of *L. edodes,* designated K2-S, which is a peptide containing α-mannan with and estimated molecular weight of 60-95 kDa (Fujii et al., 1978). Biologically active high molecular weight compounds other than polysaccharides have been isolated from mushrooms. Lentin is an antifungal protein (27.5 kDa) isolated from fresh shiitake mushrooms that inhibits HIV-1 reverse transcription and it possesses antiproliferative effects on leukemia cells (Ngai and Ng, 2003). Some organosulfur compounds are responsible for the distinctive flavor of shiitake mushrooms. Lenthionine [1,2,3,5,6-pentathiepane] is the major sulfur-rich compound in *L. edodes*, displaying antibacterial and antifungal activities (Morita and Kobayashi, 1967), and it also has been reported to inhibit platelet aggregation (Shimada et al., 2004). Finally, other studies evaluating the chemical composition of *L. edodes* have been done, for example it has been found tocopherols and phenolic compounds with a high antioxidant activity, along with free sugars and fatty acids (Carneiro et al., 2013).

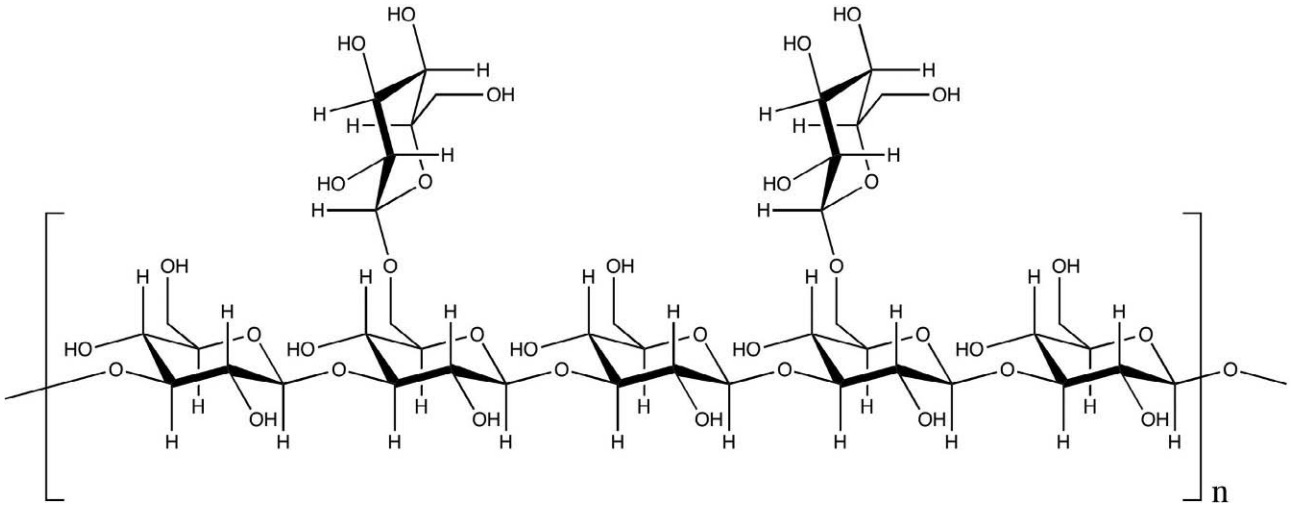

FIG. 5.3.2 Chemical structure of the polysaccharides lentinan found in *Lentinula edodes*.

Since the identification of the first 6-branched β-1,3-glucan (named grifolan) isolated from *G. frondosa* (Ohno et al., 1984), a myriad of similar glucans with antitumour activity has been purified from maitake extracts (Adachi et al., 1989; Fang et al., 2012; Iino et al., 1985; Kodama et al., 2002; Nanba et al., 1987; Ohno, Iino, Suzuki, et al., 1985; Ohno, Iino, Takeyama, et al., 1985; Ohno, Suzuki, et al., 1985). Besides those, a polysaccharide from the fruiting body of maitake, named MT-α-glucan, was determined to have anti-diabetic effect (Hong et al., 2007). In addition, heteropolysaccharides myeloid zinc finger and growth factor protein were found to have antitumor and immunostimulatory activities, respectively (Masuda et al., 2009; Meng et al., 2017). From the cultured mycelia of *G. frondosa* GF 9801 other heteropolysaccharides with protein portion, coded as GFPS1b and GFG-3a, exhibited potent anti-proliferative activity (Cui et al., 2013; Cui et al., 2007). The sterol constituents of *G. frondosa* have been studied and more than 20 different compounds have been identified, most of them related to ergosterol (Ishizuka et al., 1997). More recently a new antimicrobial compound, a furanone designated as grifolaone A, was identified from the culture medium of *G. frondosa* (He et al., 2016).

RELATION OF REISHI, SHIITAKE AND MAITAKE WITH HUMAN HEALTH

Many bioactive compounds have been reported in *G. lucidum*, being triterpenes and polysaccharides, the most active molecules related to prevent and treat several kind of illnesses. At the moment, more than 300 different triterpenes (mainly ganoderic acids, ganoderic alcohols, and their derivatives) have been isolated from the genus *Ganoderma* (Xia et al., 2014). Within the potential therapeutic uses of *Ganoderma* is remarkable its anti-cancer, anti-neurodegeneration or anti-human immunodeficiency virus (anti-HIV). Triterpenes are potent anti-oxidant molecules but also can directly interact with diverse molecular targets altering cellular processes including cell cycle, apoptosis or angiogenesis (Ferreira et al., 2010; Stojkovic et al., 2014). Polysaccharides of this species are predominantly high molecular weight heteropolymers with proposed neurological benefits and immunomodulatory activity (Mantovani and Sica, 2010; Matsuzaki et al., 2013).

Promising results of the anti-cancer activity of *G. lucidum* have been arisen by performing diverse laboratory and preclinical studies *in vitro* and using animal models. In these studies, triterpenes and polysaccharides, especially β-D-glucans, from *G. lucidum* have inhibited the cell proliferation and tumor growth and to induce the tumor cell death by addressing numerous cancer particular targets. The molecular mechanisms are mainly associated with the inhibition of transcription factors such as activator protein 1 (AP-1), nuclear factor kappaβ (NF-κβ) and signal transducer and activator of transcription 3 (STAT3) signaling pathways (Jiang et al., 2008; Li et al., 2012; Weng et al., 2008; Yao et al., 2012). However, clinical trials in patients with cancer in order to test *G. lucidum* are very scarce and with important limitations since long-term survival rates have not been registered by these studies. Up to date, a Cochrane revision based on the only 5 clinical trials that met the inclusion criteria comprising 373 patients, concluded that there is insufficient data to validate the *G. lucidum* use as the first chosen treatment for cancer (Jin et al., 2016). However, in patients treated with *G. lucidum* together with chemotherapy or radiotherapy were more probable to react positively respect to the conventional treatments alone.

Neurodegenerative diseases are a group of diseases, in which dementia, Alzheimer's disease (AD) and Parkinson's disease can be included, and which are directly associated with a risen longevity of the world's population. Several studies reported neuroprotection associated to *G. lucidum*. However, until to date no clinical trials have been designed to determine the effectiveness of *G. lucidum* intake. Studies performed *in vitro* evidenced an anti-inflammatory neuroprotection of *G. lucidum* extracts or triterpenes by attenuating the activation of microglial cells (Ding et al., 2010; Yoon et al., 2013; Zhang et al., 2011) or Aβ-induced synaptotoxicity and apoptosis (Lai et al., 2008). A diet supplemented with *Ganoderma* extract to senescence-accelerated mice reduced brain amyloid and improved the antioxidant defenses when contrasted to the control mice (Wang et al., 2004). Oral administration of *Ganoderma* polysaccharides improved the cognitive function and neural progenitor proliferation and enhanced neurogenesis in a mouse model of AD (Huang et al., 2017). In another study, *G. lucidum* polysaccharides were effective using a rat model of traumatic spinal cord injury reporting beneficial effects in biochemical, histopathological, and ultrastructural analyses (Gokce et al., 2015).

HIV is a fatal and not curable status well-known as acquired immunodeficiency syndrome (AIDS). Diverse triterpenoids of *G. lucidum* have been shown to exert anti-HIV-1 protease activity that can contribute to the delay of the progression of this illness (Akbar and Yam, 2011; el-Mekkawy et al., 1998; Min et al., 1998). A protein isolated from *G. lucidum* exhibited laccase activity and inhibitory activity towards HIV-1 reverse transcriptase (Wang and Ng, 2006). Additionally, other studies reported promising effects against different viruses such as papillomavirus, enterovirus or herpes (Donatini, 2014; Hijikata et al., 2007; Zhang et al., 2014).

The shiitake mushroom (*L. edodes*) is a highly consumed mushroom worldwide being a common ingredient in Chinese and Japanese cuisine. The major biological active compounds in *L. edodes* are polysaccharide compounds and specially lentinan and krestin, beta-1,3-glucans. However, consumption of high amounts of raw or undercooked shiitake mushrooms can result in gastrointestinal discomfort or shiitake dermatitis to lentinan (Levy et al., 1998; Nguyen et al., 2017).

L. edodes polysaccharides have been reported to exert diverse bioactivities being the most studied the antitumor and immunomodulatory activities. *In vitro* studies evidenced that polysaccharide fractions from shiitake mushroom are capable to inhibit the proliferation of a broad range of carcinogenic cells and also to induce tumor cell apoptosis (Jeff et al., 2013; Ren et al., 2012; Zhang et al., 2015). However, *L. edodes* extract alone to treat clinical cancer seems to be useless (deVere White et al., 2002). Nevertheless, *L. edodes* polysaccharide has promising results in diverse animal models when used as a coadjuvant with other conventional forms of cancer treatment such as chemotherapy (Ren et al., 2014). In a pilot study with patients following chemotherapy, the quality of life and immune function got better with an orally administered *L. edodes* mycelia extract (Yamaguchi et al., 2011). The immunomodulatory activity of the polysaccharide and extracts from shiitake mushroom is considered a central point for the anti-cancer effects of shiitake. In a randomized dietary intervention in healthy subjects, the regular consumption of shiitake increased *ex vivo* peripheral blood mononuclear cells proliferation and expression of activation receptors and also increased salivary immunoglobulin A production (Dai et al., 2015). Another study supplementing with β-glucan from *L. edodes* mycelium provoked a raise in the amount of circulating B-cells in elderly subjects (Gaullier et al., 2011).

An interesting potential use of *L. edodes* is associated with its antimicrobial capacity, especially with anticariogenicity activity. Dental caries affects more than 60% of children and the majority of adults being the most prevalent oral disease worldwide (Avinash et al., 2016). Data available from the literature indicate that *L. edodes* appears to have wide antimicrobial action beside both gram-positive and gram-negative bacteria (Alves et al., 2012). Moreover, the capability of shiitake extracts has also been investigated in order to improve oral health, showing a significant bactericidal effect against *Streptococcus mutans, S. sobrinus* and *Prevotella intermedia*, strongly implicated in dental caries and gingivitis (Shouji et al., 2000; Signoretto et al., 2013; Signoretto et al., 2014). Moreover, a number of *in vitro* works carried out to elucidate the biofilm inhibitory effect of *L. edodes* evidenced significant reductions in the biofilms on the tooth surface (Ciric et al., 2011; Signoretto et al., 2014; Zaura et al., 2011). Another study using a rat model fed with a cariogenic diet containing shiitake extract (0.25%) and infected with *S. mutans*, showed a significant decrease in caries score respect to animals fed without shiitake extract (Shouji et al., 2000). A clinical trial tested in 30 young subjects a shiitake mushroom extract formulated as a mouth rinse during 11 days, reported a reduction in plaque and gingival indexes as well as in total bacterial and specific oral pathogens counts respect to controls (Signoretto et al., 2011). A reduction in the metabolic activity of dental biofilm was also evidenced in a similar clinical trial (Lingstrom et al., 2012).

Similar to *L. edodes*, the polysaccharides and, specifically the β-glucans, obtained from *G. frondosa* (maitake extracts) are suggested to be the most bioactive compounds of the mushroom. The main components of maitake extracts are a mixture of glucan/protein in a ratio ranging from 80:20 to 99:1 (Mayell, 2001). These extracts are recognized for their non-specific immunomodulatory effects which can be associated to the antiviral and antitumor activity of the maitake mushroom. *G. frondosa* extracts can be used together with conservative medical therapies so as to treat cancer. In addition, potential uses for the management of neurodegenerative diseases or as lipid lowering agent have also been suggested.

The improvement of the immune system has been indicated as the main reason of the anticancer effects of maitake. *G. frondosa* extracts potentiated the action of immune cells including macrophages, natural killer (NK) cells and cytotoxic T-cells that may attack carcinogenic cells (Harada et al., 2003; Kodama et al., 2003; Wu et al., 2013). This effect is also favored in increases of diverse cytokines (interleukin (IL)-1 and 2), lymphokines and interferon-γ (IFN-γ) (Adachi et al., 1994; Svagelj et al., 2008). Administration of β-glucan extracts from *G. frondosa* has been reported to induce antitumor immune response and reducing tumor volume in animal models (Inoue et al., 2002; Masuda et al., 2013). Also, maitake can be capable of providing protective effects against chemotherapeutic immusuppression induced by cisplatin (Masuda et al., 2009). A phase I/II trial in breast cancer patients showed that oral intake of a polysaccharide extract from *G. frondosa* for 21 days increase the production of IL-2, IL-10, TNF-α and IFN-γ by subsets of T cells (Deng et al., 2009). Other clinical trial on patients with myelodysplastic syndromes evidenced that maitake extract consumption for 12 weeks increased endogenous neutrophil and monocyte function and enhanced the reactive oxygen species response to *Escherichia coli ex vivo* (Wesa et al., 2015).

Another interesting potential of maitake mushroom is its lipid lowering effect. In a rat model of obesity the supplementation of the diet with maitake dried powder inhibited the amount of lipids accumulated in the liver and reduced serum lipids concentration so altering the lipid metabolism (Kubo and Nanba, 1996). Glucans extracted from *G. frondosa* administered to diabetic mice resulted in a significant a hypoglycemic and hypolipidemic effect and also increased serum insulin (Lei et al., 2012). Also, an inhibitory activity of different components of the maitake against α-amylase and α-glucosidase, digestive enzymes related to type 2 diabetes, were reported (Su et al., 2013). Finally, it is interesting to mention an open trial in patients with polycystic ovary syndrome reporting the induction of ovulation, associated with insulin resistance, by maitake extract consumption for 12 weeks (Chen et al., 2010).

CONCLUSIONS

Mushrooms have been used as food sources and traditional remedies and medicine for ancient times. However, studies to determine the specific effects of mushrooms on health and which compounds are responsible for their biological effect have gained noticeable interest in recent years. Reishi, shiitake and maitake are three mushrooms very important in oriental culture and are increasingly used in Western countries. Recent studies evidenced very interesting data about the potential use of these mushroom, their extracts and/or specific compounds against many diseases. Studies developed in cell cultures and animal models seem to support these effects derived from millennial medicinal traditions. However, clinical studies are in preliminary stages and most of them are not able to evidence clear therapeutic effects of the mushrooms. It should be noted that promising results can be observed when used as a coadjuvant of conventional therapies. In order to determine their therapeutic uses, it will be necessary to carry out more clinical studies to evidence the therapeutic potential, doses and real applications of mushrooms.

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Chapter 5.4

Ophiocordyceps sinensis

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INTRODUCTION

With changing lifestyle and food habits the risk of various life threatening diseases, including hypertension, diabetes, anxiety and cancer have been increased worldwide (Pereira et al., 2005). Although the pharmaceutical arsenal of synthetic drugs to treat these aging-associated diseases has rapidly enlarged, their potential side effects have provoked the interest of people in natural therapies (Tuli et al., 2013). Therefore, natural remedies, such as herbal medicines as dietary supplements are becoming more and more popular worldwide both in public communities as well as among researchers (Yue et al., 2013).

Medicinal mushrooms are well known for their ability to protect from many diseases (Tuli et al., 2013). The genus *Ophiocordyceps*, belongs to Ophiocordycipitaceae family and represents over 140 species that grow on insects (Kirk et al., 2008). The caterpillar fungus, *Ophiocordyceps sinensis* (earlier known as *Cordyceps sinensis*) is used in Chinese traditional medicine system and later was popularized and identified in other countries (Yue et al., 2013; Jiraungkoorskul and Jiraungkoorskul, 2016; Nakamura et al., 2015). It is an entomopathogenic fungus growing parasitically on larvae of a particular species of moth, Lepidoptera (Jiraungkoorskul and Jiraungkoorskul, 2016; Jang et al., 2016; Zhang et al., 2016). The larvae of the host insect feeds on roots of alpine plants and in winters, they usually die due to fungus infection (Li et al., 2011) and the fruiting body of the fungus comes out from the exoskeleton of the dead larvae in spring (Fig. 5.4.1). It is one of the most expensive natural products due to the possibility of being used as a remedy to wide range of conditions, especially as dietary supplements to strength vital force of body (Cannon et al., 2009). Indeed, *O. sinensis* and its products have been shown to display beneficial effects on cardiovascular, nervous, renal, gastrointestinal, hepatic, respiratory, immunological, and sexual systems (Jiraungkoorskul and Jiraungkoorskul, 2016, Xu et al., 2016). This mushroom can reveal antioxidant, antiinflammatory, antimicrobial, antifungal, antinociceptive, hypoglycaemic, hypolipidemic, antidiabetic, antiosteoporotic, anticancer, antimetastatic, and immunomodulatory properties and may even improve the athletic power, memory and learning abilities (Yue et al., 2013).

Considering the above, the present chapter describes geographical distribution and its availability including trends and trade, therapeutic effects and the underling molecular mechanisms, major bioactive components with their structural features and interaction and toxicology profile of *O. sinensis*. Also the potential clinical evidences of this mushroom as nonvitamin nonmineral nutritional supplement both in prevention as well as treatment of modern age-associated diseases are discussed.

GEOGRAPHICAL DISTRIBUTION

O.sinensis is considered as endemic to Tibetan Plateau and surrounding Himalaya (Winkler, 2009) in the alpine ecosystems of Bhutan, China, Nepal, Tibet and India (Yue et al., 2013;Tuli et al., 2013; Negi et al., 2014; Nakamura et al., 2015; Jiraungkoorskul and Jiraungkoorskul, 2016; Jang et al., 2016; Zhang et al., 2016). The common name of species varies from place to place. For instance, in China it is popularly known as "DongChongXiaCao", in Tibet as "YartsaGunbu" (winter worm-summer grass) and in India as "Keera Jhar" or "Keera ghas" (Insect herb) (Sharma, 2004; Winkler, 2009; Negi et al., 2014). Some of the regions of occurrence along with the altitudinal range are listed in Table 5.4.1.

Nonvitamin and Nonmineral Nutritional Supplements. https://doi.org/10.1016/B978-0-12-812491-8.00069-2

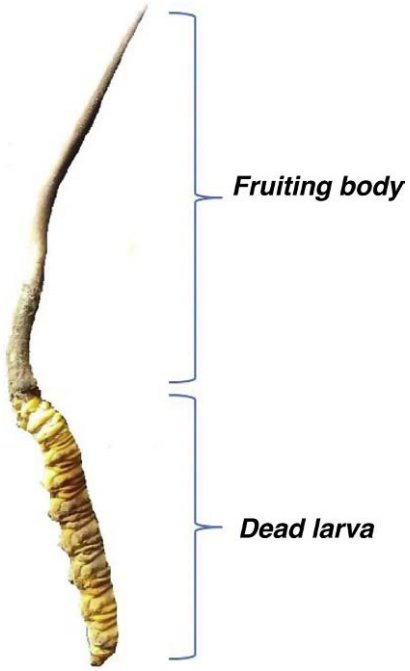

FIG. 5.4.1 *O. siniensis* fruiting body and dead larva. (Photo Credit: *Pradeep Kumar Sharma)*

TABLE 5.4.1 Distribution of *Ophiocordyceps sinensis* in Different Countries

S. No.	Country	Region of Occurrence	Altitudinal Range (m asl)	References
1	Bhutan	Namna (North Western Bhutan), Bumthang Valley (North Central Bhutan), and Bumdeling Wildlife Sanctuary	4200–5200	Balfour-Browne (1955), Kobayasi (1980), Cannon et al. (2009)
2	China	Xinjiang, Yunan, Jilin, Shanxi, Shaanxi, Hubei, Zhejiang, Jiangxi, Guizhou, Taiwan, Guangdong, Guangxi, Sichuan and Hainan Province, and Lhasa and Shannan in Tibet	2260–5000	Li et al. (2011)
3	India	Uttarakhand (Darma valley, Choudas valley, Ralamdhura, Panchahuli base Dharchula-Munsyari regions of Pithoragarh district and Pindari catchment in Bageshwar district, Garhwal region) Sikkim, Arunanchal Pradesh, and Himanchal Pradesh	3200–4800	Negi (2003), Negi et al. (2006, 2009, 2014), Kuniyal and Sundriyal (2013), Sharma (2004), Winkler (2009)
4	Nepal	Dolpa, Darchula, Jumla, Bajura, Kalikot, Mugu, Humla, Rukum, Bajhang, Manang, Mustang, Gorkha, Lamjung, Dhading, Rasuwa, Dolakha, Sindhupalchowk, Solukhumbu, Sankhuwasabha, and Taplejung districts	3540–5050	Shrestha and Sung (2005), Adhikari (2008), and Devkota (2008, 2010)

BIOACTIVE COMPOUNDS

The *O. sinensis* extracts, concentrates or powders have found to exhibit health benefits. In fact *O. sinensis* has been designated as nutraceutical mushroom (Smith et al., 2000), due to be a rich source of novel biologically active chemical constituents with diverse structural architecture (Fig. 5.4.2). It mainly contains proteins, peptides, amino acids, polyamines, nucleosides, polysaccharides, sterols, steroids and fatty acids, which have been associated with different pharmacological properties. A list of active bio-molecules is summarized in Table 5.4.2. Nucleosides, polysaccharides and sterols are major bioactive compounds of genus '*Ophiocordyceps*' and are known to be predominantly involved in providing a broad spectrum of therapeutic potential.

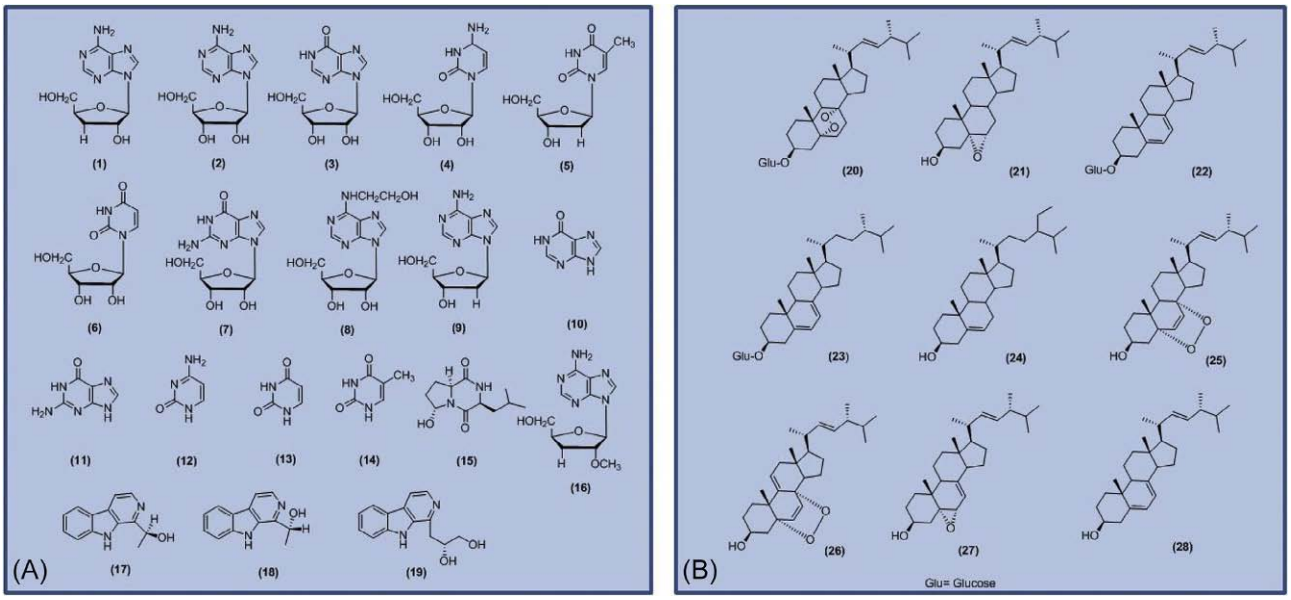

FIG. 5.4.2 Structural features of some of the bioactive compounds identified in *O. sinensis*. (A) Nucleosides and nitrogenous bases, and (B) Steroids. *(Source: Prasain, J.K., 2013. Pharmacological effects of cordyceps and its bioactive compounds. Stud. Nat. Prod. Chem. 40, 453.)*

TABLE 5.4.2 List of Major Bioactive Compounds of *Ophiocordyceps sinensis*

S. No.	Class	Name of the Compound
1	Nucleosides	Cordycepin (1), Adenosine (2), Inosine (3), Uridine (4), Thymidine (5), Cytidine (6), 6-Hydroxyethyladenosine (8), Guanosine (7), 20-Deoxyadenosine (9), Hypoxanthine (10), Guanine (11), Uracil (13), Cytosine (12), Thymine (14), Cordysinins A (15), Cordysinins B (16), Cordysinins C (17), Cordysinins D (18), Cordysinins E (19)
2	Steroids	5a,8a-epidioxy-24(R)-methylcholesta-6,22-dien-3-D-glucopyranoside (20), 5a,6a-epoxy-24(R)-methylcholesta-7,22-dien-3-ol (21), Ergosteryl-3-O-D-glucopyranoside (22), 22-dihydroergosteryl-3-O-b-D-glucopyranoside (23), Sitosterol (24), 5a,8a-epidioxy-22E-ergosta-6,22-dien-3-ol (25), 5a,8a-epidioxy-22E-ergosta-6,9,22-trien-3-ol (26), 5a,6a-epoxy-5-ergosta-7,22-dien-3-ol (27), Ergosterol (28)
3	Cyclopeptides	Cordyheptapeptide A, Cycloaspeptides A, Cycloaspeptides C, Cycloaspeptides F, Cycloaspeptides G, bisdethiodi(methylthio)hyalodendrin
4	Polysaccharides	CS-F10, CPS-2, CME-1, CS-PS, Cordysinocan, SCP-1, PS, CS-Pp, EPS
5	Fatty acids	Lauric acid, Myristic acid, Pentadecanoic acid, Palmitoleic acid, Palmitic acid, Linoleic acid, Oleic acid, Stearic acid, Docosanoic acid, and Lignoceric acid
6	Sterols	Ergosterol, Cholesterol, Campesterol, and Beta-sitosterol

Source: Prasain, J.K., 2013. Pharmacological effects of cordyceps and its bioactive compounds. Stud. Nat. Prod. Chem. 40, 453; Tuli, H.S., Sandhu, S.S., Sharma, A.K., 2014. Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. 3 Biotech 4 (1), 1–12.

Nucleosides

Nucleosides i.e., thymine, adenosine and cordycepin are found to be major biochemical markers in a range from 138.5–174.2, 79.6–186.5 and 31.3–91.2 μg/g, respectively (Xie et al., 2010), which are used for quality control of *O. sinensis* and are believed to be active components. The nucleosides contents varied among natural and cultured *O. sinensis*. Adenosine and cordycepin (3′-deoxyadenosine) are pharmaceutically active components that exhibit multiple pharmacological actions such as immune-modulator, antioxidant, among others, and are a key principle component, ensuring authenticity of *O. sinensis* (Li et al., 2006a,b).

Polysaccharides

Polysaccharides are the major class of bioactive fraction of *O. sinensis*, which are major contributors to most of the biological health benefits. Using polysaccharides as marker for quality control is a challenge because of macro molecular mass and structural complexity. The polysaccharides with their structural features isolated from natural and cultured *O. sinensis* are represented in Table 5.4.2. Wild and cultured *O. sinensis* consist of major monosaccharides such as rhamnose, ribose, glucose, fructose, among others (Guan et al., 2010), and are found in the range of 3 to 8 mg/g of dry weight of *O. sinensis* (Li et al., 2006b).

Sterols

The sterols are also an important class of compounds isolated from *O. sinensis* that possess potent health benefits. Ergosterol is the major sterol found in *O. sinensis* and it is present in two forms (free and esterified), each having different functions. Cholesterol, campesterol and β-sitosterol including ergosterol in natural (wild) *O. sinensis* were determined using pressurized liquid extraction (PLE), trimethylsilyl (TMS) derivatization and GC–MS analysis, and found in the range of 68.8 to 134.3 μg/g (Yang et al., 2009). These phytosterols helps in treating colon, prostate and breast cancer and their bioactivities are helpful in elucidating therapeutic indications of *O. sinensis*.

THERAPEUTIC EFFECT

O. sinensis has diverse biological activities which are supported by various clinical studies. *O. sinensis* in its wild and cultivated forms exhibit wide spectrum of pharmacological activities, such as, improve functions of renal, hepatic, nervous and cardiovascular systems as well as it was found effective against cancer and immunological disorders (Fig. 5.4.3). Primarily polysaccharides, nucleosides and its derivatives (or modified), and cyclosporine like secondary metabolites are active fractions which exhibit potent health benefits. Some of the therapeutic effects of *O. sinensis* are discussed below.

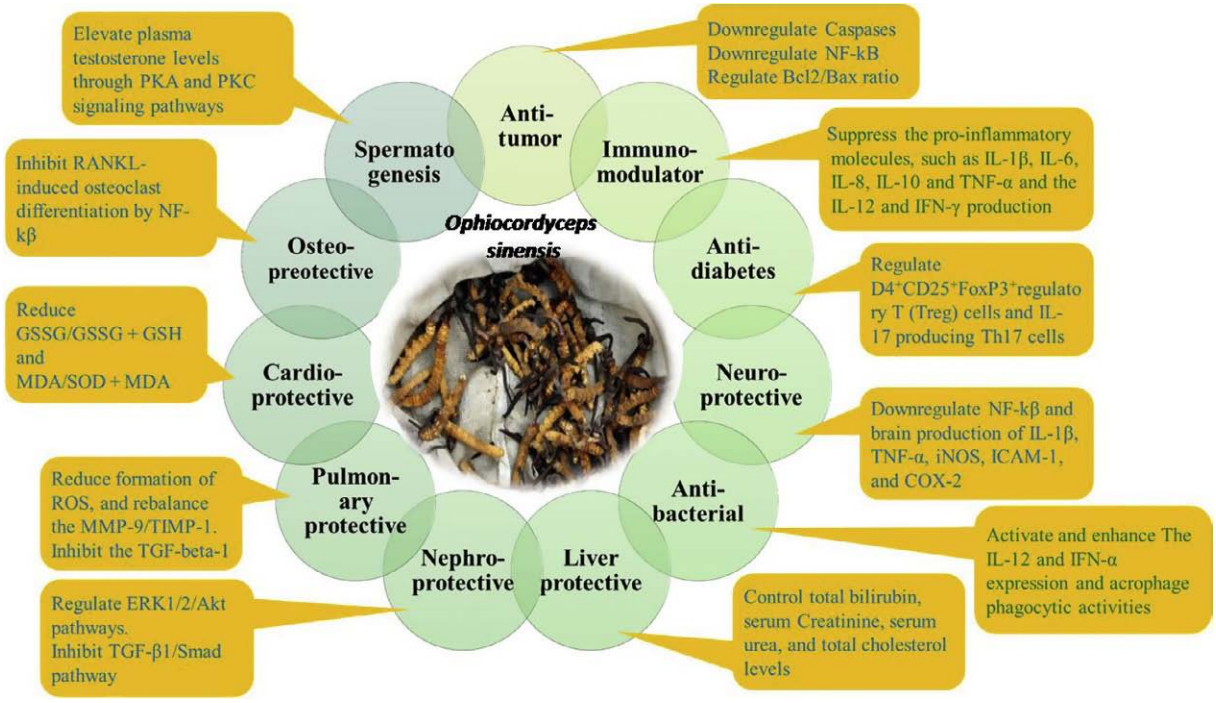

FIG. 5.4.3 Therapeutic effects of *O. siniensis*.

Immune-Modulator

Immunomodulation during acute and chronic inflammation leads to several manifestations, including cancer, which provokes urgent treatment implementation (9-11). *O. sinensis*, suppressed the pro-inflammatory molecules, such as IL-1β, IL-6, IL-8, IL-10 and TNF-α production and furthermore enhanced the IL-12 and IFN-γ production in activated BALF cells (Kuo et al., 2001). *O. sinensis* stimulate macrophages with upregulation of TNF-α, IL-12 and iNOS, whereas IL-10 of Ana-1 expression was downregulated (Chen et al., 2012). A pure compound (H1-A) isolated from *O. sinensis* reported to show good immune-modulatory effect by improving the survival of lupus mice (Yang et al., 1999; Lin et al., 1999).

The exo-polysaccharides obtained from submerged fermentation of *O. sinensis* have contributed to immunomodulatory activity by enhancing cytokine synthesis (IL-6 and IL-10), TNF-α and phagocytosis and also upregualted CD11b expression (Kuo et al., 2007).

Anticancer Effects

Medicinal mushrooms are very promising for cancer therapy and *Ophiocordyceps* has been effectively used as an anti-tumor herb in Chinese medicines, due to their apoptosis activation and anti-metastasis activities (Yang et al., 2006).

Apoptosis Induction

O. sinensis significantly activates apoptotic pathways in several cancers. Importantly, it can control both intrinsic and extrinsic pathways of apoptosis, which is a fundamental phenomenon opted by current cytotoxic drugs that are being utilized in chemotherapy. Its modulatory effect on Bcl2/Bax, mitochondrial membrane potential, caspases 3, 9, and 8 has been noticed. It also up-regulates the Fas-receptor and down-regulates the NF-κB protein expression (Yang et al., 2003; Buenz et al., 2004). The expression levels of these proteins are found to be involved in promoting apoptosis in cancer.

Anti-Metastasis

Metastasis is the intrinsic behavior of human malignancies associated with the majority of cancer-related deaths. Tumor and stroma cells secret many growth factors that cause tumor cell migration, and are found to be targeted as cancer preventive mechanisms by a variety of chemotherapeutic agents (Bal et al., 2015). It is found that water extract of *O. sinensis* exerts anti-metastatic activity by inhibiting the HGF-accelerated tumor invasiveness in mouse melanoma cells (Kubo et al., 2010). The methanolic extract from the fruiting bodies of *O. sinensis* showed inhibitory effect on tumor cell lines (Kuo et al., 2006). Antitumor sterols isolated from cultured *O. sinensis*, were found to inhibit the growth of cancer cells in an *in vitro* experiment (Kodama et al., 2000). Also the role of ethanolic extract of *O. sinensis* in treating cancer patient having immune disorders has been reported (Xu et al., 1992).

Aphrodisiac Effect

Recently aphrodisiac activity has been reported in *O. sinensis*, and termed as Himalayan Viagra (Kashyap et al., 2016). Wang et al. (1998) reported that *O. sinensis* contains a factor that stimulates corticosteroid production in animal model. The hot water extract of *O. sinensis* improve the sexual function in rats (Ji et al., 2009) and stimulate the steroidogenesis, significantly by inducing testosterone production via PKA and PKC signal transduction pathways (Hsu et al., 2003; Huang et al., 2004; Chen et al., 2005; Wong et al., 2007).

A clinical study reported that a supplement of *O. sinensis* given to 22 males, showed to increase sperm count (33%) and decreased incidence of sperm malformations (29%) (Guo, 1986). In another study involving 189 patients of both genders with decreased libido and desire showed improvement of symptoms and increase of desire up to 66% after treatment with *O. sinensis* (Wan et al., 1988). Further, *O. sinensis* supplementation caused increased level of thymus hormone, improved activity of adrenal glands and 300% improvement in sperm count (Huang et al., 1987). Also, improvement of libido and desire at 86% in woman was observed (Dong and Yao, 2008).

Anti-Fatigue and Improves Stamina

Many studies were conducted to prove that *O. sinensis* reduces fatigue and boosts stamina for athletes when taken as a dietary supplement. Extract of mycelium of *O. sinensis*, mainly containing carbohydrate (78.9%), was orally administered to mice to determine the swimming endurance capacity (Koh et al., 2003). *O. sinensis* was found to prolong swimming time (75 to 90 min) of test groups as compared to the control indicating the extract has effect on recovery from exhaustion with lessening of fatigue (Koh et al., 2003). In similar studies, *O. sinensis* was given to mice groups for six weeks and found higher improvement in swimming capabilities (Xiao et al., 1999). In mice administered with CordyMax Cs-4 (mycelial fermentation product), the level of β adenosine triphosphate (ATP) in liver was increased, which suggests a higher hepatic bioenergy status on improving physical endurance (Dai et al., 2001).

Anti-Inflammatory Effect

O. sinensis was found to be effective against inflammatory reactions (Wang et al., 2015). As such, *O. sinensis* mycelia extract was tested for its anti-inflammatory response by their inhibitory effect on superoxide anion generation and elastase release by human neutrophils (Yang et al., 2011). For the first time, Cordysinins A-E, have been identified and found to display significant inhibition (Yang et al., 2011). Cordymin, a purified peptide from *O. sinensis* was found to lower pro-inflammatory cytokines levels, and prevent inflammatory reaction (Qian et al., 2012). Also, *O. sinensis* mycelium showed neuroprotective activity through anti-inflammatory response (Liu et al., 2011). The *O. sinensis* extract was found to downregulate expression of NO synthase, which results in inhibition of inflammatory mediators such as nitric oxide (NO) (Rao et al., 2007).

Nephro-Protective Effect

O. sinensis exerts its antifibrotic effect by attenuating the expression of α-SMA and FSP1, therefore inhibits the TGF-β1/Smad pathway which is known to be associated with the renal fibrosis. In addition, the expression of E-cadherin upregulated and results in inhibition of the Epithelial Mesenchymal Transition (EMT) upon *Ophiocordyceps* treatment (Liu and Shen, 2003; Pan et al., 2013; Bal et al., 2015). Evidences suggest that *O. sinensis* possesses a bidirectional regulatory function for the treatment of glomerulonephritis to human mesangial cells (HMCs) through the ERK1/2/Akt pathways (Wang et al., 2015). Further in immunoglobulin-A nephropathy (IgAN) animal model, the mice fed with *O. sinensis* in diet had resulted in significant reduction of hematuria and proteinuria together with clinical and histopathologic improvement (Ding et al., 2010).

High doses of cyclosporine A (CsA) is considered harmful in the long term treatment of kidney allograft recipients, however, treatment with *O. sinensis* in combination with low doses of CsA, results in less incidences of complications in the treatment group (Ding et al., 2009, 2010). Moreover, the preventive action of *O. sinensis* on nephrotoxicity induced by aminoglycoside was evaluated and found effective in rat model (Li et al., 1996). Gentamicin-induced kidney damage patients when treated with *O. sinensis* at a dose of 4.5 g per day for 6 days, presented an improved kidney function (89%) as compared to control group (45%) (Holliday and Cleaver, 2004).

Anti-Diabetic Effect

In an *in vivo* pharmacology study, CordyMax CS-4 were administered to Wistar rats for 17 days and showed: 1) reduction in fasting blood glucose, 2) 37% decrease in fasting plasma insulin, 3) rise in glucose-insulin index, and 4) improvement in glucose tolerance (Zhao et al., 2002). Type-1 diabetes is associated with an imbalance of regulatory T (Treg) cells, that treated with the application of *O. sinensis*, resulted in the alteration of T lymphocyte and improvement in diabetic condition in mice (Shi et al., 2009; Kan et al., 2012). Comparison of anti-diabetic activity of wild and cultured *O. sinensis* in nicotinamide and streptozotocin induced diabetes in male Wistar rats was examined (Lo et al., 2006). These rats were orally administered with different forms of *O. sinensis* products along with placebo (STZ group), and found that the blood glucose concentration was significantly lower in treated group as compared to STZ group (Lo et al., 2006).

TOXICITY AND INTERACTIONS

Not many evidences are available on the toxicity and/or interactions of *O. sinensis* with other drugs and food supplements. When streptozotocin-induced hyperglycemic rat treated with *Ophiocordyceps* and Vanadium (imitate the action of insulin) together, results in co-effect with the reduction in blood glucose level and improvement in swimming and climbing

behavior (Guo et al., 2011). Evidences indicate that the *Ophiocordyceps* consumption results in neprotoxicity induction in rats, which may be due to increased level of oxidative stress markers such as GSSG/GSH ratio and also a reduction in antioxidant enzyme activity (Zhou and Yao, 2013).

Fermented *O. sinensis* was also tested for acute toxicity and safety in Wistar rats (Chang et al., 2016). After treated with different doses (0-5g/kg body weight) of *O. sinensis* for two weeks, no significant difference in the blood biochemistry level and complete blood count (CBC) were reported (Chang et al., 2016). Furthermore, heart, liver and kidney organ investigation by H&E stain reveals no impairment (Chang et al., 2016). Meena et al. (2013) studied the toxicity of cultured mycelia of *O. sinensis*, and found that it is safe and no toxic effects have been seen up to a dose of 2g/kg body weight. However, there was a significant increase in food intake, body weight gain and hematological parameters like white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and lymphocytes in *O. sinensis* treated groups were reported (Meena et al., 2013).

TRENDS OF TRADE

O. sinensis has been used as a dietary supplement and for its aphrodisiac properties for centuries, but few decades ago, after economic liberalization in China, it has been exposed to global market (Winkler, 2009). This increases the global trade of *O. sinensis*, mainly due to its aphrodisiac and energy boost up properties (Holliday and Cleaver, 2008; Winkler, 2010), resulting in one of the world's highest-priced biological commodities known till date (Stone, 2008). The increase in trade and high demand of this fungus is also attributed to its hyped fame as 'Himalayan Viagra' all over the world (Shrestha and Bawa, 2013), which increased its harvesting and its trade by the local people (Fig. 5.4.4).

Globally, the production of the caterpillar fungus ranges between 83.2 tons/year to 182.5 tons/year and it varies in Tibetan plateau and various other parts of Himalaya. As such, the highest recorded in Tibetan plateau (China) (80–175 tons/year) followed by Nepal (1.0–3.2 tons/year), India (1.7–2.8 tons/year) and Bhutan (0.5–1.5 tons/year) (Winkler, 2009). The price of *O. sinensis* is around 20000 US $/Kg, which contain near about 3600 to 4200 samples and it varied with the quality of the product and market type (Negi et al., 2014). The market price of *O. sinensis* has increased every year. As such, in Tibet, the market price climbed by ~900% during 1997 to 2008 (Winkler, 2009), while a higher increase in price (up to 2300%) from 2001 to 2011 in Nepal was recorded (Shrestha and Bawa, 2013) and in India the price increased six folds (US $ 3333/kg in 2008 to US $20,000/kg in 2012) (Negi et al., 2014). Moreover, the species has been used globally in a range of products from cosmetics to dietary supplements, revitalizing and anti-aging products (Table 5.4.3). The increased price and market demand has posed pressure on harvesting and natural populations of *O. sinensis*, which need utmost attention for its sustainable utilization.

FIG. 5.4.4 *O. sinensis*: (A) In natural habitat; (B) and (C) Collected mature samples for trade. *(*Photo Credit*: A and B- Pradeep Kumar Sharma, C-Vivek Rawat)*

TABLE 5.4.3 List of Products Having *Ophiocodyceps sinensis* as an Active Ingredient

S. No.	Product Name	Approx. Price ($)	Uses	Manufacturing Company
1	Organo Gold Red Tea	33/box	Revitalizing drink	Organo Gold Enterprises Inc., Canada
2	CordyMax CS-4	53.30/120 capsules	Dietary supplement for stamina and vitality	Nu Skin, Pharmanex, Inc., USA
3	Le'JOYva Gourmet Arabica Black Instant Healthy Coffee	29.99/pack	Revitalizing drink	Le'JOYva Inc., USA
4	Cordyceps Soap	9.79/unit	Skin nourishment	Full and Fill Bio, Thailand
5	Cordyceps organic mushroom beverage	3/unit	Sexual tonic, lowers blood glucose levels and reduce blood cholesterol levels	Full and Fill Bio, Thailand
6	Mountain Fresh Organic Cordyceps Homeopathic Cream	108.92/unit	Helps to brighten and whiten the face	Mountain Fresh, UK
7	YanWo drink with rose bud and Cordyceps extract	60.22/unit	Revitalizing drink, antiaging, smooth skin	QiYun B.V, UK
8	New China Cordyceps sinensis whitening cream	17.65/unit	Removes dark spots and sun spots	Smile, China
9	Cordyceps Mushroom Extract Powder	0.50/gram	Dietary supplement	Real Mushrooms, Canada
10	Cordyceps Capsules	44.96/pack (120 capsules)	Dietary supplement	Host Defense Inc., USA
11	Now Foods Cordyceps	12.34/750 mg	Dietary supplement	Now Foods Inc., USA
12	Mdrive Elite performance	79.99/box (90 capsules)	Dietary supplement	Dream Brands, Inc., USA
13	Kala health Cordyceps	97.95/ box (600 capsules)	Dietary supplement	Kala Health Inc., USA

Source: amazon.in, amazon.com, amazon.uk, alibaba.com, aliexpress.com.

CONCLUSION

As discussed above, the different clinical studies have supported the physiology improving abilities of the *O. sinensis*. In many studies, it has been found effective as dietary supplements from boosting stamina and aphrodisiac effect to anti-proliferative capability, thus increased the life span. Future studies may be carried out using synergistic approaches between synthetic chemotherapeutic drugs and *O. sinensis*. In addition, a deep insight into the disease-associated signaling pathways regulated by *O. sinensis* may need further exploration. However, in recent years *Ophiocordyceps* market price and demand has been increased exponentially due to its wide therapeutic applications. This leads to overexploitation and early immature removal of *O. sinensis*, which reflected in decreasing supply in many areas (Shrestha and Bawa, 2013; Negi et al., 2014). For sustainable utilization of *O. sinensis* as dietary supplement and for other therapeutic effects, effective measures need to be taken. As such, the artificial culture of *O. sinensis* needs to be encouraged to meet up the increasing market demand. Also, more studies on composition, efficacy and clinical trials of artificial cultured products contribute to more extensive medicinal use of *O. sinensis*.

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Part VI

Future Trends in Food Supplements

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Chapter 6.1

Challenges and Foresight of Food Supplements

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In the last decades, medicine has faced a considerable development regarding new therapeutics and new drugs. However, today there is an increasing number of people reluctant to synthetic compounds who look for alternatives. Moreover, is also consensual that a healthy dietary pattern has a major role in the prevention of diseases (Dietz et al., 2016; Della Valle et al., 2017). The knowledge of the potential positive and negative influence of food in the health status is of major importance and it is the propulsive engine conducive to much of the research in the area of food science carried out nowadays. Therefore, the maxim of Hippocrates dated from around 5th century BC "Let food be your medicine and medicine be your food" is more actual than ever. In fact, the recognition of the value of foods as therapeutics has led to the use of foods, functional foods and nutritional supplements as medicine (Council of Europe, 2005; FDA, 2004; European Parliament and Council, 2006; Kiely et al., 2010).

Food supplements started to focus exclusively in the deficiency of nutrients due to depletion of foods availability. Nowadays supplements are a vast area that focuses on complement the diet while preventing diseases (reduction of the risk of diseases) and maintaining good health in order to improve the quality of life and wellbeing in all aspects. Therefore, even the most traditional medical bodies are changing their established points of view.

Regarding supplementation is important to emphasis that the consumption of food components can have different impact in an individual than the consumption of the whole food. In fact, different epidemiological studies stated that is not possible to extrapolate the benefits of foods to food supplements or the opposite (van het Hof et al., 2000). Non-vitamin and non-mineral (NVNM) nutritional supplements discussed in this book are not used to correct a deficiency state, which can occur in a period of enhanced biological demand but it can also have a pharmaceutical action, which could not be attained when consuming the food by itself.

The dietary supplements market is increasing. According to a Market Research report (Zion™, 2017), the global dietary supplements market valued at USD 132.8 billion in 2016 and is expected to reach USD 220.3 billion in 2022.

Supplementation has increased popularity among general population due to: (i) the interest for phytochemicals, because they are natural and considered safe; (ii) increased life-spans and higher education; (iii) less use of pharmaceuticals and desire for self-medication; (iii) increase cost of medical care; (iv) the guidelines for development, approval and retail are less strict than for pharmaceuticals; (v) reports on the efficacy of these products.

The multi-billion-dollar industry of NVNM nutritional supplements has the tendency to increase the number of standardized pharmaceuticals introduced in the national healthcare systems of different countries.

In United States, supplements, which include botanical products, vitamins and minerals, amino acids, and tissue extracts, are regulated by the FDA under the Dietary Supplement Health and Education Act (DSHEA) of 1994 enacted by the US Congress and they are defined as "*an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of any of the aforementioned ingredients*" (FDA, 2004). On the other hand, European Union (EU) Directive on Food Supplements (2002/46/EC) defines food supplements as *"…foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles and other similar forms of liquids and powders designed to be taken in measured small quantities"* (EC, 2002). Although the term NVNM nutritional supplement has not been used in

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the act, extracts, juice, whole plants parts processed in different ways and natural products isolated from natural matrices that are added to regular food to improve their nutritional value or taken separately to decrease the risk of diseases, ageing and improve quality of life are NVNM dietary supplements.

NVNM Nutritional supplements present high concentrations of biologically active compounds, which can be beneficial to human health, but they can also be associated to adverse biological effects due to contamination or interactions. Although the awareness regarding the safety of food supplements (WHO, 2004), in USA, a wide range of dietary supplements have been found to be contaminated with toxic plant material, heavy metals, bacteria or adulterated with pharmaceutical analogues (Cohen, 2009).

Unscrupulous manufacturers can put in the market contaminated dietary supplements in retail stores, supermarkets, speciality stores, pharmacies and in the internet, in this case, making it more difficult for the authorities to detected them. Many times, the labels of the NVNM nutritional supplements do not contain any information about the levels of the active or actives substances.

According to Cohen (2009), physicians should maintain a high index of suspicion for supplement-induced adverse effects, even when the components on the label are not known to cause the observed effects.

Although NVNM supplements has a long tradition of use, there is still scarce data. In this line, the European Food Safety Authority (EFSA 2012, 2009) has made available a Compendium of Botanicals reported to contain toxic, addictive, psychotropic or other substances of concern to provide guidance for safety assessment of botanicals and botanical preparations used as plant supplements. This compendium order botanicals, contain their chemicals of concern, makes remarks on adverse/toxic effects and lists the references. However, it does not include toxicological endpoints for individual plant compounds and preparations. In Europe, there is an increasing interest in the enrichment of the information regarding these supplements and the European Union is making efforts in order to fund research projects in this field. PlantLIBRA (PLANT food supplements: Levels of Intake, Benefit and Risk Assessment) is one of these projects, which received funding from the European Union's Seventh Framework Programme and developed the ePlantLIBRA (2014). This is a database which contains information about plant- and plant-food supplements globally, specifically bioactive compounds in botanicals and herbal extracts with putative health benefits and adverse effects (Pumb et al., 2016).

Although the use of multivitamin and multimineral supplements is consistent due to the amount of evidence supporting their positive health influence. However, in the case of NVNM nutritional supplements there is much less evidence of their putative benefit effects and most of these regards to non-plant based nutritional supplements.

Therefore, there is the need of evaluating the recommended doses in order to avoid subtherapeutic or toxic doses, i.e., it is important to establish a cut-off point for active substances included in food products as well as possible interactions with other drugs, supplements or foods and possible adverse reactions. More *in vivo* studies are required to be able to have a broader view of the drawbacks and advantages of supplementation in a case-to-case basis (Kusar and Pravst, 2014).

The groups more prone to present adverse reactions to supplements include those that concomitantly take other medicines, immune compromised, elderly and kidney or liver insufficient people.

The mechanisms of the interaction of *NVNM nutritional supplements* with other supplements, drugs or foods depend on drug factors (pharmacokinetics of the drug, pharmacodynamics or drug/body interaction and physical/biochemical interactions of *NVNM nutritional supplements* and drugs) and supplements' factors (or instance in the case of plant based supplements several factors can contribute for variability such as species, geographical origin, maturity, part of the plant, manufacturing processes, storage conditions, and seasonal variability) (Nafiu et al., 2018).

In the future, it is of major importance that consumers are not exposed to unacceptable risks due to the lack of scientific based health benefits of food supplements or due to their contamination.

In this regard, each NVNM nutritional supplements should have a marker (a bioactive compound) that allows to standardize the products among different manufactures. Moreover, it would be very valuable to indicate the levels of possible contaminants and of metabolites in order to fully understand the toxicological profile.

In light of the available knowledge, NVNM foods supplements are an excellent source of a multitude of putative health benefits but there are concerns regarding their safety and quality that cannot be disregarded. To assure quality is important to follow good agricultural practices (GAP) and good collection practices (GCP) published by WHO (WHO, 2004). Quality also concerns to the effective concentration on bioactive agents and to the interaction of these with other supplements, foods or drugs. In this case, statements about safe use shall be provided in the label of the product. Also recommendations on the use by certain groups of the population (e.g. pregnant, breast-feeding, infants) shall be given. On the other hand, measures shall be taken to avoid any safety concerns due to chemical or microbiological contamination (e.g., with elements, pesticides, mycotoxins, bacteria, sand, soil, radioactivity, drugs, other plants, illegal substances).

Large randomized, double-blind clinical studies need to be conducted on NVNM nutritional supplements, to provide more evidence on the clinical efficacy and safety of these products. The final aim is to assure protection of health and

to correspond to the expectation of consumers in what regards to safety, composition, quality, physiological effects and transparent information that does not encourage over-consumption and allows an informed choice.

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NONVITAMIN AND NONMINERAL NUTRITIONAL SUPPLEMENTS

Edited by

Seyed Mohammad Nabavi and Ana Sanches Silva

Nonvitamin and Nonmineral Nutritional Supplements synthesizes multiple characteristics of featured supplements, including recent findings, to present a comprehensive guide for informing nutritional practices. The book focuses on non-essential nutrients, animal extracts, yeast and fungi extracts, and plant and algae extracts used as supplements, providing an understanding of the positive and negative aspects of each supplement. With these insights into the impact of dietary supplementation on human health and discussing future trends and challenges of dietary supplements, *Nonvitamin and Nonmineral Nutritional Supplements* offers an essential resource for students and researchers concerned with nutritional health.

This book:

- provides reliable information on available supplements to inform nutritional practices;
- presents each supplement's sources, availability, health benefits, drawbacks, and possible interactions with other supplements, food, or drugs; and
- serves as a guide to non-essential nutrients, plant and algae extracts, animal extracts, including bee products and shark cartilage, as well as supplements from yeast and fungi.

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