Renal, Metabolic and Urologic Disorders

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Part 1

Medicinal Plants for the Treatment of Metabolic Disorders

Nova Medicine & Health

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MEDICINAL PLANTS FOR THE TREATMENT OF METABOLIC DISORDERS

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RENAL, METABOLIC AND UROLOGIC DISORDERS

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PART 1

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PREFACE

This book provides a complete review of multiple metabolic disorders and the use of phytochemicals for their prevention and treatment. Moreover, some plant species used for the extraction of these chemical components are listed and described. When describing metabolic disorders, authors describe main health alterations of each one, providing the most current figures and statistics worldwide. Medicinal plants used in traditional medicine for treatment and scientific information related to their potential to prevent or treat each conditions are also discussed. Authors also include a complete scientific background check, complete analysis of all phytochemicals recommended for each disorder, a description of the mechanism(s) of action and in vivo and/or in vitro studies. Within this volume, a series of 16 species, recommended to treat or prevent the previously described disorders, are presented with their botanical information, traditional use in different cultures and countries, and the available scientific information validating their health benefits.

Chapter 1 - Diabetes mellitus (DM) is a multifactorial disease; it represents a set of autoimmune, metabolic, and genetic disorders, which include hyperglycemia and insulin resistance. There are two types of DM; type 1, which is due to the acute destruction of insulin-producing cells in the pancreas, and type 2, in which insulin action or secretion becomes insufficient, favoring hyperglycemia. The incidence and severity of DM are increasing worldwide and represent a burden for society in terms of economy and welfare. Plant products have been used for a long time to treat diabetes; even today, most of the world's population use herbal products for the treatment of DM. Unlike other diseases, DM needs treatment throughout life, and despite the existence of a large number of antidiabetic drugs and injectable insulin, anti-diabetic therapies are not entirely effective, particularly in the long term, mainly because of adverse reactions and loss of efficacy over time. About 2000 ethnomedicinal plants have been tested against DM, proving to be safe and effective when appropriately consumed, together with a change in lifestyle, which includes increasing physical activity, ingesting high-quality meals, and reducing stress. From these plants, more than 1000 have been scientifically tested, and around 120 are subject to additional studies. In this chapter, the authors address those plants in which scientific trials have already been conducted, providing relevant information for the development of Phyto-pharmacological products with enhanced efficiency and safety standards. Moreover, since more than one active ingredient is present in the plants, almost all of them show additional pharmacological properties; in most cases, these associated activities are also beneficial.

Chapter 2 - Medicinal plants have progressed through history as a reliable source of bioactive compounds with a potentially positive effect on health. The advantages of this type of medication are its efficacy, safety, and acceptance by a majority of consumers. Medicinal plants play an essential role in health systems in many parts of the world. Its use is varied: directly as plant parts (leaves, stems, roots, fruit, etc.) or through alternative processes such as infusions or preparations (macerated, ointments, etc.) destined for ingestion or application on the affected area. These effects derive from the presence of phytochemicals such as sterols, alkaloids, polyphenols, and carotenoids, among others. In consequence, these phytochemicals are potent agents against metabolic diseases such as diabetes, obesity, and hypercholesterolemia. The latter pathology is characterized by high blood cholesterol levels produced by an increase in the concentration of lipoproteins, usually low-density lipoproteins (LDL) with normal or low levels of high-density lipoproteins (HDL). Hypercholesterolemia is considered a risk factor for cardiovascular diseases. Because of this, diverse research has been developed that reports the close relationship between medicinal plant consumption and a reduction in cholesterol levels.

Chapter 3 - Common beans (*Phaseolus vulgaris*) are especially consumed in Asia (46%) and Mesoamerica (34%), but less in Africa (17%) and other parts of the world. Usually, their dried seeds are cooked before consumption, which inactivates readily antinutritive peptides like protease-inhibitors. As legumes, common beans are known for their high protein content (15-30%), from which by normal digestion derive bioactive peptides with, among others, antimicrobial, antihypertensive, or immunomodulatory effects. Recently, a <3 kDa peptide fraction exhibited potential antidiabetic effects by inhibiting α-amylase and α-glucosidase. Additionally, from the total fiber content (14-19%), about 1/3 are soluble fibers (like pectin, gums, inulin-type fructans, and some hemicelluloses), which contribute to a slow postprandial intestinal glucose-uptake - a property, which supports an antidiabetic action. Common beans also have a high content of polyphenols (up to 1%), especially flavonoids, which are well known for their antioxidant capacity, thus protect against oxidative stress and related diseases like cardiovascular diseases. Moreover, these polyphenols are antimutagenic and antiproliferative hence exhibit anti-cancer properties. This review highlights the complementary and overlapping modes of action of common bean constituents and that dietary plants produce not only nutrients but also phytochemicals; thus they have not only nutritive value but also a health-promoting effect.

Chapter 4 - Watercress (*Nasturtium officinale* L.) is a perennial plant that belongs to the family of the *Brassicaceae* and is cultivated for its leaves, which are principally used as salad greens or garnishes. It is used for culinary purposes by people almost all around the world. It has a strong flavor and is rich in vitamins, mainly vitamin C, but also A, B1, B2, and E. It also has minerals, gluconasturtiin, and phenolic compounds, and others phytochemicals. Watercress can inhibit tumorogenesis by modulating the metabolism of carcinogens. Watercress is one of the most important herbal medicines used for the treatment of some diseases like type 1 diabetes mellitus, type 2 diabetes mellitus, bronchitis, diuresis, and others.

Chapter 5 - Watercress (*Nasturtium officinale* L.) is a perennial plant that belongs to the family of the *Brassicaceae* and is cultivated for its leaves, which are principally used as salad greens or garnishes. It is used for culinary purposes by people almost all around the world. It has a strong flavor and is rich in vitamins, mainly vitamin C, but also A, B1, B2, and E. It also has minerals, gluconasturtiin, and phenolic compounds, and others phytochemicals. Watercress can inhibit tumorogenesis by modulating the metabolism of carcinogens. Watercress is one of

the most important herbal medicines used for the treatment of some diseases like type 1 diabetes mellitus, type 2 diabetes mellitus, bronchitis, diuresis, and others.

Chapter 6 - *Cinnamon* is a common species well appreciated due to its unique flavor and taste, it is consumed in tea preparations from harvested sticks or as specified in pulverized. It is largely accepted worldwide for many centuries, its flavor is mainly attributed to its chemical composition where cinnamaldehyde plays an important role not only as a flavor source, it also has a remarkable antioxidant effect that scavenges Radical Oxygen Species (ROS) responsible of cancer. Other compounds as trans-cinnamic acid and polyphenols as proanthocyanidins confer to cinnamon important biological activities such as antifungal (against *Aspergillus flavus*), antibacterial (against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*), antiosteoporotic, emmenagogue, reductor agent of blood glucose, total cholesterol and triglycerides levels enhancing agent of HDL cholesterol levels, anti-inflammatory agent and other interesting activities. Cinnamon also has been used in the treatment of nausea, diarrhea, diabetes, frigidity, cough, rheumatism, neuralgia, vaginitis. Nevertheless, secondary effects may be observed if it is consumed in larges amounts due to the presence of coumarins, although there are more benefits than secondary effects. Also, emerging technologies are using microencapsulation techniques to preserve cinnamon characteristics because many bioactive compounds are volatile.

Chapter 7 - The Roselle (*Hibiscus sabdariffa* L.), also is known as jamaica rose, Abyssinian rose, roselle flower, sarent, and alleluia, is a hibiscus of the family of malvaceae. Hibiscus is successfully cultivated in Mexico, Central America and Southeast Asia, including South China. *Hibiscus* is known for its use as a traditional drink in Mexico. This plant is rich in a variety of phytochemicals or nutraceutical compounds such as phenolic compounds such as anthocyanins and procyanidins, strong antioxidants that are the cause of intense red color and which represent a potential alternative for the replacement of synthetic dyes in foodstuffs. Additionally, it has a significant content of vitamins A and C, a large number of minerals, citric, and malic acid. The antioxidants found in *Hibiscus* makes it a food that can help to combat various diseases. It could eliminate alcohol discomfort by stimulating the action of the liver and blood vessels, the absorption of certain minerals, lowers blood pressure, and because of this, it is used as a cardiac tonic, diuretic, antiseptic, analgesic, anti-inflammatory, antimicrobial, astringent, healing, digestive, depurative, emollient, sedative, mild laxative, weight reducer, detoxifier, antioxidant, toner, stimulant, aphrodisiac, and vasodilator.

Chapter 8 - The *Salvia* genus is widely distributed throughout the world, found in temperate, subtropical, and tropical regions. *Salvia* species have been traditionally used in folk medicine since ancient times due to their medicinal properties against some ailments. Currently, there are thousands of bioactive compounds derived from different sage species, such as phenolics, flavonoids, and terpenoids, which have been isolated and studied to be used as potential biopharmaceutical agents against different noncommunicable diseases. Furthermore, *Salvia* species are of commercial interest in the pharmaceutical and food industries due to their antioxidant capacity. This chapter provides relevant information on the *Salvia* genus and some of the species that make up this genus as well as its compounds and its effect related to metabolic dysregulation, including metabolic syndrome, cancer, and neurogenerative diseases.

Chapter 9 - *Beta vulgaris* L. belongs to the Chenopodiaceae family, and it's generally known as beetroot or garden beet and has several varieties with bulb colors ranging from yellow to red. It is considered as one of the ten most essential vegetables around the World, due to the presence of essential components such as fiber, minerals, vitamins, nitrate, ascorbic acid,

carotenoids, phenolic acids, polyphenols and betalains making its consumption highly beneficial to a human body. Traditionally, beetroot is usually consumed in juice, or boiled for salads, and processed for different uses as food, however nowadays it is recognized as a functional food, due to the properties it has shown as an antioxidant, anti-inflammatory, and for its anticoagulant activity among others. The objective of this chapter is to provide a brief overview of the phytochemicals and bioactivities present in beetroot and their possible benefits on human health.

Chapter 10 - Barberry (*Berberis vulgaris*) is a widely distributed species in Europe, Africa, Asia, and North America. This species has been used in traditional medicine for the treatment of gastritis and peptic ulcers, gallstones, kidney stones, and liver problems, rheumatism, diarrhea, and skin infections, but also to improve immune function. Within this chapter, its applications on diabetes –T2DM-, obesity, hypertension, dyslipidemias, hyperuricemia, cancer, Alzheimer's, and Parkinson's diseases, will reviewed. Berberine appears to have the most dramatic effects on blood insulin levels, cholesterol levels, inflammatory processes, and tumorigenesis. As these underlie or are components of a variety of metabolic disorders, berberine has the potential to have wide-reaching utility in clinical settings. Despite the lack of clinical studies, its relevance in traditional medicine has located berberine as a well-known supplement for the aid of these and other health conditions.

Chapter 11 - Phenolic compounds are associated with a reduction of risk factors related to metabolic syndrome. Cocoa bean shell is one of the main byproducts generated from cocoa processing and is an important source of phenolic compounds. This chapter discusses (i) the optimum parameters to extract polyphenols from cocoa bean shell and (ii) the development of a method for the identification of phenolic acids in cocoa bean shell infusion using capillary electrophoresis. Extraction was performed using reflux, and ultrasound-assisted extraction and the phenolic acid profile was validated according to the guidelines established by the International Council for the Harmonization of Technical Requirements for the Registration of Pharmaceutical Products for Human Use (ICH). Results showed that the optimum conditions to obtain polyphenols were in reflux using water as a solvent for 5 minutes (262.76 ± 20.66 mg GAE/ 100 g). Further, satisfactory results were obtained for the phenolic profile. Nevertheless, no phenolic acids (gallic, syringic, benzoic, chlorogenic, and caffeic) were detected in the infusion of cocoa bean shell. This study revealed that cocoa bean shell represents a high potential of use in the food industry, principally in the field of functional food development and also this work provides a new alternative, which allows identifying quickly and effectively the phenolic acids that may be present in an infusion.

Chapter 12 - The social and economic cost to treat type-2 diabetes (T2DM), one of the major public health problems in the world, is high. There is an urgent need to develop novel strategies to prevent and find new culturally friendly low-cost therapeutic alternatives to treat the disease. Several studies have shown that peptides derived from legume proteins have metabolic properties, including hypoglycemic, lipid-lowering, anti-carcinogenic, anti-inflammatory, and anti-thrombotic. People who consume diets rich in legumes significantly decrease total serum cholesterol levels compared to control groups; also, peptides derived from legumes show inhibitory properties of enzymes involved in the pathophysiology of T2DM and cardiovascular diseases such as angiotensin-converting enzyme and dipeptidyl peptidase IV inhibition. These studies demonstrate the importance of legumes in the diet and their potential as functional foods that could contribute to the prevention and treatment of metabolic diseases, including diabetes. For several years, the authors' research group has developed studies with

Andean crops to determine health beneficial properties in these foods that can be applied in the prevention and treatment of the most prevalent diseases in Ecuador, including cardio-metabolic diseases. Here the authors discuss clinical and molecular evidence for the use of the legume *Lupinus mutabilis* Sweet in the treatment of T2DM. Current evidence indicates that consumption of legumes including Andean *L. mutabilis* can contribute to the prevention and treatment of T2DM. It is important to educate and promote the consumption of legumes by the general population.

Chapter 13 - Genus *Allium* belongs to a group of species of Liliaceae Family. This valuable genus has widely been used in the traditional medicine of several cultures to treat some types of human diseases. Recent investigations performed with genus *Allium* showed several pharmacological activities such as antidiabetic, antihypertensive, anti-inflammatory, antiobesity, antioxidant, antihypercholesterolemic, anticancer (prostate, colorectal, breast and esophageal), immune system activator, antifungal and bactericide. Organosulfur and polyphenols compounds are considered secondary metabolites that are responsible for several pharmacological activities. Therefore, the main purpose of the present chapter is to show current research concerning successful, useful, and representative studies that demonstrate the pharmacological actions of the genus *Allium*.

Chapter 14 - The use of plant products and derivatives for prevention and treatment of metabolic syndromes has increased in recent years. In this context, the effect of extraction temperature, particle size, and solid/solvent ratio on aqueous extraction of diterpenoid glycosides from *Stevia rebaudiana* Bertoni leaves is discussed in this chapter, in order to establish ideal conditions that provide further applications to the aqueous extract such as nutraceuticals products focused on dealing with health problems. A 2^3 full factorial design of 8 experimental runs was applied to describe the concentration of stevioside as a function of assessed extraction parameters. The extraction was carried out in stainless steel digestor equipment. Organic solvents were used for clarification of raw extracts. UV-Vis screening of raw and clarified extracts was performed in a Synergy HTX multi-mode microplate reader. Rebaudioside A, and stevioside identification, and quantification were performed in a High-Performance Liquid Chromatography (HPLC) Perkin Elmer Series 200. The data analysis and graph plotting were performed in MINITAB 17.0 statistical software. The result obtained indicates that particle size and solid/solvent ratio mainly influence on aqueous extraction of stevioside. Additionally, the highest achieved stevioside concentration (3.30 g/100 g dried leaves) showed that natural sweeteners could be obtained at 60°C, 250µm, 1/10 kg/L by digestion, and the extraction conditions found possibly could be applicable on an industrial scale with the aim of substitute artificial sweeteners.

Chapter 15 - *Nephelium lappaceum* L., commonly known as achotillo, rambutan, or Chinese sucker is a fruit native to Asia whose cultivation has spread in many countries. In addition to its use as food due to the chemical components it possesses, various therapeutic properties are attributed to it. Among these are its use to combat obesity and diabetes is, which is why it is also considered a source of nutraceutical products. The objective of this work was to study two varieties of rambutan grown in Ecuador and to evaluate their pharmacognostic and phytochemical characteristics and their use as a source of anti-obesity nutraceutical products. The aerial parts of two varieties of adult plants in the flowering-fruiting state from the province of Los Rios were used. The organoleptic characteristics and dimensions of the organs; histological analysis of leaves, and fruit bark, physicochemical parameters, phytochemical screening and content of phenols and flavonoids were evaluated. Macroscopically the varieties

presented slight differences in the dimensions and coloration; however, histologically there were no differences. The physicochemical parameters showed a discrepancy between the varieties and different plant organs.

The content of phenols and flavonoids established the difference between the two varieties. Differences among the organs of the two varieties that underwent qualitive and quantitive analysis were found and thus it was established that the bitter type had the highest concentrations of the active ingredients responsible for battling obesity.

Chapter 16 - Several species of ferns from the *Polypodium* genus are known under the generic name "Calahuala." These ferns grow in many tropical places all over the world and the rhizomes or leaves are empirically used in traditional medicine for the treatment of many diseases. *Polypodium* genus has been the object of scientific interest especially for the properties shown by its extracts in preventing the harmful effects of UV radiation and the effectiveness in the treatment of psoriasis and skin problems; however, many of other biological properties remain scientifically undocumented. The objective of this chapter is to provide a general overview of the *Polypodium* genus and its reported beneficial properties to human health. The authors also highlight some of its biological properties such as the tumor inhibition that needs to be supported with scientific evidence.

Chapter 17 - Epilepsy is a life-shortening brain disorder that currently affects ~ 1% of the worldwide population. Despite the availability of several antiepileptic drugs (AEDs), severe side effects such as cognitive and affective disorders, teratogenicity, hepatotoxicity, among others, have been reported after chronic administration. Also, some patients remain refractory to the available AEDs. Such is the case of metabolic epilepsy. Hence, there is a current need for the discovery of novel active principles with minimal or no adverse side effects. Nature is an exciting source of potential drug candidates for the treatment of pharmacoresistant epilepsy (PRE) due to the highly diverse and complex chemical structures of bioactive vegetal compounds. In this context, the authors analyzed the rhizome powder of *Curcuma longa*, commonly known as turmeric. Until the authors' study, the anticonvulsant properties of turmeric were exclusively attributed to its curcuminoids. For the first time, the authors revealed the anticonvulsant properties of turmeric oil and its main bisabolene sesquiterpenoids, ar-turmerone, α-, β-turmerone, and α-atlantone. Thus, the present chapter discusses the botanical aspects of turmeric, the chemical composition and phytopharmacological aspects of turmeric, curcumin and turmeric oil as well as the authors' results obtained from the anticonvulsant activity characterization of turmeric oil and ar-turmerone. The authors' findings support further characterization of the anticonvulsant properties of these active compounds and demonstrate the usefulness of the zebrafish and mouse models for searching novel AEDs. Also, the potential therapeutic application of turmeric oil and ar-turmerone for the treatment of metabolic epilepsy is discussed.

Chapter 18 - *Capsicum* spp. have been consumed regularly all over the world not only for their nutritive properties but also for its physiological and pharmaceutical uses. *Capsicum* spp. is a good source of different phytochemicals including capsaicinoids, capsinoids, flavonoids, phenolic compounds, carotenoids, among others. The present chapter elucidates the mechanism of the different phytochemicals of *Capsicum* spp. face to obesity, cancer, diabetes, hypertension, hypercholesterolemia, and hypertriglyceridemia. *In vitro* and *in vivo* studies showed that various phytochemicals of *Capsicum* spp., especially capsaicin have anti-obesity activity, anti-diabetic activity, anti-hyperlipidemic activity, anti-hypertensive activity, and anti-

carcinogenic activity. Therefore, *Capsicum* sp., is a promising medicinal plant to develop natural drugs against metabolic disorders.

Chapter 19 - Metabolic Syndrome (MetS) is defined as a set of risk factors (obesity, hyperglycemia, high triglyceride levels, low HDL cholesterol levels, and hypertension), which are considered as precursors of cardiovascular and metabolic diseases. The objective of this work was to determine the prevalence of Metabolic Syndrome in adolescents with obesity in a public junior high school in San Juan del Río. The anthropometric measurements as indicators of obesity (Waist Circumference, WC); and measurements of blood pressure, fasting glucose, HDL cholesterol, and triglycerides were made in 75 adolescents of both genders, with an average age of 13.07 years. The prevalence of MS was 21.33% (7 men and nine women). The age group with the highest prevalence was 14 years, with 6 cases. High blood pressure was detected in 26.67% of participants, while altered levels of triglycerides, HDL cholesterol, and glucose were found in 21.33%, 17.33%, 53.33%, respectively. Obesity in children and adolescents has become a growing risk factor for the development of multiple complications for health, including the metabolic syndrome that has had a significant increment in recent years. So, it is important to implement appropriate activities in health and specific action plans aimed at guiding adolescents and teaching the administrative school staff to know the impact of this and other metabolic diseases.

Chapter 20 - Parkinson's disease (PD) is a progressive neurological disorder that presents several motor and non-motor features in a variable degree, which directly affects the patient's health-related quality of life, affecting mainly adults and the elderly. Parkinson's disease patients lose 60 to 80 percent of the dopamine-producing cells in the substantia nigra, an important area of the brain that regulates muscle movement. On the other hand, the non-motor alterations are more significant, among which neuropsychiatric, sensory or gastric-intestinal, cognitive (like depression and anxiety) symptoms stand out. Modest personality changes can also be present over time in this kind of patient. PD is considered a multifactorial disease resulting from both environmental factors and genetic susceptibility. Several studies have demonstrated that age is an unequivocal risk factor for this disease as the incidence increases with age increasing. The etiology of the PD is not clear; for that reason, the disease remains incurable. Levodopa is the most used medication for the treatment of PD, used to simulate the dopamine effect. Different studies have identified compounds with antiparkinson activity in *Mucuna pruriens*, *Nardostachys jatamansi*, *Withania somnifera*, *Gingko biloba*, and *Bacopa monnieri*, species that represent a potential alternative for the treatment of this disease.

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Chapter 1

DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus (DM) is a multifactorial disease; it represents a set of autoimmune, metabolic, and genetic disorders, which include hyperglycemia and insulin resistance. There are two types of DM; type 1, which is due to the acute destruction of insulin-producing cells in the pancreas, and type 2, in which insulin action or secretion becomes insufficient, favoring hyperglycemia. The incidence and severity of DM are increasing worldwide and represent a burden for society in terms of economy and welfare. Plant products have been used for a long time to treat diabetes; even today, most of the world's population use herbal products for the treatment of DM. Unlike other diseases, DM needs treatment throughout life, and despite the existence of a large number of antidiabetic drugs and injectable insulin, anti-diabetic therapies are not entirely effective, particularly in the long term, mainly because of adverse reactions and loss of efficacy over time. About 2000 ethnomedicinal plants have been tested against DM, proving to be safe and effective when appropriately consumed, together with a change in lifestyle, which includes increasing physical activity, ingesting high-quality meals, and reducing stress. From these plants, more than 1000 have been scientifically tested, and around 120 are subject to additional studies. In this chapter, we address those plants in which scientific trials have already been conducted, providing relevant information for the development of Phyto-pharmacological products with enhanced efficiency and safety standards. Moreover, since more than one active ingredient is present in the plants, almost all of them show additional pharmacological properties; in most cases, these associated activities are also beneficial.

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DIABETES MELLITUS DEFINITION

Diabetes mellitus is described as a series of conditions that include hyperglycemia and insulin resistance; it is divided into type 1 and type 2. Type 1 diabetes mellitus (T1-DM), previously known as juvenile or insulin-dependent diabetes, accounts for about 5 to 10% of all diabetes cases. It is due to acute destruction of the β-cells of the pancreas (responsible for producing insulin) through autoimmune diseases, leading to a sudden and total insulin deficiency (Figure 1) (American-Diabetes-Association 2019).

Type 2 diabetes mellitus (T2-DM) is known as non-insulin-dependent diabetes or adult diabetes (although it is present in overweight children, too), which accounts for the majority of cases of diabetes. In this type, insulin secretion remains, but not in adequate concentrations to maintain blood glucose levels under a healthy range, it is due not only to the decrease of insulin secretion, but also to a reduced response to insulin, known as insulin resistance, and occurs in several situations, usually obesity and overweight. In people with T2-DM, both insulin resistance (insufficient action of the insulin) and insulin deficiency (inadequate insulin secretion) favor rising blood glucose levels (WHO 2013).

TYPES OF DIABETES

Type 1

T1-DM involves the destruction of pancreatic β-cells leading to diabetes; in this type of diabetes, it is necessary to administer insulin to survive and to prevent the development of ketoacidosis, coma, and death (American-Diabetes-Association 2019). T1-DM mediated by the immune system is the classic type, which can occur at any age, and results from autoimmune destruction mediated by the same pancreatic β-cells. The process is characterized by the presence of islet cell autoantibodies (ICA), antibodies to glutamic acid decarboxylase (anti-GAD), islet-2 antigen (IA2), or insulin autoantibodies that identify the autoimmune process associated with the destruction of β-cells. Other autoimmune disorders, such as Grave's disease, Hashimoto's thyroiditis, and Addison's disease may be related to T1-DM (Atkinson and Maclaren 1994). The rate of β-cell destruction is quite variable, usually rapid in children and slower in adults. Typically, T1-DM requires insulin therapy from the time of onset in both adults and children, but a slow progressive form, latent autoimmune diabetes in adults (LADA), is also possible and is well described (Tuomi et al. 1993). Blood glucose levels in LADA patients can be controlled initially by changes in lifestyle and oral hypoglycemic agents and, therefore, can be masked as T2-DM. However, if compared with the typical T2-DM patient, LADA patients are thinner and show a faster progression of the disease. Importantly, markers of autoimmunity (most commonly anti-GAD antibodies) are present in these patients, and therefore, LADA is classified as type 1 autoimmune diabetes mellitus (Pieralice and Pozzilli 2018). Some forms of T1-DM do not show a known etiology or evidence of autoimmunity. Some of these patients present permanent insulinopenic and are prone to ketoacidosis (McLarty et al. 1990).

Type 2

T2-DM, also known as “non-insulin-dependent diabetes” or “adult diabetes,” accounts for 90-95% of all diabetes cases. This form includes people who show a relative (rather than absolute) insulin deficit and peripheral insulin resistance. At least initially, and often during their lifetime, these patients may need insulin treatment to survive (American-Diabetes-Association 2019). The World Health Organization defines T2-DM as the inability of the body to respond adequately to the action of insulin produced by the pancreas, representing around 90% of all cases of diabetes worldwide. It occurs more frequently in adults but is also seen in young people (Mathers and Loncar 2006).

The symptoms of T2-DM may be identical to those of T1-DM, including increased thirst, frequent urination, fatigue, slow wound healing, recurrent infections, and tingling or numbness of the hands and feet. However, the onset of T2-DM is usually gradual without the acute metabolic disorders that appear in T1-DM, making it very difficult to determine the exact time of onset. The causes of T2-DM are not fully understood, but there is a strong link between overweight and obesity as well as with advanced age, ethnic origin, and family background. Some risk factors include 1an excess of adiposity (obesity), malnutrition and inadequate diet, lack of physical activity, prediabetes or impaired glucose tolerance (ATG), smoking, and a history of gestational diabetes mellitus (GDM) with exposure of the fetus to high levels of glucose during pregnancy. Among the dietary factors, recent evidence also suggests that there is an association between the high consumption of sugary drinks and the risk of T2-DM (Salvador et al. 2008, Roglic and Unwin 2010, Cho et al. 2018).

Other factors include low consumption of fruits and vegetables, whole grains, and dietary fiber, as well as high energy intake in the form of saturated fats (Mozaffarian 2016). In general, according to the latest research, moving away from this type of food in favor of dietary patterns such as, but not limited to, the Mediterranean diet is highly recommended (Forouhi and Wareham 2014, Ley et al. 2014).

The most crucial part of treating T2-DM is a healthy lifestyle, which involves switching to a healthy diet, increasing physical activity, a plan to quit smoking, and a healthy body weight (Basu et al. 2013). If the attempts to change the lifestyle are not enough to control blood glucose levels, a pharmacological prescription to treat hyperglycemia becomes necessary, with metformin being the most used initial treatment worldwide (Marin-Penalver et al. 2016).

Obesity is widely associated with an increased risk of developing insulin resistance and T2-DM (Reaven 1988). In obese individuals, adipose tissue releases higher amounts of non-esterified fatty acids, glycerol, hormones, proinflammatory cytokines, and other factors involved in the development of insulin resistance (Perley and Kipnis 1966). When the dysfunction of pancreatic insulin-producing β-cells accompanies insulin resistance, blood glucose levels cannot be controlled (Kahn et al. 1993). In addition to adipocyte-derived factors, increased release of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and additional products from macrophages and other cells in adipose tissue might also have a role in the development of insulin resistance (Fain et al. 2004). TNF-α and IL-6 act through classical receptor-mediated processes to stimulate both the c-Jun amino-terminal kinase (JNK) and the IκB kinase-β (IKK-β)/nuclear factor-κB (NF-κB) pathways, resulting in upregulation of potential mediators of inflammation that can lead to insulin resistance. Pathways involving the suppression of cytokine signaling (SOCS) proteins (Ueki, Kondo, and Kahn 2004, Mooney et al. 2001) and inducible nitric oxide synthase (iNOS)

(Perreault and Marette 2001) may be involved in mediating cytokine-induced insulin resistance. Secretion of these pro-inflammatory proteins, particularly MCP-1 by adipocytes, endothelial cells, and monocytes, increases macrophage recruitment and thereby contributes to a feedforward process (Weisberg et al. 2003). Also, the distribution of body fat is a critical determinant of insulin sensitivity. While simple obesity is usually associated with insulin resistance, insulin sensitivity also varies markedly in thin individuals due to differences in the distribution of body fat (Carey et al. 1996, Cnop et al. 2002). Lean individuals with a more peripheral distribution of fat are more sensitive to insulin than thin individuals with a central fat distribution, this is, in the abdominal and chest areas (Baccanari et al. 1975).

INSULIN RESISTANCE IN T2-DM

The main characteristic of T2-DM is insulin resistance, a condition in which cells do not respond adequately to insulin (Boucher, Kleinridders, and Kahn 2014). This deficiency in insulin signaling is caused by different alterations, such as mutations and/or post-translational modifications of the insulin receptor, the insulin receptor substrate (IRS), or effector molecules located downstream. Among the most common alterations in insulin resistance are the decrease in the number of insulin receptors and their catalytic activity; the increase in the phosphorylation state in Ser/Thr residues of the insulin receptor and the IRS; the increase in the activity of phosphatases of Tyr residues, mainly PTP-1B, involved in the dephosphorylation of the receptor and the IRS (Hubbard 2013); the decrease in the activity of the PI3K and Akt kinases, and defects in the expression and function of GLUT-4. These alterations reduce the incorporation of glucose in muscle and adipose tissue and promote metabolic alterations (Manning and Cantley 2007).

A significant factor that contributes to the development of insulin resistance is the hyperphosphorylation of Ser/Thr residues of the IRS proteins (Montagnani et al. 2002). The hyperphosphorylation of the IRS reduces its interaction with PI3K, altering the phosphorylation and activation of the Akt kinase (Dimmeler et al. 1999). Additionally, it has been reported that the phosphorylation of Ser/Thr residues of the IRS accelerates its degradation. Agents such as proinflammatory cytokines, saturated fatty acids (SFA), amino acids, endothelin 1, angiotensin II (Ang II), and hyperinsulinemia (Lee and Ragolia 2006) increase the activity of kinases, such as several isoforms of PKC, the stress kinase JNK, mTOR, PKA, and MAPK, which phosphorylate IRS (Odegaard and Chawla 2013). Scientific evidence indicates the importance of the increased phosphorylation of the IRS in clinical studies performed with obese patients, where the expression of IRS-1 decreases around 54%. Biochemical and genetic evidence indicates that the hyperphosphorylation of Ser/Thr residues in the IRS-1 can reduce up to 50% Tyr phosphorylation stimulated by insulin (Zick 2005). This level of inhibition is sufficient to cause glucose intolerance progressing to T2-DM, especially if pancreatic β-cells fail to provide adequate compensatory hyperinsulinemia. Moreover, hyperinsulinemia itself may aggravate Ser/Thr phosphorylation of IRS-1 through the activation of the PI3K/Akt, PKC-τ/-λ, or mTORC1/p70S6k pathways, which participate in the regulation of insulin signaling (Kido et al. 2000).

Inflammation and Insulin Resistance

Inflammation refers to a physiological process of protection; it is presented to control physical, chemical, or biological aggressions, and is characterized by a high number of leukocytes and/or an increase in the levels of proinflammatory cytokines (Ye 2013). A large amount of experimental and clinical evidence has been presented indicating that obesity induces alterations in adipose, liver, and muscle tissue leading to a low-grade chronic inflammatory response, which contributes to insulin resistance and systemic metabolic dysfunction.

Obesity is defined as the presence of an excessive amount of body fat or adipose tissue, which is manifested by an increase in body weight associated with a greater distribution of visceral adipose tissue (Shoelson, Lee, and Goldfine 2006). In obese patients, an increase in the levels of inflammatory markers and a correlation between these markers and the presence of abdominal adiposity has been detected (Visser et al. 1999, Park, Park, and Yu 2005). In a state of obesity (mainly visceral obesity), there is an increase in the accumulation of lipids, particularly in adipose tissue, which causes hypertrophy and hyperplasia, and the alteration in the secretion of adipokines and proinflammatory cytokines, as well as the aberrant release of free fatty acids (FFAs) (Xu et al. 2003). The FFAs and proinflammatory cytokines act in metabolic tissues, such as liver and muscle, modifying the inflammatory response, as well as the metabolism of lipids, thus contributing to insulin resistance. Also, obesity increases the infiltration of macrophages in adipose tissue, and this contributes substantially to the production and secretion of cytokines (Weisberg et al. 2003). The proinflammatory cytokines secreted in adipose tissue and by macrophages include resistin, tumor necrosis factor α (TNF-α), interleukins (IL) 6, 18 and 1β, monocyte chemotactic protein 1 and Ang II (Trayhurn and Wood 2004, Shoelson, Lee, and Goldfine 2006, Ouchi et al. 2011). These factors contribute to the local and systemic state of inflammation associated with obesity and, as in the case of TNF-α, IL-6, IL-18, IL-1β, and Ang II, directly induce insulin resistance (Morino, Petersen, and Shulman 2006, Kalupahana and Moustaid-Moussa 2012). In the case of cytokines such as TNF-α, IL-6 and IL-1β, insulin resistance is induced through multiple mechanisms, such as the activation of Ser/Thr kinases, the decrease in the expression of IRS-1, GLUT-4 and gamma receptor activated by the peroxisome proliferator, or the expression and activation of SOCS-3 (Senn et al. 2003, Steppan et al. 2005, Plomgaard et al. 2005, Kwon and Pessin 2013, Boucher, Kleinridders, and Kahn 2014). It has been reported that the activation of the TLR-4/NF-κB pathway in macrophages by AGL action promotes the synthesis and secretion of cytokines such as IL-6, TNF-α IL-1β, and IL-18, which contribute to the inflammatory state of adipose tissue during central obesity (Suganami et al. 2007). These data reinforce the hypothesis that the immune system plays a crucial role in the development of insulin resistance as a consequence of T2-DM (Watanabe, Nagai, and Takatsu 2013).

Gestational Diabetes

Diabetes is usually the medical situation complicating pregnancy, which in turn results in several complications to the infant and the mother. Pregnancy is associated with decreased sensitivity or insufficient secretion of insulin, even in normoglycemic women (American-Diabetes-Association 2019). Thus, diabetes in pregnancy can become a severe problem if not

managed properly. Based on the current literature and reports, maternal diabetes can be classified into three major (Kalter 2011):

1. Pregestational diabetes mellitus (preexisting T1-DM or T2-DM)
2. Gestational diabetes mellitus
3. Diabetes mellitus in pregnancy (T1-DM or T2-DM occurring first during pregnancy)

Pregestational Diabetes Mellitus

This term denotes a previous diabetic condition. It has long been known that the severity of maternal diabetes greatly influences the incidence of maternal and fetal complications (Gilmartin, Ural, and Repke 2008, Alfadhli 2015). When assessing severity, the following factors should be considered: duration of diabetes, age of the mother at the onset of diabetes, presence or absence of vascular complications, and treatment methods. On these concepts of preconceptionally factors, a method of clinical classification was established in 1949 and later modified in 1965 and 1971. This classification tried to predict the pregnancy outcome according to various metabolic, obstetric, and other risk factors and was classified from A (better) to F (worst), according to Warrner and Cornblath (1969).

Women who have pregestational diabetes (both T1-DM and T2-DM) are considered high-risk pregnancies. Pregestational diabetes, especially T1-DM, is responsible for a high morbidity rate since the physiology of glucose metabolism alters during pregnancy (Kitzmiller et al. 2008). A reduction in fasting glucose levels has been observed as a result of increased renal clearance and decreased gluconeogenesis. This situation increases the risk of women suffering from T1-DM, especially in the first trimester of pregnancy. Insulin requirements and insulin resistance in pregnant women increase as pregnancy progresses due to placental hormones like cortisol, hPL, and progesterone (Abourawi 2006). For instance, pregnant women who are not suffering from preexisting DM enhance their postprandial insulin secretion by 50%, however, pregnant women with DM fail to produce a sufficient amount of insulin to counteract increased blood glucose levels due to absence of pancreatic β cells (T1-DM) or reduction of a β cell/insulin resistance state (T2-DM). Thus, pregnant women with T1-DM will require higher doses (up to three times) of insulin compared to their normal dose, while T2-DM women will need an adequate quantity of insulin or oral hypoglycemic agents to attain normoglycemia during pregnancy. Once the baby is delivered, women with diabetes can return to their pre-pregnancy state (Kitzmiller et al. 2008, Patel, Hameed, and Banerjee 2014, Lambert and Germain 2010, Wahabi et al. 2012).

Higher frequency of congenital abnormalities, macrosomia, preeclampsia, stillbirth, infections, and other complications in the fetus, associated with preexisting DM, are also responsible for long-term complications in the mother (Rosca 2017). The risk of maternal complications associated with GDM is higher in the presence of preexisting microvascular diseases like retinopathy and nephropathy. Diabetic ketoacidosis is a common, severe problem, which is found in women with T1-DM, and also responsible for the high perinatal mortality rate. Diabetic retinopathy and renal impairment are linked to preexisting diabetes. Miscarriage is another problem in pregestational diabetic women. Diabetic ketoacidosis due to pregnancy-induced lipolysis is more common in pregnant women with T1-DM (Buchanan, Xiang, and Page 2012, Kitzmiller et al. 2008, Lambert and Germain 2010).

When T2-DM is the pre-existing condition, both insulin resistance and secretion are the predominant features, since they are usually present at the time when diabetes is clinically

manifested (Rosca 2017). Insulin levels may be normal or even elevated at the time of diagnosis; however, in the setting of insulin resistance, these levels are inadequate to maintain normoglycemia. This relative insulin deficiency is what differentiates diabetic insulin-resistant individuals from normoglycemic insulin-resistant individuals. Indeed, it is noteworthy that, to date, the majority of the genes associated with T2-DM are related to insulin secretion, and not to insulin resistance (Billings and Florez 2010). Initially, and often throughout their lives, these patients do not need insulin treatment to survive (Kissebah et al. 1982). T2-DM is often asymptomatic and is not diagnosed for many years because hyperglycemia is usually not severe enough to cause noticeable symptoms (Kissebah et al. 1982). However, these patients have an increased risk of developing macrovascular and microvascular complications (Kalter 2011). Although the specific etiologies of T2-DM are not known, autoimmune destruction of the pancreas does not occur. Most patients with T2-DM are overweight or obese, and obesity itself causes insulin resistance. Many diabetic patients who are not obese may have a higher percentage of body fat distributed predominantly in the abdominal region (Kissebah et al. 1982). The risk of developing T2-DM increases with age, obesity, and lack of physical activity. It occurs more frequently in women with hypertension or dyslipidemia, and its frequency varies among different ethnic subgroups.

T2-DM is often associated with a strong family predisposition, but the genetics of the disease is complex and not clearly defined (Meigs et al. 2008) It has been shown that some patients with a clinical picture compatible with T2-DM have antibodies similar to those found in T1-DM (Juszczak et al. 2016). Independently of its type, DM causes a series of systemic complications, in general, cardiovascular diseases associated with a decrease in the quality of life (Chawla, Chawla, and Jaggi 2016).

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a common metabolic disorder in pregnant women and can be described as any situation of glucose intolerance detected in pregnancy for the first time. Based on the current understanding and analysis of a large amount of data on DM in pregnancy, the World Health Organization suggested that GDM should include pregnant women with diabetes and women with intermediate hyperglycemia (IGT/IFG) (WHO 2013). Though, the American and Canadian Diabetes Associations do not have any guideline to screen GDM and diabetes in pregnancy separately. Nearly all women with GDM ($\geq$ 90%) come back to normal glucose levels after delivery; however, those women are at risk of developing abnormal glucose tolerance and diabetes in the future (Engelgau et al. 2003, Sen, Chakraborty, and De 2016).

GDM is also responsible for maternal and perinatal complications and metabolic disorders in the offspring. Long-time consequences of DM during pregnancy include amplified adiposity and high body weight at birth. The extent of adverse outcomes of GDM in the mother and the fetus is often linked to obesity, as the majority of the adverse effects can be seen in obese pregnant women with GDM compared to non-obese women (Boney et al. 2005, Simmons 2011, Jain, Pathak, and Kotecha 2014).

Pathophysiology of GDM

Insulin resistance and hyperinsulinemia are common problems during pregnancy. In this phase, the release of placental diabetogenic hormones, including cortisol, growth hormone (GH), human placental lactogen (hPL), and progesterone, increase. As pregnancy progresses,

several hormones like cortisol, human chorionic somatomammotropin (hCS), prolactin, progesterone, and estrogen levels increase, causing insulin resistance in peripheral tissues. Secretion of prolactin and estradiol occur at week 10 and 26 of gestation, respectively, showing a weak to very weak diabetogenic potency. The levels of hCS (moderate diabetogenic potency) and cortisol (very strong diabetogenic potency) reach a peak at week 26, while at week 32 a peak of progesterone (strong diabetogenic potency) is observed (Newbern and Freemark 2011).

The human placental lactogen during pregnancy also rises to 30-fold and is responsible for the release of insulin. Some studies have shown that hPL can induce peripheral insulin resistance, even though the consequences have been inconsistent (Sonagra et al. 2014). An increase in maternal adipose deposition reduced exercise and the rise in caloric consumption are common situations in pregnancy. The physiological, metabolic alterations during pregnancy are essential for enough supply of energy and nutrients to the fetus but are also responsible for a "diabetogenic" environment. The human placental lactogen is responsible for the breakdown of triglycerides, which contribute to maternal energy, while fetal nutrition is based on carbohydrates (Plows et al. 2018). These changes in the hormonal environment induce an insulin resistance state, as such hormones are antagonizing insulin action, creating glucose intolerance (Mecacci et al. 2015).

In the late phase of pregnancy, the ability of insulin to restrain lipolysis in the body is also decreased, which is more common in women with GDM. This situation causes a few-fold increase in postprandial FFAs, increased production of hepatic glucose, and severe insulin resistance (Buchanan, Xiang, and Page 2012). Insulin-induced glucose uptake into the skeletal muscle is reduced in pregnancy. C-peptide response to intravenous glucagon is also considerably decreased in pregnant women with impaired glucose tolerance (IGT) in pregnancy (Jacobs et al. 1994), while the concentration of proinsulin in serum is increased (Dornhorst et al. 1991). Thus, GDM develops when maternal pancreatic β cells are insufficient to compensate for the increased demand for insulin, or when insulin resistance increases during pregnancy (Nicholls et al. 1994).

Pre-obese women are more susceptible to develop diabetes in pregnancy, since they have elevated plasma triglycerides, plus elevated non-esterified fatty acids, and reduced plasma adiponectin levels. Obesity is a significant risk factor, as maternal body mass index is directly proportional to GDM (Shalayel, Al-Noaemi, and Ahmed 2011, Marcinkevage and Narayan 2011, Fadl et al. 2014, Agarwal 2015, Noctor and Dunne 2015).

The role of the placenta is complex in diabetic pregnant women. The human placenta expresses several adipokines like tumor necrosis factor-α (TNF-α), resistin, and leptin (Perez-Perez et al. 2018). Current investigations have shown that these cytokines exhibit a vital role in the control of insulin action and the development of DM. They also link inflammation to metabolic alterations by increasing insulin resistance in the mother (Vrachnis et al. 2012). Depending on nature and extent, the placenta undergoes an assortment of functional and structural changes in diabetic conditions (Kaufmann, Mayhew, and Charnock-Jones 2004, Desoye and Hauguel-de Mouzon 2007).

Generally, women with GDM go back to a normal state after pregnancy. Although the prevalence is lower, DM in pregnancy also exists. This type of DM in pregnancy should be screened by the WHO criteria (or by other recognized criteria), which is like the diagnosis of DM in different individuals (WHO 2018).

DIABETES MELLITUS AND THE PROGRESSION OF CARDIOVASCULAR DISEASE

The risk of developing cardiovascular disease (CVD) in people with diabetes mellitus has increased alarmingly due to an association between insufficient insulin action and a variety of atherogenic mechanisms occurring in a diabetic patient. In this context, insulin resistance is defined as the reduced response in insulin signaling (Leon and Maddox 2015). Insulin resistance is the pathophysiological basis not only of T2-DM but also of the metabolic syndrome, which is a predictor of CVD. Conditions such as hypertension, obesity, and dyslipidemia are common components of metabolic syndrome and T2-DM; this means that most people with T2-DM are obese or overweight (Cornier et al. 2008). Another mechanism that increases cardiovascular damage in diabetes is oxidative stress due to the production of superoxide anions and lipid peroxidation products, together with the lower availability of antioxidants occurring in diabetic patients (Matough et al. 2012). Oxidative stress, along with sustained hyperglycemia, promotes the formation of advanced glycation end-products (AGE). AGEs, which are produced by the non-enzymatic glycosylation of proteins, can affect or alter the physiological functions of normal proteins (Ighodaro 2018). Through these mechanisms (that have not yet been entirely determined), chronic hyperglycemia causes endothelial or mitochondrial dysfunction, metabolic disorders, and damage to the extracellular matrix, causing a functional deterioration in the myocardium, which is known as cardiomyopathy. The mortality due to heart failure has increased more than ten times in diabetic patients with cardiac insufficiency (MacDonald et al. 2008).

KEY FACTS ABOUT DIABETES MELLITUS

Globally, the number of adults afflicted with DM has increased from 108 million in 1980 to 422 million in 2014, 8.5% of the adult population worldwide. Global age-standardized diabetes prevalence increased from 4.3% in 1980 to 9.0% in 2014 in men, and from 5.0% to 7.9% in women (Zhou et al. 2016). In 2013, the global diabetic population was 382 million people, but this figure may reach 430 million by 2030, and 592 million by the year 2035 (Guariguata et al. 2014). There are no less than 300 million people with pre-diabetes worldwide, and the number will rise to 418 million by 2025 (Manaf, Tjandrawinata, and Malinda 2016). Every year, 2–11% of the pre-diabetic population converts to T2-DM. In 2012, diabetes was the direct cause of 1.5 million deaths; more than 80% occurred in low- and middle-income countries (WHO 2018).

By escalating the risks of cardiovascular and other diseases, higher-than-optimal blood glucose caused an additional 2.2 million deaths. Levitt (2008) reported that 10.8 million people had diabetes in sub-Saharan Africa in 2006; and that this figure may rise to 18.7 million by the year 2025, an increment of 80%, which exceeds the globally predicted increase of 55%. DM may increase to 5.4% of the global population by the year 2025 (Parikh, Parikh, and Kothari 2014), becoming the 7th leading cause of global mortality by the year 2030 (WHO 2013).

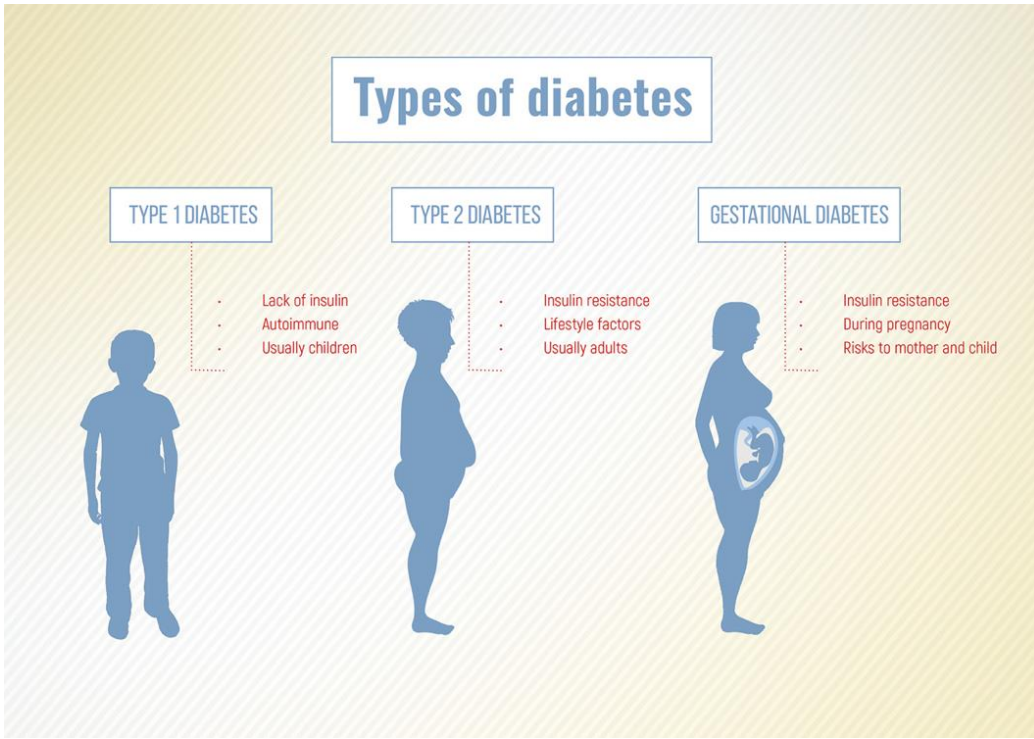


Figure 1. Classification of diabetes. All forms of diabetes are characterized according to their known etiologies (immunologic, genetic, or otherwise)(elaborated by Ovando Gonzalez Ivan).

By 2040, the prevalence of DM in adults is expected to rise to 10.4% of the global population. The burden of DM will be higher in developing countries where it will increase by 13%, from 62% in 1995 to at least 75% by the year 2025 (King, Aubert, and Herman 1998, Boyle et al. 2001). In sub-Saharan Africa, about 10.8 million people developed DM in 2006 (Levitt 2008); Nigeria had 4 million diabetics in 2013 (Salihu Shinkafi et al. 2015); in South Africa, more than half a million people and more than 8% of the black population suffers from DM (Steyn et al. 2013), and about 4.0% in a sample of 1928 individuals aged 25 years and older in Lusaka, Zambia, had DM (Nsakashalo-Senkwe et al. 2011).

There are three main groups of drugs used in the control of diabetes (Ezuruike and Prieto 2014, Pfeiffer and Klein 2014). The first group of drugs increases endogenous insulin availability; these are the sulphonylureas such as glibenclamide, the glinides, insulin analogs, glucagon-like peptide-1 (GLP-1) agonists, and dipeptidyl peptidase IV inhibitors (Eriksson et al. 2016). The second group enhances the sensitivity of insulin, the thiazolidinediones (glitazones), which are agonists of the peroxisome proliferator-activated receptor-gamma (PPAR-γ) and the biguanide metformin (Dawed et al. 2016, Mardinoglu, Boren, and Smith 2016). Metformin, marketed under the tradename Glucophage is the first-line medication for the treatment of T2-DM. The third group is composed of the α- glucosidase inhibitors, which reduce the digestion of polysaccharides and their bioavailability. One of them is acarbose, used to treat T2-DM and prediabetes in some countries (Sun et al. 2016). While sulphonylurea derivatives and glitazone compounds are potent synthetic anti-diabetic drugs, they often fail to restore the glycemic index (Iwaki et al. 2003, Russell-Jones and Khan 2007). They also produce adverse side effects such as inhibition of hepatic regeneration and induce obesity and

osteoporosis with increased risk of fracture (de Souza et al. 2001, Rzonca et al. 2004, Turmelle et al. 2006, Meier et al. 2016). Despite being effective in the normalization of blood glucose levels, the currently used PPAR-γ agonists from the thiazolidinedione type have serious side effects, discovering novel ligands an urgent task.

Summarizing:

- The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014.
- The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014 (Ezuruike and Prieto 2014).
- Diabetes prevalence has been rising more rapidly in the middle- and low-income countries.
- Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation.
- In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012.
- Almost half of all deaths attributable to high blood glucose occur before the age of 70. WHO estimates that diabetes will be the seventh leading cause of death in 2030 (Mathers and Loncar 2006).
- A healthy diet, regular physical activity, maintaining normal body weight, and avoiding tobacco use are ways to prevent or delay the onset of type-2 diabetes.
- Diabetes can be treated, and its consequences avoided or delayed with diet, physical activity, medication, and regular screening and treatment for complications.

At present, few antidiabetic drugs are derived from natural products; for example, metformin, a hypoglycaemic biguanide compound, is obtained from *Galega officinalis* L.; a precursor of acarbose is isolated from bacteria belonging to the genus *Actinoplanes*, and 4-hydroxy-isoleucine is extracted from *Trigonella foenum graecum* L. (Yin et al. 2014). Still, out of an estimated 250,000 higher plants, less than 2500 species have been screened for pharmacological efficacy against DM (Arumugam, Manjula, and Paari 2013). The prospects of discovering new antidiabetic plants are immense, although adverse interactions with allopathic drugs are a potential drawback. The major problem is that existing medications have limited efficacy, narrow tolerability, increased side effects and complications; these concerns occasionally compel patients to take four different drugs per day (Meier et al. 2016, Turner et al. 2016).

Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, the search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been admired for their therapeutic properties in the traditional systems of medicine (Arumugam, Manjula, and Paari 2013). This traditional medicine (herbal) is used for the treatment of diabetes in developing countries where the cost of conventional medicines is too high for the population (Jung et al. 2006).

Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many medicinal plants are useful to manage diabetes successfully. One of the great advantages of

medicinal plants is that these are readily available and have low side effects. Plants have always been an excellent source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. Ethnobotanical information reports that there are more than 800 plants with antidiabetic potential (Alarcon-Aguilara et al. 1998).

MEDICINAL PLANTS USED IN TRADITIONAL MEDICINE TO TREAT DIABETES

Traditional medicine is characterized by knowledge, skills, and practices based on the theories, beliefs, and experiences of natives from different cultures, used in the maintenance of health and/or the prevention, diagnosis, improvement or treatment of diseases (WHO 2013). In traditional medicine, many plants have been used empirically to treat numerous pathological disorders, including DM and its complications. The use is related to folk culture diffused from generation to generation and, currently, there is a wide variety of plants used because of their possible hypoglycemic effects (Trojan-Rodrigues et al. 2012, Arumugam, Manjula, and Paari 2013).

Prevalence surveys indicate that, by 2017, there were 425 million people with DM in the world, and in 2045, this number will reach 629 million people (Cho et al. 2018). Despite the high economic cost to produce anti-diabetic treatments ($727 million in 2017) (IDF 2017), there is still a large part of the population without access to pharmacotherapy. In traditional medicine, a great variety of plants with possible anti-diabetic properties can be used; approximately 800 plants with antidiabetic effects have been identified (Arumugam, Manjula, and Paari 2013), and more than 200 bioactive compounds (such as (alkaloids, glycosides, terpenes, and flavonoids) have been described with this potential (Bailey and Day 1989). Nevertheless, the main problem is the further development of such compounds into clinically useful medicines, phytomedicines, or adequate nutritional supplements, which would be of direct benefit to the patients. In this context, it is essential to remember that the modern drug metformin (a biguanide) is a derivate of an active natural product, galegine, a guanidine isolated from *Galega officinalis L*., which was used in the medieval times to relieve the intense urination in diabetic people (Witters 2001).

Plants are known to be excellent sources of anti-DM medicines (Figure 2) (Marles and Farnsworth 1995). A thorough review of the literature (Grover, Yadav, and Vats 2002, Sabu and Kuttan 2002, Yeh et al. 2003, Patel et al. 2012, Jung et al. 2006, Prabhakar and Doble 2008, Joseph and Jini 2011, Campbell-Tofte, Mølgaard, and Winther 2012, Coman, Rugina, and Socaciu 2012, Fatima, Agrawal, and Singh 2012, Chang et al. 2013, Ehsan et al. 2013, El-Abhar and Schaalan 2014, Kooti et al. 2016, Sen et al. 2016, James et al. 2017) shows that there are more than 1700 plants used around the world to treat or control DM by different cultural groups in traditional medicine. Different levels of pharmacological evaluation and/or bioassays have been carried out in more than 1000 of these traditional anti-DM plants. Most of the studies on these plants are not complete enough to determine their therapeutic value, showing very marginal or no activity to substantial anti-hyperglycemic, hypoglycemic, or anti-DM activities. However, based on the conducted studies, more than 120 plants show promising results for the development of anti-DM medicine. In some cases, the same active molecules are distributed in many plants; the known mechanisms of action and active molecules of the anti-DM plants are

diverse. These plants are attractive sources for the development of novel safe and effective medicines, including a combination of drugs and polyherbal formulations for DM and its complications (Figure 2).

Long before the birth of conventional therapies for DM, plant-based crude medicinal preparations were used to manage DM. Although these ancient methods of treatment were originally based on trial and error, experience, and empirical knowledge, their long-term existence proves that these medicines have some level of efficacy in most of the cases. Scientific studies on these herbal medicines also support this belief; even today, medicinal plants play a prominent role in the control of DM, particularly in rural areas and ancestral pockets of the world. Thus, in the present chapter, we will approach plants that have been pharmacologically tested and have been shown to have some value in the treatment of DM.

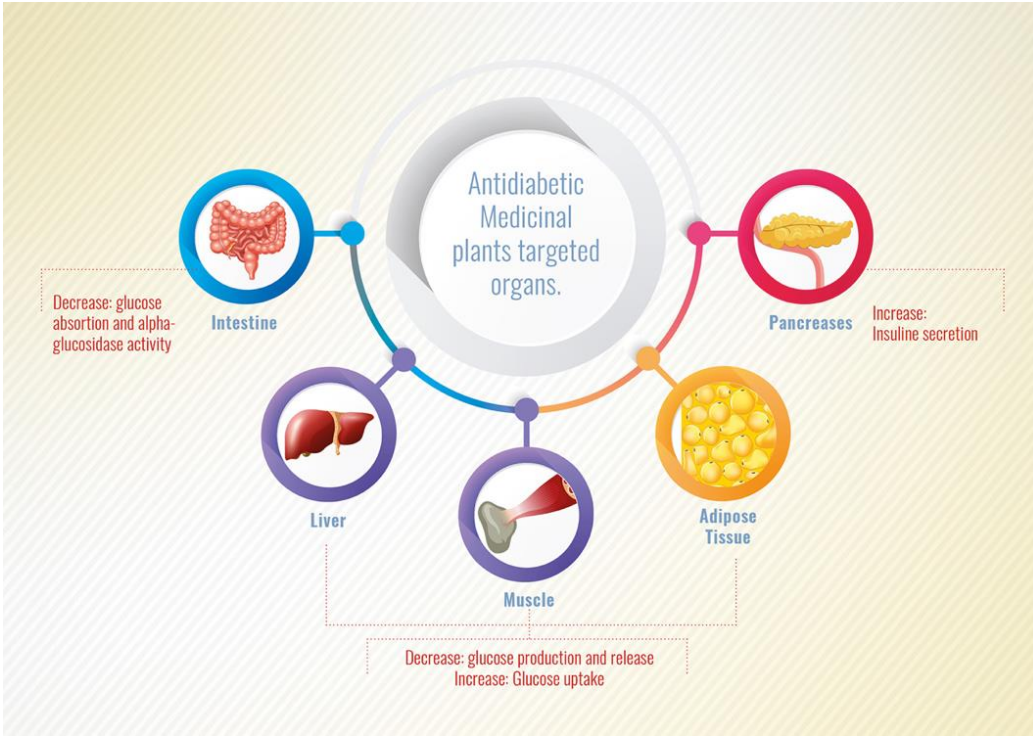


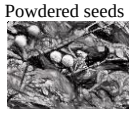
Figure 2. Target organs and mechanisms of action of medicinal plants for treating diabetes (elaborated by Ovando Gonzalez Ivan).

Plants with Anti-Diabetic Potential

At present, a large number of medicinal plants with potential use for the treatment of diabetes mellitus have been registered (Table 1), but only a small amount of them have received scientific and medical evaluations for effectiveness. Traditional treatments have been considered over the years as alternative treatments; alternative herbalists prescribe some of them or taken by patients as supplements in conventional therapy. For this reason, plant remedies have been the raw material of compounds with pharmacological activity for the

development of new molecules with antidiabetic activity. Hypoglycemic action of some treatments has been confirmed in animal models and insulin-independent diabetic patients, and several components have been identified, but traditional therapies are undoubtedly an inexhaustible source for the development of new oral hypoglycaemic agents and simple dietary supplements (Alarcon-Aguilara et al. 1998).

Table 1. Some medicinal plants in which antidiabetic effects have been demonstrated *in vitro* and/or *in vivo* studies

Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Abelmoschus esculentus	Malvaceae	Seed 	Africa	The hypoglycemic effect, stabilization of blood glucose levels by normalizing the speed of reabsorption	(Sabitha et al. 2011)
Abelmoschus moschatus	Malvaceae	Aerial parts 	Egypt, India	Insulin sensitizer, increases insulin signaling enhancing IR1-associated PI3 kinase and GLUT4	(Xu et al. 2017, Liu, Tzeng, and Liou 2010, Liu et al. 2005)
Abies balsamea	Pinaceae	Aerial parts (balsam) 	USA	Insulin sensitizer improves glucose uptake, decouples the production of mitochondrial energy, which activates AMPK	(Eid and Haddad 2014)
Abies pindrow	Pinaceae	Aerial part 	Himalaya (Afghanistan, India, Nepal)	Insulin secretagog, contains triterpenoids, maltol, and pinitol, the latter is anti-DM agent	(Hussain et al. 2004, 20)
Abroma augusta	Malvaceae	Leaves 	Asia	Reduces hyperglycemia, hyperlipidemia, membrane disintegration, oxidative stress, vascular inflammation	(Islam, Rahman, and Islam 2012)
Acacia arabica or *nilotica*	Fabaceae	Powdered seeds 	Africa, India	Hypoglycemic, secretagog effects by inducing insulin secretion from beta cells	(Wadood, Wadood, and Shah 1989)
Achyranthes aspera	Amaranthaceae	Powder Aerial parts 	Unclear, probably South-east Asia, Africa or Oceania	Hypoglycemic, antioxidant activity, hepatic healing and regeneration	(Zambare et al. 2011)
Achyrocline satureioides	Asteraceae	Aerial parts 	South America	Hypoglycemic, active compounds such as prenylated dibenzofuran, acirofuran, reduce blood glucose levels and increase hepatic gluthatione	(Kadarian et al. 2002)
Aegle marmelose	Rutaceae	Root bark 	Asia	Contains phenolic compounds, antioxidant activity, promotes insulin secretion, decreases liver glycogen	(Das, Padayatti, and Paulose 1996, Sharma, Satapathi, and Roy 2007)
Agrimony eupatoria	Rosaceae	Aerial parts 	Europe, North Asia, Brazil, North Africa	Anti-hyperglycemic, stimulates insulin secretion, insulin-like activity (transport and oxidation of glucose)	(Gray and Flatt 1998)

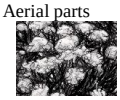
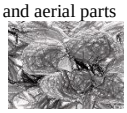
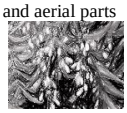
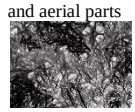
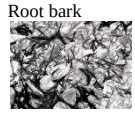
Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Allium cepa	Amaryllidaceae	Aerial parts 	Central Asia and Mediterranean region	Anti-hyperglycemic, antioxidant, hypolipidemic, normalizes hepatic hexokinase, glucose 6-phosphatase and HMG CoA reductase activities	(Karunanayake et al. 1984, Das, Padayatti, and Paulose 1996)
Allium sativum	Amaryllidaceae	Aerial parts 	Asia	Hypoglycemic, antioxidant activity, inhibits platelet aggregation and inflammation	(Ramawat, Dass, and Mathur 2009, Taj Eldin, Ahmed, and Elwahab H M 2010)
Aloe barbadensis	Asphodelaceae	Exudate of the leaves 	Africa, Canary Islands	Hypoglycemic, promotes insulin release, antioxidant, promotes wound healing and closure	(Yimam et al. 2014)
Aloe vera	Asphodelaceae	Leaf pulp 	Africa, Arabic peninsula	Hypoglycemic, inhibits inflammation, antioxidant, promotes wound healing and closure	(Yimam et al. 2014)
Anacardium occidentale	Anacardiaceae	Leaves 	Brazil	Hypoglycemic, reduces diabetes-related functional and morphological alterations in kidneys	(Tedong et al. 2006)
Annona squamosal	Annonaceae	Seeds, leaves and aerial parts 	Mexico, Central and South America	Hypoglycemic and antidiabetic, increases insulin synthesis, improves glucose utilization, inhibits hepatic gluconeogenesis	(Tedong et al. 2006)
Areca catechu	Arecaceae	Aerial parts 	Asia	Hypoglycemic, phenolic compounds, antioxidant activity, contains saturated and unsaturated fatty acids	(Ghate et al. 2014)
Artemisia herba alba	Asteraceae	Leaves or barks 	Mediterranean regions of Africa, Asia, and Europe	Regulates blood glucose levels, flavonoids decrease carbohydrate hydrolysis and glucose absorption	(al-Shamaony, al-Khazraji, and Twaij 1994)
Astragalus membranaceus	Fabaceae	Root 	China	Reduces blood glucose, increases plasma insulin levels, increases glutathione peroxidase activity, decreases glycation, increases activity of Na+ K+ ATPase	(Yu et al. 2006)
Averrhoa bilimbi	Oxalidaceae	Seeds, leaves and aerial parts 	Probably Indonesia	Hypoglycemic, reduces hepatic glucose-6-phosphate activity	(Pushparaj, Tan, and Tan 2001)
Azadirachta indica	Meliaceae	Seeds, leaves and aerial parts 	India and Burma	Hypoglycemic, antioxidant and anti-inflammatory activities, wound healing and hepatoprotective effects	(Sunarwidhi, Sudarsono, and Nugroho 2014, Khosla et al. 2000)
Bauhinia candicans	Leguminosae	Leaves 	North of Argentina	Hypoglycemic, increases peripheral metabolism of glucose	(Fuentes, Arancibia-Avila, and Alarcon 2004)

Table 1. (Continued)

Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Bauhinia forficate	Leguminosae	Leaves 	South America	Hypoglycemic, the leaves contain a range of compounds including flavonoids, alkaloids, and glycosides	(Pepato et al. 2002)
Biophytum sensitivum	Oxalidaceae	Seeds, leaves and aerial parts 	Tropical Africa and Asia	Hypoglycemic, stimulation of insulin synthesis or release from beta cells	(Ananda et al. 2012)
Bixa orellana	Bixaceae	Aerial parts 	Mexico, Central and South America	Increases insulin levels, hypoglycemic, stimulates peripheral use of glucose	(Chang et al. 2013)
Boerhaavia diffusa	Nyctaginaceae	Root and the whole plant 	Africa, Asia, North and South America, South Pacific	Hypoglycemic, probably by recovery of beta cell activity or extrapancreatic action	(Nalamolu, Boini, and Nammi 2004)
Bombax ceiba	Malvaceae	Leaves 	India, South Asia	Hypoglycemic, reduces dyslipidemia, antioxidant activity and protection of pancreatic beta cells	(Grover, Yadav, and Vats 2002)
Brassica juncea	Brassicaceae	Aerial parts 	South of Asia	Hypoglycemic, stimulation of glycogen synthetase and suppression of glycogen phosphorylase	(Khan, Abraham, and Leelamma 1996)
Brassica nigra	Brassicaceae	Seeds, leaves and aerial parts 	Eurasia	Hypoglycemic, antioxidant activity, reduces glycosylated proteins and serum glucose	(Anand et al. 2007)
Bridelia ndellensis	Euphorbiaceae	Seeds, leaves and aerial parts 	Africa, probably South Asia	Anti-hyperglycemic, stimulates insulin secretion, no apparent effects in glucose uptake	(Sokeng et al. 2013)
Bryonia alba	Cucurbitaceae	Root and the whole plant 	Central and Southern Europe	Hypoglycemic, trihydroxiocitedecadienoic acids obtained from the roots restore lipid metabolism	(Karageuzyan et al. 1998)
Bumelia sartorum	Sapotaceae	Root bark 	Brazil	Hypoglycemic, increases plasma insulin levels, increases glucose uptake in muscle and adipose tissues, inhibits hepatic gluconeogenesis	(Ruela et al. 2013)
Caesalpinia bonduc	Fabaceae	Seeds, leaves and aerial parts 	Caribbean and tropical America	Anti-hyperglycemic, blocks glucose absorption, reduces cholesterol and LDL levels	(Kannur, Hukkeri, and Akki 2006)

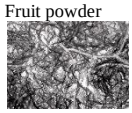
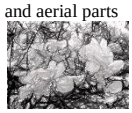
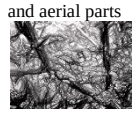
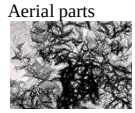
Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Cajanus cajan	Fabaceae	Seeds 	Africa or India	Hypoglycemic, contains flavonoids and stilbenes, antioxidant and anti-inflammatory activities, contains genistein, reduces cholesterol levels	(Amalraj and Ignacimuthu 1998)
Capparis decidua	Capparaceae	Fruit powder 	Mediterranean region	Hypoglycemic, antioxidant and hypolipidemic activities, inhibition of alpha-amylase and alfa-glucosidase activities	(Sharma et al. 2010)
Casearia esculenta	Salicaceae	Root 	India	Hypoglycemic, anti-hyperglycemic, reduction of glucose-6-phosphate and fructose-1,6-biphosphate activities, increases hepatic hexokinase activity	(Prakasam, Sethupathy, and Pugalendi 2002)
Cassia auriculata	Fabaceae	Seeds, leaves and aerial parts 	Southeast Asia	Hypoglycemic, improves hepatic hexokinase and phosphofructokinase activities, reduction of glucose-6-phosphate and fructose-1,6-biphosphate activities	(Fatima, Agrawal, and Singh 2012)
Catharanthus roseus	Apocyaceae	Leaves 	Madagascar	Antidiabetic, increases glucose utilization in the liver by increasing glucokinase activity	(Vega-Avila et al. 2012)
Cocculus hirsutus	Menispermaceae	Leaves and roots 	India, Pakistan, tropical Africa	Anti-hyperglycemic effect due to the action of alkaloids from the root, anti-inflammatory activity	(Badole et al. 2006)
Citrullus colocynthis	Cucurbitaceae	Fruit, bark 	Mediterranean Basin (Turkey and Nubia) and Asia	Hypoglycemic effect due to the action of saponins and glycosidic components of the bark	(Abdel-Hassan, Abdel-Barry, and Mohammeda 2000)
Coccinia indica	Cucurbitaceae	Fruit, leaves 	India	Hypoglycemic effect due to stimulation of glycogen synthetase activity and the reduction of phosphorylase activity	(Motiwala et al. 2015)
Combretum micranthum	Combretaceae	Seeds, leaves and aerial parts 	Benin, Senegal, Mali, Gambia	Hypoglycemic, anti-inflammatory, relieves hypertension, polyphenolic compounds, inhibition of PEPCK expression and glucose production	(Bierer et al. 1998, Welch et al. 2018)
Dioscorea dumetorum	Dioscoreaceae	Aerial parts 	Tropical Africa	Hypoglycemic effect due to an alkaloid (dioscoretin) present in the extract, aqueous extract controls hyperlipidemia, hypercholesterolemia and hypercetonemia	(Karageuzyan et al. 1998)
Elephantopus scaber	Asteraceae	Aerial parts 	Tropical Africa, Asia, India, north of Australia	Hypoglycemic, improves insulin sensitivity, hepatoprotective, antioxidant, anti-inflammatory activities, wound healing effects	(Subramoniam 2016)
Eucalyptus globulus	Myrtaceae	Seeds, aerial parts 	Australia	Enhancement of insulin secretion, improves transport of 2-deoxyglucose, glucose oxidation and incorporation into glycogen	(Dey and Mitra 2013)

Table 1. (Continued)

Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Eugenia uniflora	Myrtaceae	Leaves 	Argentina	Hypoglycemic, improves insulin sensitivity, inhibits glucose intake in the intestine, controls triglyceride levels, inhibits maltase, sucrase, and lipase activities	(Arai et al. 1999)
Ficus bengalenesis	Moraceae	Bark 	India	A fraction of leucopelargonidin-3-0-alpha-L rhamnoside isolated from the bark exerts hypoglycemic effect, improving insulin sensitivity	(Pochhi and Muddeshwar 2017)
Ficus hispida	Moraceae	Seeds, leaves and aerial parts 	Asia, Australia	Hypoglycemic, antioxidant activity, increase of glucose receptors, cardioprotective effect	(Grover, Yadav, and Vats 2002, Ali and Chaudhary 2011)
Garcinia kola	Clusiaceae	Seeds, leaves, fruit 	Africa	Hypoglycemic effects due to a mixture of bioflavonoids attached to C-3/C-8, the kolaviron fraction inhibited the activity of aldose reductase	(Iwu et al. 1990)
Gymnema sylvestre	Asclepiadaceae	Leaves 	India, South of China	Hypoglycemic, strong antioxidant activity, reduces levels of lipid peroxidation, decreases glutathione peroxidase activity in liver and serum glutamate transaminase pyramide	(Arumugam, Manjula, and Paari 2013)
Helicteres isora	Sterculiaceae	Fruit 	Asia, India	Antioxidant and moderate antidiabetic activity, reduces tryglyceride levels, hypolipidemic and sensitizing activity to insulin	(Chakrabarti et al. 2002)
Hibiscus rosa-sinesis	Malvaceae	Aerial parts 	Tropical Asia	Hypoglycemic, the leaf extract acts as tolbutamide may be by stimulation of pancreatic beta cells or an increase in glycogen deposition in the liver	(Sachdewa and Khemani 1999)
Hypoxis hemerocallidea	Hypoxidaceae	Aerial parts 	Southern Africa	Hypoglycemic, enhanced antioxidant activity, blood glucose-lowering mechanisms similar to that of metformin	(Ojewole 2006)
Inula racemosa	Asteraceae	Roots 	Asia	Lowers plasma insulin and glucose levels counteracts adrenaline-induced hyperglycemia	(Tripathi, Tripathi, and Upadhyay 1988)
Ipomoea batatas	Convolvulaceae	Tuberous roots 	Central or South America	Reduces hyperinsulinemia, reduction in insulin resistance, improves glucose and lipid metabolism	(Olowu, Adeneye, and Adeyemi 2011)
Lantana camara	Verbenaceae	Leaves 	Tropical America	Leaf juice once a day shows hypoglycemic effect, but also shows hepatotoxicity	(Sharma, Satapathi, and Roy 2007, Chakrabarti et al. 2002)

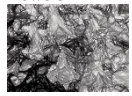
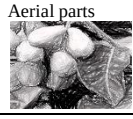
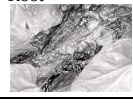
Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Lepidium sativum	Brassicaceae	Aerial parts 	Ethiopia	Potent inhibitor of renal glucose reabsorption, reduces blood sugar in blood	(Eddouks and Maghrani 2008)
Liriope spicata	Liliaceae	Seeds, leaves, fruit, root 	East Asia	Antidiabetic effects due to components in the water extract which includes crude polysaccharides (LS1 and LS2) from the root	(Chen et al. 2009)
Mangifera indica	Anacardiaceae	Seeds, fruits, leaves 	India	The antidiabetic effect due to the intestinal reduction of the absorption of glucose, antioxidant and anti-inflammatory activities	(Shah et al. 2010)
Momordica charantia	Cucurbitaceae	Seeds, fruits, leaves 	South of India Organic	Suppresses postprandial hyperglycemia by inhibiting the activity of alfa-glycosidase, the most beneficial component is present in the methanol fraction	(Uebanso et al. 2007)
Morinda lucida	Rubiaceae	Fruit, leaves 	Africa	Hypoglycemic effects, antioxidant and anti-inflammatory activities, cardioprotective effects	(Olajide et al. 1999)
Murraya koenigii	Rutaceae	Leaves 	India	Hypoglycemic, enhances insulin secretion and glucose uptake, inhibits alpha-amylase activity, reduces cholesterol levels	(Grover, Yadav, and Vats 2002, Handral, Pandith, and Shruthi 2012)
Ocimum sanctum	Lamiaceae	Aerial parts 	India	Hypoglycemic, antioxidant, prevents the increase of plasma glucose	(Vats, Yadav, and Grover 2004)
Panax ginseng	Araliaceae	Roots 	Korea, China Russia	Anti-hyperglycemic, ginsenosides may regulate insulin production and improve insulin resistance, anti-inflammatory effects, side effects may be observed	(Liu, Liu, and Cheng 2005)
Picrorrhiza kurroa	Plantaginaceae	Aerial parts 	Nepal	Anti-hyperglycemic reduces blood urea levels and serum lipid peroxides, inhibits reduction of body weight and leucopenia	(Joy and Kuttan 1999)
Punica granatum	Lythraceae	Flowers 	Iran, India	Hypoglycemic, antioxidant, anti-inflammatory effects,	(Grover, Yadav, and Vats 2002, Schubert, Lansky, and Neeman 1999)
Terminalia chebula	Combretaceae	Aerial parts 	India, Burma	Antidiabetic and renoprotective, antioxidant activity	(Nalamolu and Nammi 2006)
Tinospora cordifolia	Menispermaceae	Root 	India, Burma, Sri Lanka	Anti-hyperglycemic, insulin secretion stimulated by AFTC, palmatin, jatrorizine and magnoflorin	(Mishra et al. 2016)

Table 1. (Continued)

Plant name	Family	Part used	Origin	Antidiabetic effect	Reference
Trigonella foenum	**Fabaceae**	**Leaves and seeds** 	**Eastern Mediterranean, Western Asia, India**	**Anti-hyperglycemic, insulinotropic effect due to change in the activity of the enzymes metabolizing glucose and lipids in liver and kidney**	**(Avalos-Soriano et al. 2016)**

FUTURE PERSPECTIVES

DM is currently one of the most common chronic noncommunicable diseases in contemporary societies; it is a life-threatening condition in which insulin is either absent, insufficient, or unable to remove sugar from the blood. This condition affects millions of people worldwide; many of them live in low-income countries without access to antidiabetic drugs. Medicinal plants follow different mechanisms to lower blood sugar; some of them perform additional antioxidant or immune functions, becoming appropriate remedies to improve symptoms and/or complications in diabetic patients.

DM describes a group of metabolic disorders characterized by high blood glucose levels. People with DM have an increased risk of developing a series of severe health problems that are life-threatening, which translates into higher medical care costs, reduced quality of life, and increased mortality (Baena-Diez et al. 2016). In 1980, the World Health Organization estimated that there were 108 million people with diabetes, and this number increased alarmingly in the 2014 estimates ('Worldwide Trends in Diabetes since 1980: A Pooled Analysis of 751 Population-Based Studies with 4·4 Million Participants' 2016). The International Diabetes Federation (IDF) estimated that the global prevalence was 151 million in 2000 (Whiting et al. 2011), 194 million in 2003, 246 million in 2006 (IDF 2017), 285 million in 2009, 366 million in 2011 (Whiting et al. 2011), 382 million in 2013 (IDF 2017), and 415 million in 2015 (Ogurtsova et al. 2017). In 2017 the IDF estimated that 424.9 million people between 20 and 79 years old, or 451 million people between 18 and 99 years old lived with diabetes. Finally, it was predicted that the number of people with diabetes between 20 and 79 years old would increase to 629 million or to 693 million in people between 18 and 99 years old by 2045. These estimates confirm the great and growing burden of diabetes in the world, but with considerable variation between regions and income groups (Cho et al. 2018).

Despite the availability of a large number of antidiabetic agents and pharmacological therapies focused on reducing cardiovascular risk, morbidity, and mortality, the economic consequences of DM continue to be a great burden for patients, society, health care systems, and the economy of the countries. Many of the pharmacological interventions have been developed based on the current understanding of the pathophysiology of DM; current therapies include sulfonylureas, biguanides, and thiazolidinediones. However, all these agents have undesirable side effects, and, finally, all of them fail to restore glycemic control (Laville and Andreelli 2000); in particular, thiazolidinediones induce obesity (de Souza et al. 2001) and cause osteoporosis (Rzonca et al. 2004). Such side effects discourage the accurate monitoring and adherence of medication protocols by patients, with the progression of DM and its associated complications. For this reason, it is desirable to find new antidiabetic agents that improve the response to insulin, restore pancreatic damage, and improve cardiovascular

diseases. From an ethnopharmacological perspective, it is important to understand that this disease is at the interface of conventional and local (or traditional) biomedical treatment. Many studies have been carried out on glucose-lowering plants, and a great variety of compounds (alkaloids, glycosides, terpenes, flavonoids, etc.) have been isolated, but the main bottleneck is the further development of these compounds into clinically useful medicines (especially phytomedicines) or adequate nutritional supplements, which would be of direct benefit to patients.

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Chapter 2

THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF HYPERCHOLESTEROLEMIA

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ABSTRACT

Medicinal plants have progressed through history as a reliable source of bioactive compounds with a potentially positive effect on health. The advantages of this type of medication are its efficacy, safety, and acceptance by a majority of consumers. Medicinal plants play an essential role in health systems in many parts of the world. Its use is varied: directly as plant parts (leaves, stems, roots, fruit, etc.) or through alternative processes such as infusions or preparations (macerated, ointments, etc.) destined for ingestion or application on the affected area. These effects derive from the presence of phytochemicals such as sterols, alkaloids, polyphenols, and carotenoids, among others. In consequence, these phytochemicals are potent agents against metabolic diseases such as diabetes, obesity, and hypercholesterolemia. The latter pathology is characterized by high blood cholesterol levels produced by an increase in the concentration of lipoproteins, usually low-density lipoproteins (LDL) with normal or low levels of high-density lipoproteins (HDL). Hypercholesterolemia is considered a risk factor for cardiovascular diseases. Because of this, diverse research has been developed that reports the close relationship between medicinal plant consumption and a reduction in cholesterol levels.

Keywords: hypercholesterolemia, phytochemicals, medicinal plants

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INTRODUCTION

Cholesterol is a steroid that is an essential component of the cell membrane, and it is a precursor of steroid hormones, several vitamins, and bile. A fraction of cholesterol is obtained from food, but the highest proportion is synthesized in the liver from where it passes to the bloodstream transported by lipoproteins (Zárate et al. 2016). Their density distinguishes five groups of lipoproteins: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL); these have different sizes and composition. Hypercholesterolemia is a condition that is characterized by elevated blood levels of cholesterol. It is due to an increase in the levels of lipoproteins, and in general, represents high total or LDL cholesterol (LDL-C) levels or normal or low HDL cholesterol (HDL-C) levels (Liu et al. 2011). Cholesterol levels can be determined by multiple factors such as diet, genetics, and environmental factors (Matias-Perez, Perez-Campos, and Garcia-Montalvo 2015). Table 1 shows serum cholesterol values.

An increase in the blood concentration of LDL is associated with an elevated risk of atherothrombotic infarct or ischemic heart disease (Salazar Ruiz et al. 2015). Atherosclerosis is the most frequent cause of coronary heart disease, and it is considered a "cholesterol-dependent" arterial disease since the accumulation of LDL in the subendothelial space is one of the first steps in the development of atheromatous lesions (Ascaso and Carmena 2015) and because of the components involved in its development. The lipid alteration is considered a fundamental inducer of meta-inflammation in atherogenesis since 1) LDL and oxidized LDL (oxLDL) are inflammatory stimuli; 2) atherosclerosis does not develop in animal models or patients who do not have a significant level of these molecules, and 3) multiple inflammatory molecules such as C-reactive protein and interleukin 6 (IL-6) can regulate the atherosclerotic process (León-Pedroza et al. 2015). According to data from the World Health Organization, it is estimated that 7.4 million people died from coronary artery disease (WHO 2019). In Mexico, the economic burden of hypercholesterolemia represents, in an average case, per year, $258,761.37 MXP, which translated to approximately 45,075 diagnosed and treated cases per year represents an economic impact on the health system of more than 115 billion MXP (Baeza-Cruz et al. 2018).

Table 1. Normal serum cholesterol values

Level of risk	TC mg/dL	LDL mg/dL	HDL mg/dL
Low risk	<200	100 to 129	≥60
Greater risk	200 to 239	130 to 159	41 to 59
High risk	≥240	160 to 189	<50
Very high risk	≥190		<40

TC: Total cholesterol.
LDL: Low-density lipoprotein.
HDL: High-density lipoprotein.

BIOACTIVE COMPOUNDS WITH ANTI-HYPERCHOLESTEROLEMIC EFFECT

The medicinal properties of different plants are due to the presence of several constituents such as sterols, polyphenols, and carotenoids, for example.

Plant Sterols

Among the plant sterols, there are two types: sterols and stanols. Stanols are less abundant in plants than sterols; therefore, when we speak of "plant sterols" or "phytosterols," we are usually referring to the former, although the term "plant sterols" can refer to both (Meco López, Pascual Fuster, and Solà Alberich 2016). These compounds possess a structure chemically like cholesterol.

It is believed that they inhibit cholesterol absorption and actively participate in its reduction. The consumption of 2 to 2.5 g/day of products enriched with sterols/stanols could reduce plasma LDL-C by 10 to 14% (Mannu et al. 2013). Phytosterols are important components of whole cereals, dried fruit, seeds, and derived oils (Cofan Pujol 2014). Párraga-Martínez et al. (2015) evaluated the effect of plant stanol ethers in 182 people with hypercholesterolemia. Ninety-one subjects consumed one bottle of liquid yogurt per day with 2 g of stanols (experimental group) and 91 subjects consumed un-supplemented yogurt (control group) for 12 months. When the modification of TC was compared [with 95% confidence intervals (CI)], a greater reduction in the intervention group was found both at 3 months, (15.5 [95% CI, 5.3-25.8] mg/dL; $p = 0.003$) and at 12 months (18.1 [95% CI, 8.1-28.2] mg/dL; $p < 0.001$). The reduction in LDL-C was also greater in those who consumed stanols at 3 months (13.3 [95% CI, 3.8-22.8] mg/dL; $p = 0.006$) and 12 months (13.7 [95% CI, 3.2-24.1] mg/dL; $p = 0.011$).

In another study that included 54 people (30 women and 24 men with a mean age of 38.8 ± 7.3 years), 2 glasses of milk per day (350 mL) with 2.24 g of plant sterols were administered during a period of 3 weeks in the experimental group and the same amount of skimmed milk without sterols was delivered to the control group. Two phases of three weeks each were carried out with a washout period of two weeks, with every participant going through both stages. Blood extractions were performed at the beginning and the end of each stage. A reduction of 9.73%, 12.5%, 3.15% and 13.2% for TC, LDL-C, triglycerides (TG), and non-HDL cholesterol, respectively, were found, without a significant difference in TG (Mauro-Martín et al. 2016).

Fat-based products such as margarine, spreadable, and dairy products, like milk and yogurt, represent the matrix of commonly used foods that can be enriched with phytosterols. However, there is evidence of the effectiveness of phytosterols in all food formats with no apparent difference in the reduction of LDL-C in foods with a high, low or no-fat content (Trautwein et al. 2018).

Polyphenols

Polyphenols (Figure 1) are secondary metabolites of plants. They are structurally formed by at least one aromatic ring and are joined by one or more hydroxyl groups. Polyphenols are abundant in fruits, vegetables, and herbs. They can neutralize free radicals. Although they are not considered as nutrients, they form an essential part of the human diet (Procházková, Boušová, and Wilhelmová 2011).

Polyphenols are classified as:

- Phenolic acids: Phenolic acid is described as a phenol that possesses at least one carboxylic acid. The predominant antioxidant pathway of phenolic acids is by hydrogen atom donation. This chemical process is essential at the moment of ingestion of phenolic acids, so they produce their beneficial effect on health. They are a subclass of polyphenols (comprising more than 8,000 different compounds) (Quideau, Deffieux, Douat-Casassus, et al. 2011)
- Flavonoids: Their name comes from "flavus" which means yellow. They are the most abundant subclass of polyphenols and are characteristic of diverse red, blue, and purple colored foods. Epidemiological studies have shown the positive effect of eating foods rich in flavonoids and their direct relationship with an increase in longevity and a reduction in cardiovascular diseases with high ingestion of fat (Burr 1995, Ferrières 2004, Rosenkranz et al. 2002)
- Tannins: Tannins possess specific characteristics such as their minimal solubility in water and a molecular mass between 500 and 5000 Da. They are subdivided into proanthocyanidins or condensed tannins and hydrolyzable tannins and a minority group, phlorotannins (Isaza 2007).
- Condensed tannins are flavonoid polymer oligomers type flavan-3-ol (catechins, epicatechin, epigallocatechin, etc.) that are linked by a carbon-carbon bond (positions 4→6 and 4→8). They are also known as proanthocyanidins because their breakdown produces anthocyanidins.
- Hydrolyzable tannins are composed of a glucose core. They are also known as gallotannins, and if the presence of hexahydroxydiphenic acid is detected, they are called ellagitannins. All these compounds are phenolic acid esters (gallic and ellagic acid) with sugar and a polyalcohol.

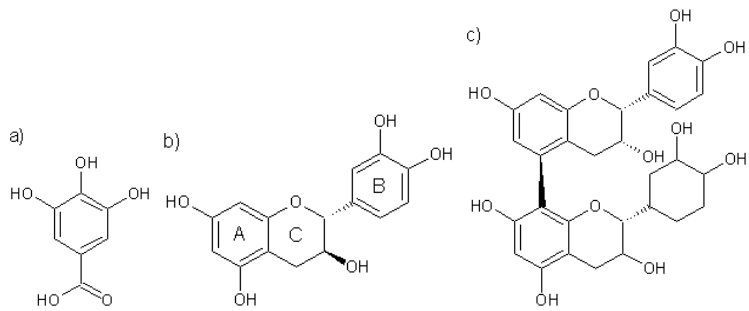

Figure 1. Chemical structure of polyphenols: a) phenolic acid (gallic acid); b) flavonoids (catechin) and c) tannins (procyanidin B1 dimer). Own authorship was created by free ChemSketch software.

- Phlorotannins are polyphenols isolated from brown algae. Their structures are composed of units of phloroglucinol with C-C and C-O bonds. They are structurally defined as dibenzo-1,4-dioxin.

Carotenes

Carotenes are naturally occurring fat-soluble organic pigments that are found in algae, plants, and other forms, such as fungi and bacteria. Regarding their physicochemical characteristics, carotenoids are responsible for the majority of green, orange, and red colors that are present in vegetables and also animals. They are divided into two broad groups: carotenes and xanthophylls. The pigments β-carotene, α-carotene, lycopene, and cryptoxanthin are found mainly in micro and macroalgae, as well as in terrestrial vegetables: carrots, papayas, melons, and oranges, among others (Armstrong and Hearst 1996)

STUDIES ON PLANTS FOR THEIR POTENTIAL TO PREVENT OR TREAT HYPERCHOLESTEROLEMIA

Traditional medicine has been used for centuries to treat several diseases because plants are rich in active biological compounds that can promote health and cure some diseases (Table 2). These compounds are usually found in several plant organs such as leaves, roots, stems, fruit, and seeds (Ceylan et al. 2019).

Black Pepper (*Piper nigrum*)

Black pepper is rich in piperine, an alkaloid that suppresses the absorption of cholesterol in the small intestine. It can modulate the activity of key enzymes involved in the hepatic homeostasis of cholesterol and regulate the expression of LDLR, stimulating the maturation of SREBP2 and facilitating its translocation from the endoplasmic reticulum to the Golgi apparatus, thus increasing the hepatic clearance of LDL-C in the blood (Zhao and Chen 2018). A reduction in total cholesterol, triglycerides, VLDL-C, and LDL-C in plasma by oral administration of black pepper extracts in an animal model has been observed, particularly ethyl acetate and aqueous extracts at a dose of 200 mg/kg for 42 days (Parim et al. 2015).

Garlic (*Allium sativum*)

Garlic is a bulbous plant; its main bioactive compound in the aqueous extract is allicin, but it contains other vital compounds such as: 1-propenyl allyl thiosulfonate; allyl methyl thiosulfonate; y-L-glutamyl-S-alkyl-L-cysteine; and ajoene (Bayan, Koulivand, and Gorji 2014). It has been found that it reduces total cholesterol and LDL-C by inhibition of cholesterol biosynthesis by inhibiting the activity of enzymes such as hydroxymethyl glutaryl-coenzyme A reductase (HMG-CoA) and lanosterol-14-dimethylase (Maza et al. 2014). Several studies

have reported a reduction in total cholesterol or LDL levels with a dose of 600 to 900 mg daily of garlic in different formulations; however, there are clinical trials (few) that have not demonstrated an effect on lipid levels.

The reason for this discrepancy could be the composition and quantity of active sulfur compounds in the different garlic preparations used, subject recruitment, the duration of the study, dietary control, lifestyle, and the lipid analysis methods (Quideau, Deffieux, Douat-Casassus, et al. 2011).

Soy (*Glycine max*)

Soy is a plant of Asian origin that belongs to the family Fabaceae; it has many phytochemicals such as phytic acid (1.0–2.2%), sterols (0.23–0.46%) and saponins (0.17–6.16%). The most abundant sterols are sitosterol, campesterol, and stigmasterol (Rizzo and Baroni 2018).

A study was carried out in 2011 with 86 volunteers organized into three groups: the first received 2000 mg/day of a *Glycine max* (EGML) leave extract; the second group received 2000 mg/day of a *Garcinia cambogia* extract; the third group received 2000 mg/day of starch (placebo) in the morning and afternoon for 10 weeks. After ten weeks of supplementation, a significant reduction of total cholesterol was observed in the EGML group in comparison to the other groups (Kim et al. 2011). The United States FDA recommends 25 g of soy protein to reduce blood cholesterol; however, consuming a higher dose could be more productive; some studies prescribe up to 40 g day (Bahmani et al. 2015).

Oak (*Quercus infectoria*)

Oak possesses many polyphenols that are associated with antioxidant activity and cardiovascular effects. It significantly reduces total cholesterol, triglycerides and LDL (Gholamhoseinian et al. 2012), in addition to preventing the oxidation of LDL, regulating the activity of HMG-CoA reductase, and originating a potent vasorelaxant effect (Park et al. 2016). The main active components of oak are tannins, and in a lesser quantity, gallic and ellagic acids (Ikram and Nowshad 1977).

Alfalfa (*Medicago sativa*)

Alfalfa contains a large variety of phytochemicals such as alkaloids, amino acids, carotenes, coumarins, minerals, flavonoids, phenolic compounds, organic acids, phytoestrogens, phytosterols, proteins, polyamides, saponins, vitamins and volatile compounds (Bora and Sharma 2011).

Several studies performed in animal models report that the ingestion of alfalfa reduces the absorption of cholesterol and the formation of atherosclerotic plaques (Cohen et al. 1990). It has been reported that the intake of 40 g three times a day for eight weeks has an anticholesterolemic effect. Saponins have been associated with a decrease in plasma HDL-C concentrations without affecting HDL concentrations, which reduces the total

cholesterol/HDL-C ratio, as well as reducing the intestinal absorption of cholesterol (Bora and Sharma 2011).

Gundelia (*Gundelia tournefortti*)

Gundelia is a member of the family Asteraceae (Compositae), which grows in semi-desert areas of Iran, Jordan, Palestine, and Syria, among others. It is considered a plant that is common in the Mediterranean region (Asadi-Samani, Rafieian-Kopaei, and Azimi 2013). This plant is traditionally used for the treatment of renal diseases as well as diabetes, to mention a few examples. But it has also been reported that it has hypolipemic activity. Through phytochemical analyses of the plant, important components have been identified, such as sterols, triterpenes, esters, and carboxylic acids. In this regard, the two most common sterols are sitosterol and stigmasterol (Abu-Lafi et al. 2019). The presence of these components gives it important properties such as its anti-atherosclerotic action, mainly due to its antioxidant property, which causes a decrease in cholesterol, LDL-C, triglycerides, VLDL-C, apolipoprotein B, oxidized LDL, and at the same time, increases HDL-C (Asadi-Samani, Rafieian-Kopaei, and Azimi 2013).

Valeriana (*Valeriana officinalis*)

Valeriana is a perennial plant that grows naturally in Europe, North and South America and parts of north Asia. It is chemically composed of monoterpenoids, sesquiterpenoids, lignans, flavonoids, and alkaloids, among others (Wang et al. 2016). Rabbits with hyperlipidemia treated with *Valeriana officinalis* have been reported. Valeriana induces a remarkable anti lipidic peroxidation reducing total serum cholesterol (TC), triglycerides (TG), LDL-C, and malondialdehyde and elevating HDL-C and superoxide dismutase (SOD). This evidence together shows that Valeriana possesses an antihyperlipidemic effect (Chen et al. 2015).

Sesame (*Sesamum indicum*)

Sesame is oleaginous; more than half of its weight is oil (55-60%). It is composed of essential fatty acids, oleic and linoleic, 16-20% is a protein with the essential amino acids, lysine, and tryptophan; the rest is carbohydrates, fibers, and others. It has been reported that sesame reduces cholesterol, triglyceride and LDL levels and increases HDL in acute and chronic models of hyperlipidemia, in addition to having antioxidant, neuroprotective, anti-inflammatory and liver-protective properties (John et al. 2015). It has been proposed that these effects occur by inhibiting lipoperoxidation and mitigating oxidative stress, by decreasing the generation of free radicals, especially reactive oxygen species, and by improving endogenous antioxidants (Nwozo, Lewis, and Oyinloye 2017).

Strawberries (*Fragaria* sp.)

Strawberries are an important source of polyphenols, particularly ellagitannins, anthocyanins, and proanthocyanins with a positive effect on health due to their antioxidant, anti-neurodegenerative, and anti-inflammatory actions (Fotschki et al. 2016). There is a particular interest in the potential reported antioxidant effects of anthocyanins and pro-anthocyanins, in addition to the beneficial effect on blood vessels. It has been reported that in adults with abdominal adiposity and high serum lipid content, the implementation of low and high doses of lyophilized strawberry are effective in lowering plasma concentrations of cholesterol and LDL, as well as the rate of lipoperoxidation. The proposed mechanism is by acting on malondialdehyde and hydroxynonenal, while parallelly increasing HDL levels, considering that due to their nature they exert a high antioxidant effect on cardiovascular risk markers (Basu et al. 2014).

Linseed (*Linum usitatissimum*)

Linseed, from the family Linaceae, is a seed that is rich in alpha-linoleic acid (ω-3 fatty acid) and it is the best source of lignans. Therefore, it performs a fundamental role in reducing the risk of cardiovascular disease by reducing free radicals (Rubilar et al. 2010). Due to its components, linseed has important antiapoptotic and antiangiogenic properties that play a fundamental role as an anti-atherosclerotic and, because of its high fiber content, reduces glucose and cholesterol levels (Kristensen et al. 2012, Wong et al. 2013, Almario and Karakas 2013).

Thus, this seed is considered with important anti-hypercholesterolemic properties by improving serum levels of HDL, cholesterol, and triglycerides together with levels of cellular antioxidant enzymes such as catalase, SOD, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S transferase (GST). These properties together allow it to have a significant anti-hypercholesterolemic role and help reduce the first atherosclerosis changes at the level of the aorta (Naik et al. 2018).

Tea (*Camellia sinensis*)

Camellia sinensis are plants of the family *Theceae*, a native of Asia but they have been naturalized in many parts of the world. The seeds of *Camellia sinensis* are used with therapeutic aims. The leaves of this shrub are used to make tea. Green tea is considered a healthy drink and it has been shown to have curative properties such as antioxidant, anti-obesity, anti-inflammatory, anti-angiogenic, and it lowers cholesterol levels; all these actions are mainly due to its polyphenol content, especially flavonoids. The main components of this plant are catechins, which are a source of polyphenols. The main catechin present is epigallocatechin-3-gallate (EGCG) contained in 50-80% (Rafieian-Kopaei and Movahedi 2017). A previous study reported that a dose of 1 or 2 g/kg of body weight for 30 days significantly reduces plasma cholesterol levels and there is an increase in HDL; therefore, *Camellia sinensis* can be used as a nutritional supplement to protect against the development of hypercholesterolemia (Luo, Li, and Liang 2013).

Table 2. Bioactive compounds from medicinal plants

Plant	Bioactive compound	References
Piper nigrum	Piperine	(Zhao and Chen 2018)
Allium sativum	1-propenyl allyl thiosulfonate, allyl methyl thiosulfonate, y-L-glutamyl-S-alkyl-L-cysteine, ajoene	(Bayan, Koulivand, and Gorji 2014)
Glycine max	Phytic acid, sitosterol, campesterol, stigmasterol	(Rizzo and Baroni 2018)
Quercus infectoria	Tannins, gallic acid, ellagic acid	(Ikram and Nowshad 1977)
Medicago sativa	Alkaloids, carotenes, coumarins	(Bora and Sharma 2011)
Gundelia tournefortti	Triterpenes, sitosterol, stigmasterol	(Abu-Lafi et al. 2019)
Valeriana officinalis	Monoterpenoids, sesquiterpenoids, lignans, alkaloids	(Wang et al. 2016)
Sesamum indicum	Sesamol, sesamolin, sesaminol	(John et al. 2015, Nwozo, Lewis, and Oyinloye 2017)
Fragaria sp.	Ellagitannins, anthocyanins, proanthocyanins	(Fotschki et al. 2016)
Linum usitatissimum	Lignans, omga-3 fatty acids	(Almario and Karakas 2013, Kristensen et al. 2012, Naik et al. 2018, Rubilar et al. 2010, Wong et al. 2013)
Camelia sinensis	Catechins	(Rafieian-Kopaei and Movahedi 2017)

CONCLUSION

Several studies and chemical analyses have demonstrated the positive effect of consuming medicinal plants on serum levels of cholesterol whether on absorption through the intestine or by modulating the activity of key enzymes needed for their homeostasis, as well as their antioxidant capacity. This effect has been attributed to phytochemicals such as sterols (sitosterol, campesterol, stigmasterol), terpenoids and polyphenols (protocatechuic acid, gallic acid, catechins); the former supplied as functional foods when added to other dietary matrices such as yogurt, milk, and margarine, among others; the latter administered in the form of extracts (aqueous or ethyl acetate, for example).

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Chapter 3

COMMON BEANS (*PHASEOLUS VULGARIS L.*) AND THEIR RELATIONSHIP WITH CHRONIC DISEASES OF METABOLIC ORIGIN

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ABSTRACT

Common beans (*Phaseolus vulgaris*) are especially consumed in Asia (46%) and Mesoamerica (34%), but less in Africa (17%) and other parts of the world. Usually, their dried seeds are cooked before consumption, which inactivates readily antinutritive peptides like protease-inhibitors. As legumes, common beans are known for their high protein content (15-30%), from which by normal digestion derive bioactive peptides with, among others, antimicrobial, antihypertensive, or immunomodulatory effects. Recently, a <3 kDa peptide fraction exhibited potential antidiabetic effects by inhibiting α-amylase and α-glucosidase. Additionally, from the total fiber content (14-19%), about 1/3 are soluble fibers (like pectin, gums, inulin-type fructans, and some hemicelluloses), which contribute to a slow postprandial intestinal glucose-uptake - a property, which supports an antidiabetic action. Common beans also have a high content of polyphenols (up to 1%), especially flavonoids, which are well known for their antioxidant capacity, thus protect against oxidative stress and related diseases like cardiovascular diseases. Moreover, these polyphenols are antimutagenic and antiproliferative hence exhibit anti-cancer properties. This review highlights the complementary and overlapping modes of action of common bean constituents and that dietary plants produce not only nutrients but also phytochemicals; thus they have not only nutritive value but also a health-promoting effect.

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Keywords: *Phaseolus vulgaris*, common bean, bioactive peptides, flavonoids, phytates, chronic diseases, obesity, cancer

INTRODUCTION

The rapid changes in diets and lifestyles that have occurred with industrialization, urbanization, economic development, and market globalization have accelerated over the last decade. Those changes are having a significant impact on the health and nutritional status of the population, particularly in developing countries. While standards of living have improved, food availability has expanded and become more diversified, and access to services has increased, there have also been significant negative consequences in terms of inappropriate dietary patterns, decreased physical activities and increased tobacco use, and a corresponding increase in diet-related chronic diseases, especially among low-income people.

The changes in the world food economy are reflected in shifting dietary patterns, for example, increased consumption of energy-dense diets high in fat, particularly saturated fat, and low in unrefined carbohydrates. These patterns are combined with a decline in energy expenditure that is associated with a sedentary lifestyle, the motorized transport, labor-saving devices in the home, the phasing out of physically demanding manual tasks in the workplace, and leisure time that is preponderantly devoted to physically undemanding pastimes. Because of these changes in dietary and lifestyle patterns, the chronic diseases of metabolic origin including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension and stroke, and some types of cancer are becoming increasingly significant causes of disability and premature death in many countries (WHO 2003, Manach et al. 2004).

According to WHO (2003), chronic diseases such as obesity, diabetes, cardiovascular disease, and cancer resulted in ~60% of the 56.5 million total reported deaths in the world. In developing countries, public health problems related to these chronic diseases are increasing.

On the other hand, the leguminous plants have been widely used by the man as food and for the maintenance of his health. These plants synthesize in their cells a great variety of phytochemical compounds like isoflavones, flavonoids, phenolic compounds, lignins, lignans, alkaloids, and cyanogenic glucosides, all of them have health benefits (Okwu 2004).

Phaseolus vulgaris is the most important food legume for human consumption in the world. Its seeds consist mainly of carbohydrates and are a good source of nitrogen and protein. The grains of this legume is rich in bioactive compounds such as enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic substances, which exhibit metabolic roles in humans and animals. The biological activities related to those compounds are antioxidant capacity, reduction of cholesterol level, decrease in low-density lipoproteins, and a protective effect against cardiovascular diseases. Also, they have shown favorable results against cancer because of the antimutagenic and antiproliferative properties of the phenolic compounds, lectins, and protease inhibitors. Additionally, *Phaseolus* has demonstrated effects on obesity and diabetes due to its content of resistant starch and α-amylase inhibitor (Aparicio-Fernandez et al. 2005).

This review explores the nutritional value of legumes and the importance of their consumption to prevent metabolic disorders in humans.

THE COMMON BEANS (*PHASEOLUS VULGARIS* L.)

As mentioned above, *Phaseolus vulgaris* L. is the most important edible legume for direct consumption in the world. There are many varieties regarding growth patterns, seed characteristics, maturation, and adaptation, accounting for more than 40,000 (Schneider 2002). This crop is produced under a diversity of systems and environments in all regions: Asia (45.75%), the Americas (34.17%), Africa (17.56%), Europe (2.29%) and Oceania (0.24%). For the 2001–2011 period, the largest producer was Brazil (16%), followed by India (15.9%), Myanmar (10.5%), China (8.9%), Mexico (5.8%) and the USA (5.6%) (FAOSTAT 2013).

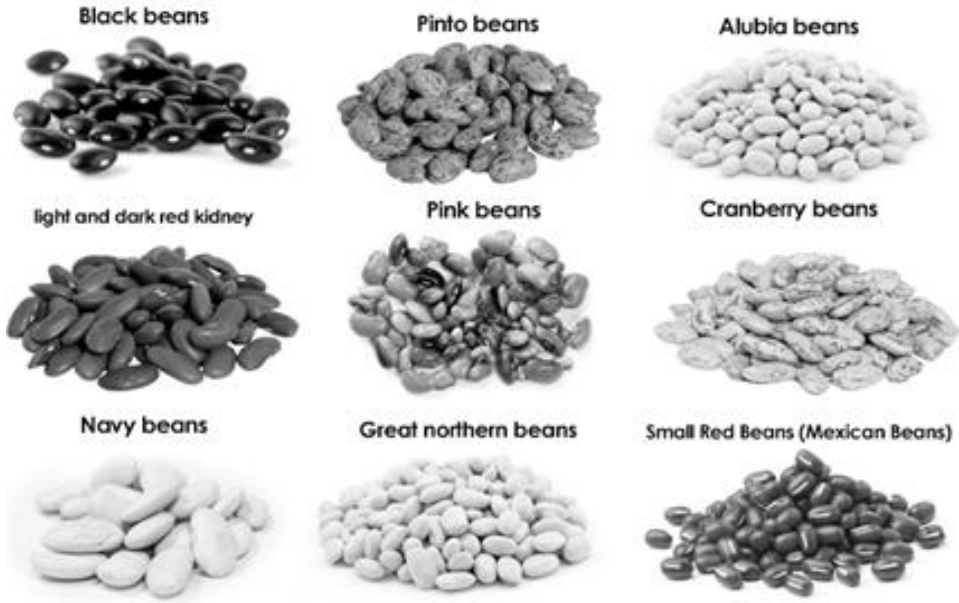

Figure 1. Major *Phaseolus vulgaris* bean varieties.

Among all the major food legumes, the common bean (*Phaseolus vulgaris L.*) is the world´s third most important bean after soybeans (*Glycine max* (L.) and peanut (*Arachis hypogaea* L.).

The common bean is primarily consumed as dry seeds (dry beans) but also as green pods (snap beans), and green-shelled seeds (Aparicio-Fernandez et al. 2005). There is a large variability in common bean seeds, due to seed color and size. The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and condensed tannins (Figure 1) (Beninger and Hosfield 2003). Also, factors such as genotype, environmental conditions, storage, and processing methods, play a critical role in the content and composition of common bean polyphenols. Besides, analytical methods, including extraction, separation, and identification, are of importance for the precise and comparable evaluation of polyphenols in common beans (Yang et al. 2018).

Studies have reported that the black bean variety contains higher amounts of bioactive components such as saponins, flavonoids, and antioxidant properties compared to other common bean cultivars (Díaz-Batalla et al. 2006, García-Lafuente et al. 2014, Yang et al. 2018). According to Guajardo-Flores, Serna-Saldívar, and Gutiérrez-Uribe (2013), the content

of phytochemical compounds (flavonoids and saponins) in black beans depends on different factors, such as anatomical seed part and germination time. In their study reported that the total content of phenols and the antioxidant capacity were higher on seed coats on the third germination day. The extracts obtained from seed coats after 3 and five germination days inhibited cancer cell lines proliferation with no cytotoxicity against control cells. Besides, they found that genistein was related to the activity against mammary cancer cells, but flavonols and saponins were more related to hepatic and colon cancers.

Common beans are rich in many other bioactive components than polyphenols such as enzyme inhibitors, lectins, phytates, oligosaccharides, soluble fiber, and peptides, which exhibit metabolic roles and have beneficial effects on human health (Campos-Vega et al. 2013, Díaz-Batalla et al. 2006). These effects may be regarded as positive, negative, or both (Champ 2002). Some adverse effects could be as antinutritional factors due to their impact on diet quality; the enzyme inhibitors can diminish protein digestibility, and lectins can reduce nutrient absorption, but both have little effect after cooking. Phytic acid can decrease mineral bioavailability; some phenolic compounds can reduce protein digestibility and mineral bioavailability and finally, the oligosaccharides may result in flatulence (Lajolo and Genovese 2002, Sandberg 2002, Chung et al. 1998, Muzquiz et al. 1999).

On the other hand, positive effects could be protective effects against cancer; phytic acid has antioxidant and protective DNA damage effects; phenolic compounds such as flavonoids and phenolic acids have antioxidant, and other specific properties and oligosaccharides may result in prebiotic activity (Mathers 2002, Lajolo and Genovese 2002, Phillippy 2003, Midorikawa et al. 2001).

Chemical Composition

Phaseolus vulgaris L. is a good source of nitrogen and protein (20–30%). One portion (90 g or a ½ cup of cooked beans) provides 7 to 8 g of protein, nearly 15% of the recommended dietary intake of protein for a 70 kg adult. *Phaseolus* seeds consist mainly of carbohydrates, where starch represents the main fraction of energy. The seed contains minerals such as calcium, magnesium, potassium, phosphorus, copper, iron, zinc, manganese, and sulfur (Paredes, Becerra, and Tay 2009). The grains beans are rich in B-vitamins such as niacin (1.85-4.01 mg $100g^{-1}$), thiamin (0.46-1.72 mg $100g^{-1}$), riboflavin (0.22-170 mg $100g^{-1}$) and ascorbic acid (5.20-55.44 mg $100g^{-1}$). Common beans are rich in lysine and phenylalanine, 6.4-7.6, and 5.3-8.2 g100 g^{-1} of protein, respectively. However, the bean is deficient in amino acids sulfur methionine and cysteine (Reyes-Moreno, Paredes-López, and Gonzalez 1993). The lipids fraction is the smallest (1.5 to 6.2 g $100g^{-1}$), constituted by a mixture of acyl-glycerides whose predominant fatty acids are mono and polyunsaturated. Finally, the beans are also a good source of fiber whose value varies from 14-19 g 100 g^{-1} of raw food, half of it can be of the soluble form. The main chemical components of beans fiber are pectins, pentosans, hemicellulose, cellulose, and lignin (Ulloa et al. 2011). Table 1 gives an overview of the chemical composition of some legume varieties.

Table 1. Chemical composition of some *Phaseolus vulgaris* varieties (g 100 g^{-1})

Legume	Moisture	Protein	Carbohydrates	Lipids	Fiber	Energy (kcal)	References
Vigna unguiculata	11.00	19.69	32.78	2.70	1.78	194	(Beninger and Hosfield 2003)
Glycine max (green seed)	8.81	40.15	11.01	20.04	15.01	231	(Redondo-Cuenca et al. 2007)
Pinto beans	10.02	15.41	44.84	1.11	15.40	245	(Rebello, Greenway, and Finley 2014)
Great Northern		14.74	37.33	0.80	12.40	209	(Rebello, Greenway, and Finley 2014)
Broad beans	73.5	6.4	18.3	1.2	5.7	112	(Kalogeropoulos et al. 2010)
Navy beans		14.98	47.41	1.13	19.10	255	(Rebello, Greenway, and Finley 2014)
Black beans		15.24	40.78	0.93	15.00	227	(Rebello, Greenway, and Finley 2014)
Black-eyed beans	72	7.1	19.3	1.2	6.7	121	(Kalogeropoulos et al. 2010)
Kidney beans		15.35	40.36	0.88	11.30	225	(Rebello, Greenway, and Finley 2014)

Phytochemical Compounds

Plants synthesize polyphenols as a protective response to environmental stress and microbial infections. These compounds are known to have antioxidant, anti-inflammatory and antimicrobial properties protecting body tissues against oxidative stress. Flavonoids limit free radicals, free radical-mediated cellular signaling, and inflammation between other features.

The two main types of polyphenols are flavonoids and phenolic acids. Figure 1 shows the main phenolic compounds from common beans (Yang et al. 2018). Some polyphenols in foods are flavonoids that themselves distributed among several classes: flavones, flavonols, flavanols, flavanones, isoflavones, proanthocyanidins, and anthocyanins. Some of the most common flavonoids are quercetin, a flavonol abundant in onion, tea, and apple; catechin, a flavanol found in tea and several fruits; hesperetin, a flavanone present in citrus fruits; cyanidin, an anthocyanin giving its color to many red fruits (blackcurrant, raspberry, strawberry, etc.); daidzein, the main isoflavone in soybean; proanthocyanidins, common in many fruits, such as apple, grape, or cocoa and are responsible for their characteristic astringency or bitterness. One of the most common phenolic acids is caffeic acid, present in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall (Scalbert et al. 2005).

Non-Nutritional Components of Legumes

Of the main chemical compounds that interfere with the utilization of the nutrients stand out trypsin inhibitors, tannins, lectins, and acid phytic. The trypsin inhibitors are commonly

considered as proteolytic inhibitors, slowing growth, and leading to pancreatic hypertrophy. Regarding tannins, in addition to decreasing protein digestibility, they limit the bioavailability of minerals such as iron and zinc, while phytic acid also reduces the assimilation of zinc (Ulloa et al. 2011). Luckily, the culinary techniques of bean preparation for consumption, such as soaking and cooking, radically eliminate or diminish the presence of such non-nutritional factors.

Polyphenols Compounds in Common Beans (Phaseolus vulgaris)

Table 2 shows phenolic compounds identified in common beans. The polyphenols are common constituents of foods of plant origin and major antioxidants of our diet. The primary dietary sources of polyphenols are fruits and beverages. Cereals, chocolate, and dry legumes also contribute to the polyphenol intake (Scalbert et al. 2005). Among legume polyphenols, phenolic acids, flavonoids, and proanthocyanidins are particularly notable because of their potent antioxidant properties (García-Lafuente et al. 2014). These antioxidants can terminate oxidative chain reactions by eliminating free radical intermediaries and inhibiting other oxidation reactions (Jeon et al. 2012, Paredes, Becerra, and Tay 2009). Table 2 lists phytochemical compounds present in different legumes.

Table 2. Phytochemical composition of different legume seeds

Beans type	Major compounds	Reference
Navy bean	p-Coumaric acid derivatives, ferulic acid derivatives, sinapic acid, ferulic acid	(Yang et al. 2018)
Dark red kidney bean	p-Coumaric acid derivatives, ferulic acid derivatives, sinapic acid, ferulic acid, quercetin diglycosides, kaempferol diglycosides, quercetin, kaempferol	(Yang et al. 2018)
Cowpea (*Vigna unguiculata*)	Alkaloids, Flavonoids, Tannins, Saponins	(Bennink 2005, Okwu 2004)
Phaseolus vulgaris L.	Kaempferol	(Perazzini et al. 2008)
Glycine max	Alkaloids, Flavonoids, Tannins, Saponins	(Bennink 2005, Okwu 2004)
Brazilian common bean germplasm	Daidzein, genistein, kaempferol, myricetin, quercetin	(de Lima et al. 2014)
Black vean	p-Coumaric acid derivatives, ferulic acid derivatives, sinapic acid, ferulic acid, delphinidin 3-O-glucoside, petunidin 3-O-glucoside, malvidin 3-O-glucoside	(Yang et al. 2018)
Pinto Beans	Phenolic acids: *p*-coumaric acid, ferulic acid, sinapic acid	(Luthria and Pastor-Corrales 2006)

Flavonoids

Flavonoids from different seeds in special common beans have been widely studied due to their bioactivity. Flavonoid compounds in beans have been reported to have biological activity *in vitro* as well as *in vivo*. These compounds extracted from beans, mainly anthocyanins, and proanthocyanidins, have shown antioxidant and antimutagenic activities (Beninger and Hosfield 2003, Cardador-Martínez, Loarca-Piña, and Oomah 2002). In particular, the red beans were identified as having one of the highest antioxidant capacities (as measured in the ORAC assay) among over 100 common dietary fruits and vegetables examined (Wu et al. 2004).

Other studies reported flavonols quercetin and kaempferol as the most abundant flavonoids in foods, and their consumption has been related to an inverse association between lung cancer

and cardiovascular disease risk. The plausible mechanisms involved in this relationship are modulation of detoxification enzymes and inhibition of some enzymes related to cell proliferation, but the most recognized effect is their antioxidant capacity (Le Marchand 2002). Other studies suggest that the consumption of flavonoid-rich foods protects against diseases associated with oxidative stress, like coronary heart disease and cancer (Duthie, Duthie, and Kyle 2000, Lambert and Yang 2003). *In vitro*, flavonoids from several plant sources have shown free-radical scavenging activity and protection against oxidative stress (Bagchi et al. 2000), protection against irradiation-induced cell damage (Kondo et al. 2004), protection against chemical-induced cellular transformation, and selective growth inhibitory activity of cancer cells (Rafi et al. 2002, Lazzè et al. 2004).

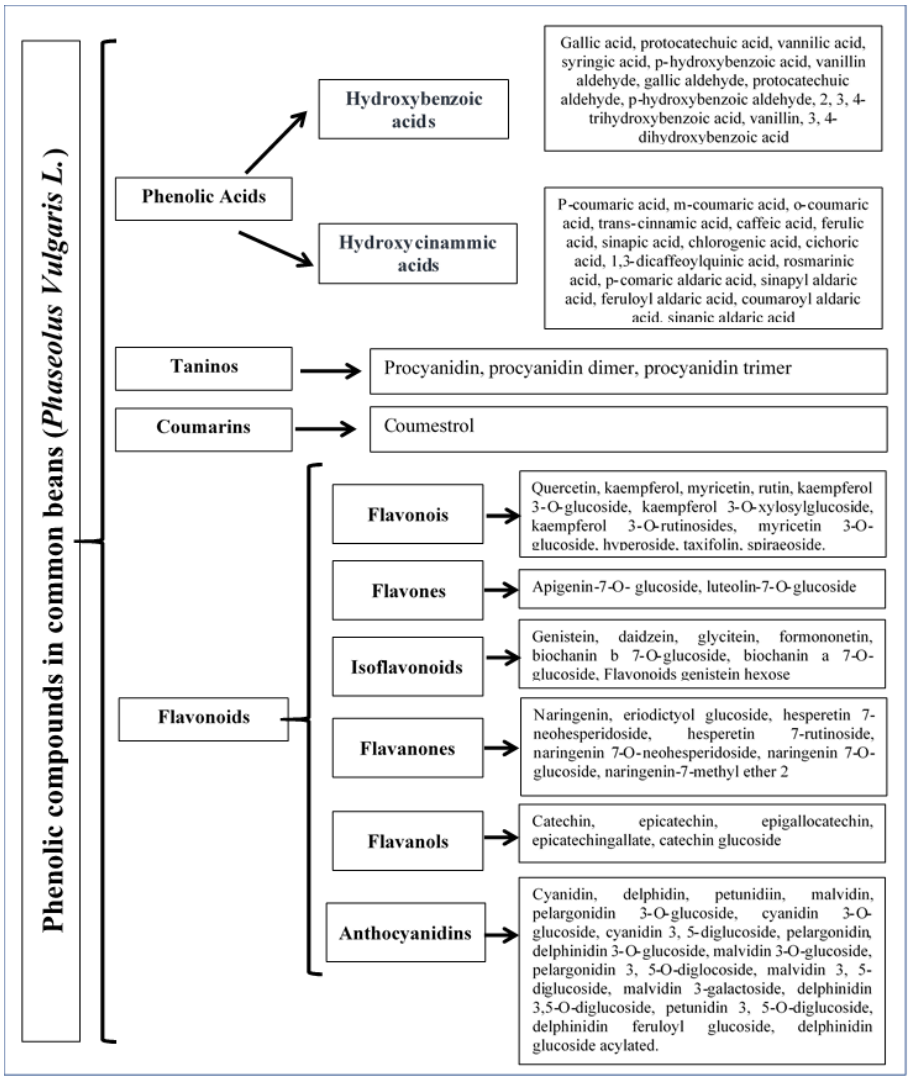

Figure 2. Common bean (*Phaseolus vulgaris* L.) phenolic compounds (Yang et al. 2018, García-Lafuente et al. 2014, Scalbert et al. 2005).

The most widely distributed group of flavonoids in beans is anthocyanidins (Figure 2, Table 3); the presence of anthocyanins has only been reported in black and blue-violet colored beans. Proanthocyanidins have been detected in different varieties of common bean mainly in the seed coat (Beninger and Hosfield 2003, de Mejía et al. 2003, Romani et al. 2004, Cardador-Martínez, Loarca-Piña, and Oomah 2002, Aparicio-Fernandez et al. 2005).

On the other hand, researchers have reported the presence of Kaempferol, which is one of the more studied phenolic compounds due to its antimutagenic and anticarcinogenic activity both *in vitro* and *in vivo* in several beans varieties (Hertog, Hollman, and Katan 1992).

Bioactive Peptides (BP)

Other important compounds in legumes are bioactive peptides, which have been defined as specific protein fragments that have a positive impact on body functions or conditions and may influence health, affecting the digestive, endocrine, cardiovascular, immune, and nervous system (Sánchez and Vázquez 2017). Currently, more than 1500 different BP has been reported in a database named 'Biopep.' BP and proteins play important roles in the metabolic functions of living organisms and, consequently, in human health. They can be classified based on their mode of action as antimicrobial, antithrombotic, antihypertensive, opioid, immune-modulatory, mineral binding, and anti-oxidants. (Kitts and Weiler 2003, Singh, Vij, and Hati 2014).

Some researchers have evidenced that the peptide fractions with the lowest molecular weight (< 3 kDa) have inhibitory activities of 16.9%–89.1% of α-amylase and 34.4%–89.2% of α-glucosidase (Mejía et al. 2019).

Amino acid composition and sequence determines the activity and properties of the peptides once that they are released from the precursor protein where they are encrypted (Fields et al. 2009).

Several studies have reported that peptides from beans were able to produce molecules with high hypoglycemic and antihyperglycemic activities. Concluding, these results provide scientific support to assure that these peptide fractions have good potential as a renewable source for natural products to be used in functional foods or pharmaceutical preparations for the prevention and/or treatment of type 2 diabetes (Mejía et al. 2019, Luna Vital et al. 2014).

Chen et al. (2019) reported that peptides with molecular weight<10 kDa have strong cellular antioxidant activity and anti-inflammatory activity suppressing TNF-α-induced IL-8 secretion in Caco-2 and HT-29 cell lines.

The presence of bioactive peptides in legumes (Beans) can contribute to increase their food protein quality value and add "functionality" to food consumed daily.

Chronic Diseases of Metabolic Origin and Its Relationship with the Common Beans (*Phaseolus vulgaris* L.)

There are two major areas of a health problem that could be significantly reduced by simply eating more beans. One area is the metabolic diseases, and the other is malnutrition.

Table 3. Phytochemical compounds and their health beneficial effects

Phytochemical	Major Compounds	Beneficial effects	Reference
Flavonols	Quercetin Kaempferol	High antioxidant capacity Modulation of detoxification enzymes Inhibition of some enzymes related to cell proliferation. Anti-allergic Anti-carcinogenic Antiviral Antiinflammatory	(Le Marchand 2002, Close and McArthur 2002, Díaz-Batalla et al. 2006, Edeoga, Okwu, and Mbaebie 2005)
Fiber	Celulose	Hypocholesterolemic effect	
Trypsin inhibitors		Confer protection against rotavirus, inhibit carcinogenesis and can be used as chemoprotective agents, that is, to protect the organism against side effects of treatments of certain diseases.	
Lectins	Phytohaemagglutinins	Decrease the growth of non-Hodgkin's lymphomas (cancer of the lymphoid tissue, which includes the lymph nodes, the spleen, and other organs of the immune system).	
Phytic acid	-	Reduces the risk of cancer, mainly of the colon and breast High antioxidant power.	
Tannins	-	Antioxidants Anticancer Antimutagen effective	
Isoflavones and Coumestrol	Genistein Daidzein Equol	Are compounds known as phytoestrogens that have been related to a risk reduction of cardiovascular disease and cancer, particularly breast and prostate cancer	(Mathers 2002, Setchell and Radd 2000)
Phenolic Acids.	Benzoic acid, cinnamic acid, ferulic, p-coumaric, and caffeic acid	Antioxidant capacity Capacity to modulate detoxification enzymes	(Díaz-Batalla et al. 2006)
Protease inhibitors		Effect on proteolytic enzymes, cell proliferation and survival, invasion metastasis. Target diseases: Cancer, HIV	(Castro-Guillén, García-Gasca, and Blanco-Labra 2010, García-Gasca et al. 2012)
Bioacive Peptides (BP) (Olive oil extraction)		Antioxidant Antihypertensive Antithrombotic Enhancement of mineral absorption/bioavailability	(Esteve, Marina, and García 2015, Malaguti et al. 2014) (Sánchez and Vázquez 2017, Zambrowicz et al. 2013)

Studies have shown the protective effect of plant-based diets on chronic diseases, and several phytochemicals have been implicated. These compounds can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of lipid and hormone metabolism, antioxidant, antibacterial, antimutagenic, and antiangiogenic effects, reduction of tumor initiation, and promotion and induction of apoptosis (Key et al. 1999, Lampe 1999).

Chronic diseases (certain types of cancer, hypertension, type II diabetes, heart disease, and other diseases of the blood system) are the most common causes of death in industrialized

countries and they significantly lower the quality of life for millions. The most critical factor in the etiology of chronic diseases is the perpetual over-consumption of foods (energy). Excess consumption coupled with inadequate physical activity results in a positive energy balance and, eventually, obesity (Bennink 2005). Obesity is a common etiologic factor in the development of chronic and metabolic diseases.

Table 4. Impact of legume intake on obesity, cardiovascular diseases, diabetes, and other important diseases

Legume type	**Impact on disease**			
	Obesity	**Cardiovascular diseases**	**Metabolic syndrome/Diabetes**	**References**
Navy beans	No effect on appetite or energy intake compared with a control	Reduced total cholesterol and LDL-C	Low blood glucose	(Mollard, Zykus, et al. 2012, Winham and Hutchins 2007)
Lupin seeds	Increase satiety and/or reduced energy intake. No effect on body weight.	Reduce blood pressure. No effect on blood lipids.	Inconsistent effects on blood glucose.	(Winham and Hutchins 2007, Lee et al. 2006, Keogh et al. 2011, Archer et al. 2004, Belski et al. 2011, Hodgson et al. 2010)
Legumes	Increase mitochondrial oxidation. Reduced appetite and/or energy intake. Reduced body weight over 4 – 8 weeks and over 3 months. No effect on body weight but waist circumference reduced at 18 months.	Reduced total cholesterol and LDL-C but reduced high-density lipoprotein cholesterol. Reduced blood pressure.	Reduced risk factors for metabolic syndrome Reduced blood glucose and HbA1c.	(Abete, Parra, and Martinez 2009, Mollard, Luhovyy, et al. 2012, Mollard et al. 2011, Leathwood and Pollet 1988, Sichieri et al. 1993, Hermsdorff et al. 2011, Bazzano et al. 2001, Jenkins et al. 2012, Messina 1999, Zhang et al. 2010, Abeysekara et al. 2012, Gortmaker et al. 2011, Thompson, Winham, and Hutchins 2012)
Pinto beans	Reduce chemically induced colon cancer in rats Low TC (mg/dL) Low LDL-C (mg/dL) Low HDL-C (mg/dL) Low TG (mg/dL) Reduce CHD			(Hughes, Ganthavorn, and Wilson-Sanders 1997, Winham, Hutchins, and Johnston 2007)
Wight (adzuki bean) (*Phaseolus angularis*)	Used in traditional Asian medicine for infections Edema Inflammation of the joints			(Suárez-Martínez et al. 2016)
Soybeans (Isoflavonoids)	Protective effect against mammary cancer			(Messina et al. 1994)

Abbreviations: TC total cholesterol, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, TG triacylglycerides, CHD coronary heart disease.

Our diet is an important factor in the presence of metabolic diseases. One central component that leads to the development of chronic diseases, elevated concentrations of blood glucose (hyperglycemia), and blood insulin (hyperinsulinemia) is the high-carbohydrate intake. The type of carbohydrate has a strong influence on the maintenance of normal blood glucose and insulin concentrations and in the presence of chronic diseases. Foods with a high glycemic index cause a more rapid and more significant rise in blood glucose and insulin than foods with a low glycemic index even though the amount of carbohydrate consumed is equal. The food-intake that have a high glycemic index can lead to diseases like type II diabetes mellitus, heart diseases, and hyperinsulinemia and hyperglycemia, which cause other metabolic disorders.

Excess body fat leads to hyperglycemia and hyperinsulinemia and vice versa. The hyperglycemia and hyperinsulinemia are hallmark features of type II diabetes, and this disease is a major contributor to the development of heart disease and other diseases of the blood system (cardiovascular diseases). Also, recent epidemiological studies suggest that hyperglycemia and hyperinsulinemia contribute to the development of certain cancers (Field et al. 2003, Leathwood and Pollet 1988). Table 4 shows the impact and beneficial effects of legume intake on obesity, cardiovascular diseases, diabetes, and other important diseases.

Obesity

Since the 1970s, the proportion of overweight and obese people in many places in the world and in special in the United States has grown at an alarming rate, causing a strong health problem. One of the consequences of obesity and overweight is the deterioration in the quality of life of individuals. This health problem has triggered the index of chronic diseases of metabolic origin, which are the cause of morbidity and mortality today. It is estimated that by 2020 three of four people suffer from obesity (Rebello, Greenway, and Finley 2014, Ezzati et al. 2006).

Obesity results from a chronic energy imbalance with complex contributing factors, including genetics, hormone levels (Schwarz 2011), behavioral patterns, and their environmental determinants (Gortmaker et al. 2011). However, helping people make small sustainable changes in lifestyle behaviors to control body weight is perhaps better than efforts to achieve more extensive changes that cannot be sustained (Hill 2008).

The prevention of this health problem can be accomplished with relatively small changes in lifestyle behaviors to control body weight. Food is the main factor of change, exercise, and low stress that will strongly help this change and achievement.

As a result of their high nutritional properties, legume consumption has shown beneficial effects on the prevention and management of obesity (Papanikolaou and Fulgoni III 2008) and related disorders, such as coronary heart disease (Bazzano et al. 2001), diabetes and the metabolic syndrome (Jenkins et al. 2012, Rebello, Greenway, and Finley 2014).

The microflora of the large intestine easily ferments soluble dietary fiber from legumes. Legume fiber consists of pectins, gums, inulin-type fructans, and some hemicelluloses. Upon dissolution in water, some soluble fibers form viscous solutions. Viscous soluble fibers delay gastric emptying, slow intestinal transit, and reduce the absorption rate of other nutrients, thereby controlling postprandial glucose and lipid levels (Tharanathan and Mahadevamma 2003). On the other hand, the viscosity is an important rheological property that is thought to control appetite by stimulating the interaction of neural and hormonal signals that mediate satiety (Rebello et al. 2013).

Regarding the insoluble fiber (lignin, cellulose, and some hemicelluloses) its digestibility is low; however, they contribute to fecal bulk, thereby promoting laxation. Therefore, a high intake of fiber, including slowly digested and resistant starch, is associated with increased satiety, improvements in the management of body weight, reduced glycaemic response and improvements in insulin resistance (Willis et al. 2009, Mcintosh and Miller 2001). The carbohydrates of bean extracts containing α-amylase inhibitors have been shown to reduce starch digestion *in vitro* and cause weight loss in humans. Thereby, the decreased digestibility of legumes effectively reduces the amount of energy that can be metabolized. Thus, legume carbohydrates, known as 'slow-release carbohydrates', when added to the diet could provide an effective tool in the management of obesity, diabetes, and hyperlipidemia (Celleno et al. 2007).

Cardiovascular Diseases (CVD)

Phaseolus vulgaris L. has shown biological activities such as antioxidant capacity, reduction of cholesterol, and reduction of low-density lipoproteins. (Suárez-Martínez et al. 2016).

A study was conducted in people over 50 years old with a normal intake and it was found that people 50 years of age or older had more risks of cardiovascular disease (CVD) than younger people. However with a diet based on pulses (common beans), the risk of CVD decreases (Abeysekara et al. 2012, Porrini et al. 1991, Anderson et al. 1984, Pittaway et al. 2007, Leterme 2002, Genest et al. 2009). According to Bazzano et al. (2001), if common beans are consumed four times or more per week, a 22% reduction in coronary heart disease and 11% reduction in cardiovascular disease could be achieved. Besides, a decrease of 5.6% in TC and 5.4% in LDL-C can be reached according to Winham and Hutchins (2007).

The current nutritional guidelines for the prevention of CVD include a diet high in fruits, vegetables and whole grains, nuts and legumes, and non-tropical vegetable oils, which is based on the Mediterranean (Med) diet, Dietary Approach to Stop Hypertension (DASH), AHA, and U.S. Department of Agriculture (USDA) dietary plans (Goff et al. 2014).

Diabetes

Diabetes is a chronic noncommunicable disease and a multifactorial disorder characterized by the inability of the body to produce insulin (type-1 diabetes) or by defects in insulin secretion and action (type-2 diabetes) (Elliott et al. 2016).

Pulses (legumes) in special common beans have a shallow glycemic index, which results in lower blood glucose and insulin response compared with other foods. Consequently brings benefits like improvement of the lipid profile, help to glycemic control, reduce systolic blood pressure and C-reactive protein (a marker of inflammation) in overweight and obese subjects (Jenkins et al. 1981, Crujeiras et al. 2007, Dumesnil et al. 2001, Jenkins et al. 2012, Hermsdorff et al. 2011).

Dietary fiber provides important health benefits in diabetes, and beans are an excellent source of dietary fiber. Besides, the consumption of dry beans in amounts from 98 to 145 g dry weight daily improves metabolic control and has beneficial long-term effects on the control of diabetes (Simpson et al. 1981, Tappy et al. 1986).

People who have diabetes must choose the sources of carbohydrates that provide a slow release of glucose after a meal. Some foods with this characteristic available are the beans,

which present the lowest glycaemic index, thus makes them a valuable source of energy for diabetic people (Leterme 2002).

Hypercholesterolemia

Data from several studies in humans indicate that consumption of dry beans reduces serum cholesterol. Generally, in carefully controlled clinical studies where the macronutrient intake was matched, and the fiber content in the bean fed group was at least twice that of the control diet, significant reductions in both total and LDL cholesterol occurred. A 1% reduction in total cholesterol corresponds to about a 2% decrease in the risk of developing heart disease (Anderson 1987, Shutler et al. 1989, Anderson et al. 1990, Simpson et al. 1981, Anderson et al. 1984).

Beans are a good source of soluble dietary fiber, containing approximately 4 g per cup of cooked beans. Soluble fiber has been shown to reduce blood cholesterol in epidemiological, clinical, and animal studies. The consumption of dietary fiber in the US is only 12-13 g/day, well below the recommended 25-35 g/day. Incorporating one cup of cooked beans into the diet would add 12 g of total fiber and 4 g of soluble fiber per day. This increase in fiber intake would be expected to modestly lower serum cholesterol and risk of heart disease, especially in hyperlipidemic individuals (Anderson, Smith, and Gustafson 1994, Fung et al. 2003, Anderson 1987, Anderson et al. 1990, Rosa et al. 1998).

According to Abeysekara et al. (2012), a pulse-based diet reduced total cholesterol and LDL-Cholesterol (LDL-C), improved blood insulin control, lows blood pressure, and reduced overweight. These results were in agreement with similar and previous studies reported by other authors (Jenkins et al. 1983, Anderson et al. 1984, Winham, Hutchins, and Johnston 2007).

Cancer

It is not yet known how the compounds of beans slow cancer. One potential mechanism is related to the regulation of blood glucose and insulin. Recent research findings suggest that high levels of blood insulin and/or high levels of blood glucose promote colon cancer. The Cancer Prevention Study by the American Cancer Society found that subjects with Type II diabetes have a higher propensity of developing colon cancer than individuals without diabetes. Type II diabetics typically have elevated blood glucose and insulin concentrations (Giovannucci 1995, 2001, McKeown-Eyssen 1994).

Data from other extensive prospective studies also suggest that subjects with Type II diabetes have an increased risk of colon cancer (Fung et al. 2003, Hu et al. 1999). Additional evidence supporting the relationship between hyperinsulinemia and the promotion of colon cancer was provided by two studies that utilized animals exposed to a colon carcinogen and subsequent injections with insulin. Insulin injections promoted both the early stages of colon cancer and the growth of colon tumors (46). As discussed above, eating beans produce low blood glucose and insulin concentrations compared to other sources of dietary carbohydrates. Taken together, these studies suggest that eating beans to keep blood insulin and glucose low may be one mechanism that slows colon carcinogenesis (Corpet, Peiffer, and Taché 1998).

On the other hand, there is evidence to suggest that increased consumption of common beans (*Phaseolus vulgaris*) could help reduce mortality from colon cancer, where Correa (1981) found an inverse correlation between bean consumption and colon cancer mortality.

Finally, the American Institute for Cancer Research (AICR) estimates that eating a healthy diet, plus staying physically active and maintaining a healthy weight could cut the cancer risk by 30–40%.

CONCLUSION

The role of functional foods in chronic disease risk reduction has been given increasing attention over the past ten years by researchers. The promotions have heightened consumer awareness about the cardiovascular benefits of some foods such as whole grains, nuts, fish, and flaxseed. While legumes or dry beans no have received yet as much publicity, several recent reviews of the literature have highlighted their positive effects on improving cholesterol, serum lipid profiles in patients with coronary heart disease (CHD), cancer, or type 2 diabetes and other chronic diseases of metabolic origin. Important factors such as obesity, elevated concentrations of blood glucose (hyperglycemia), and blood insulin (hyperinsulinemia), and excess body fat are common etiologic factors in the development of chronic diseases.

Beans have long been recognized for their protein content and, more recently, have been noted for their soluble-fiber content, but in general, there has been relatively little research and discussion about the nutritional attributes of legumes. The glaring exception to this is the soybean, which has been investigated intensively during the past 5–10 y. This is mostly because soybeans are a unique dietary source of a group of phytochemicals called isoflavones. Isoflavones are thought to exert a myriad of biological effects, and it has been hypothesized that they reduce the risk of several chronic diseases.

Finally, we can present the common beans as a valuable source of phytochemicals that are good for health. They contain polyphenols, flavonoids, saponins that knock out some kinds of tumor cells, particularly lung, colon, breast, and blood cancers. These phytochemicals also lower blood cholesterol by inhibiting either cholesterol absorption.

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Chapter 4

NASTURTIUM OFFICINALE L.: A PLANT WITH NUTRACEUTICAL POTENTIAL

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ABSTRACT

Watercress (*Nasturtium officinale* L.) is a perennial plant that belongs to the family of the *Brassicaceae* and is cultivated for its leaves, which are principally used as salad greens or garnishes. It is used for culinary purposes by people almost all around the world. It has a strong flavor and is rich in vitamins, mainly vitamin C, but also A, B1, B2, and E. It also has minerals, gluconasturtiin, and phenolic compounds, and others phytochemicals. Watercress can inhibit tumorogenesis by modulating the metabolism of carcinogens. Watercress is one of the most important herbal medicines used for the treatment of some diseases like type 1 diabetes mellitus, type 2 diabetes mellitus, bronchitis, diuresis, and others.

Keywords: Watercress, vitamins, gluconasturtiin, diabetes mellitus

INTRODUCTION

The watercress (*Nasturtium officinale* L.) is a species native to Europe and North Asia, known since the time of the Persians, Greeks, and Romans and belonging to the family of the *Brassicaceae* (Cruz, Vieira and Silva 2006). It is commonly known as watercress, cress, Watercress (English), French cress, Brunnenkresse (German), crescione d'acqua (Italian),

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cresson de fontaine (French). It has a slightly spicy smell; hence its name derives from the Latin nasus tortus ("crooked nose"). Physiologically it presents cylindrical stems, from 20 to 80cm long, with simple, fleshy bouquets, floating hairs and they grow when submerged in water, developing fibrous roots. Their stems are robust, fistulous between 10 - 50cm high, a little fleshy, and their leaves are elongated and of lamina lirada-pinnatisecta, of dark green color; they are pecioladas, fleshy, glabrous, with one to four pairs of lobes of wavy edge, oblong and rounded, with 1-5 pairs of ovados with smooth edges of 4 - 12cm of length. It presents small, white flowers grouped in long terminal bunches, four white petals of 5mm length. Its silica is short, with dark and small seeds (Maroto Borrego 1983, Palaniswamy and McAvoy 2001).

It is one of the few greens that develop in aquatic habitats in semi-warm, semi-dry and dry climates, where there are xerophytic scrub, oak forest and mixed pine (Fernández et al. 2016). This plant has a common perennial biological cycle in clear and cold slow-flowing waters, in springs, streams, flooded lands and marshes. Their shoots are cut flush with the ground, and bunches are formed, or the leaves are cut and deposited in containers (Fernández et al. 2016, Duman, Zeliha and Aksoy 2009). Today two types of watercress are known, which are the Watercress or garden and winter cress. The first is the most common and its reproduction occurs in streams, springs, and in wetlands. This is known as inedible weed, which proliferates and is collected in two or three days after its germination when it has not yet finished developing. The watercress grows in the water and is the most nutritious so they are considered the best because it has a large amount of minerals ideal for anemic people (Fernández et al. 2016). Finally, winter watercress grows easily this season in France and Europe. It has a special characteristic in its leaves and stems since it has a waxy cover (Duman, Zeliha and Aksoy 2009, Palaniswamy and McAvoy 2001).

PHYSICAL, CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF *NASTURTIUM OFFICINALE*

The edible parts of watercress (leaves and stems) can be used to season salads, cottage cheese, sandwiches, soups, and juices, alone or in combination with other vegetables. Table 1 shows the nutritional composition of watercress.

Table 1 shows that watercress is a good source of vitamins A, B_2 and C, and minerals (calcium, phosphorus, and iodine) (Warwick and Malcolm 1980); it is also rich in carotenoids, including lutein and zeaxanthin, β-cryptoxanthin and β-carotene (O'Neill et al. 2001). Its seed contains erucic acid, a rare fatty acid; glucosinolate, mainly from 2-phenyl glucosinolate (gluconasturtiin) (Engelen-Eigles et al. 2006); a sulfur component, phenolic compounds (gallic acid, chlorogenic acid, caffeic acid, rutin, quercetin) (Mazandarani, Momeji and Zarchami 2013, Aires et al. 2013), alkaloids, sterols and tannins. (Peneilla and Magno 2011).

Quercetin glycosides, including rutin (quercetin-3-O-[ramnosyl(1-6)glucoside]), have also been identified (Gill et al. 2007). Figure 1 shows the main bioactive compounds present in *Nasturtium officinale*.

Table 1. Nutritional composition of *Nasturtium officinale* R. Br.

Component	Unit	100g
Energy	Kcal	11
Moisture	%	94.5
Dietary fiber	g	0.9
Carbohydrates	g	1.3
Proteins	g	2.3
Lipids	g	0.1
Vitamins	µg	
Retinol	mg	160
Ascorbic acid	mg	43
Thiamine	mg	0.1
Riboflavin	mg	0.2
Niacin	mg	1.5
Folic acid	mg	200
Minerals	mg	
Calcium	mg	59
Phosphorus	mg	40
Iron	mg	0.2
Magnesium	mg	44
Sodium	mg	13
Potassium	mg	209

All of these phytochemicals have been associated with various biological properties, including anti-cancer and antioxidants (Gill et al. 2007), as described below. However, one factor that limits its consumption is its perishable nature, since it has a useful life of approximately seven days. Formerly, it was used against rheumatism, cracks in the skin, anti-inflammatory and in Germany it was used as a medicine against scurvy, for its vitamin C content (Yazdanparast, Bahramikia,and Ardestani 2008, Mazandarani, Momeji,and Zarchami 2013).

BIOLOGICAL BENEFITS OF *NASTURTIUM OFFICINALE*

Interest in watercress as potential food for health and disease prevention has been rekindled in the last decade due to numerous studies linking the consumption of vegetables belonging to the *Cruciferae* family (Qazi et al. 2010).

Today, it is used to help strengthen the gums and activate salivation by chewing the leaves, prevents hair loss when implementing the fresh juice of this vegetable, applied in the form of friction on the scalp, and mixed with a little cologne, it is said to prevent hair loss. The watercress has been widely used since ancient times for its benefits and medicinal properties, blood purifying, diuretic, anti-inflammatory, and antiscorbutic (Zargari, Ghorbanihaghjo and Babaei 2015, Hadjzadeh et al. 2015).

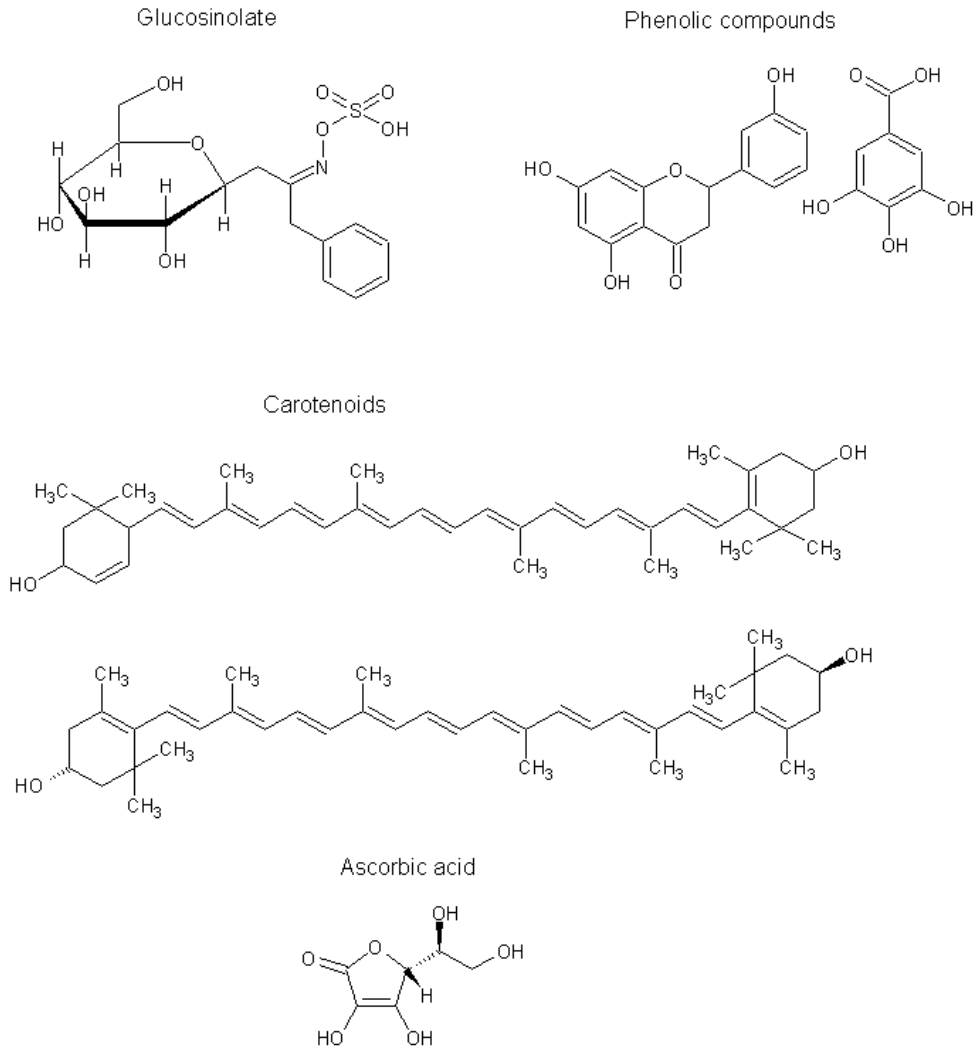

Figure 1. Phytochemicals of *Nasturtium officinale* R. Br.

It also facilitates the expulsion of acid residues from the metabolism and stimulates the production of erythrocytes (red blood cells), helping to prevent anemia, as well as skin regeneration, cell growth, intervenes in the metabolism of proteins, DNA and RNA and reduces the risk of occurrence of deficiencies in the fetus, decreases cardiovascular disease (Hadjzadeh et al. 2015, Zargari, Ghorbanihaghjo and Babaei 2015, Casanova et al. 2013). It has the peculiarity of favoring the elimination of bronchial mucus, making it more fluid due to glucosinolates (Wagner, Terschluesen and Rimbach 2013). *Nasturtium officinale* R. Br. is used to treat type 2 diabetes mellitus, bronchitis, and diuresis, scurvy, tuberculosis, influenza, asthma, nutritional supplement and also seems to have antimicrobial, anticarcinogenic, and antiestrogenic activity (Ozen 2009, Penecilla and Magno 2011). A study with aqueous extract of the aerial parts of watercress showed an anti-diabetic effect without any negative effect on the plasma lipids (Kazemi et al. 2017). Further studies are needed to determine possible mechanisms of action, establish safety profiles of the extract, and evaluate the potential value of watercress for the management of diabetic disorders.

On the other hand, *Nasturtium officinale* is popularly used as an anti-inflammatory. Therefore, Camponogara et al. (2019) investigated the anti-inflammatory activity of crude watercress extract leaves in a model of irritant contact dermatitis with croton oil-induced in mice. For this purpose, phenolic compounds (coumaric acid, rutin, and ferulic acid) were identified in the study, which reduced inflammatory cell infiltration by 62% (1mg/year) and 97% (0.1mg/year). Pro-inflammatory cytokine levels (acute model) were also reduced by 62% and 71% (MIP-2) and 44% (IL-1β). This study suggested that the use of watercress in the treatment of the inflammatory skin process can be considered as an anti-inflammatory agent. In the same way, it is suggested that the bis-tioglycosides of the cress have neuroprotective and anti-neuroinflammatory effects.

The watercress acts as an antioxidant for its high content of carotenoids and ascorbic acid, which prevents the aging of cells and protects the body against free radicals and the appearance of cancer (Hadjzadeh et al. 2015, Zargari, Ghorbanihaghjo and Babaei 2015). As mentioned above, watercress contains glucosinolates and isothiocyanates, both compounds have been reported as potent inhibitors of carcinogenesis in several animal models (Sadeghi et al. 2014, Fallah and Ebrahimi 2016). Gluconasturtiin is the most abundant in watercress and is the precursor of glucosinolates that produce phenyl isocyanate (PEITC). PEITC has been shown to inhibit some types of cancer in murine animals (Palaniswamy and McAvoy 2001). Watercress extracts are able to inhibit key stages in the route of colon carcinogenesis, including initiation, proliferation, and metastasis, suggesting that that effect may be due to a complex mixture of isothiocyanates, glucosinolates, and phenolic compounds (Boyd et al. 2006). Phenylisocyanate inhibits phase I enzymes, which are responsible for the activation of many carcinogens in animals, and induces phase II enzymes, which are associated with increased excretion of carcinogenic substances (Rose et al. 2000). Isothiocyanates also can prevent carcinogen activation through the inhibition of cytochrome P 450s in phase I enzymes, such as and through triggering quinine reductase, oxidoreductase, glutathione-S-transferase and glucuronosyltransferases in phase II in the excretion of potential carcinogens (Conaway, Jiao and Chung 1996, Wallig et al. 1998, Bianchet, Erdemli and Amzel 2008).

On the other hand, for the care of the plants, a study was carried out to investigate the effects of supplementation with exogenous sodium nitroprusside as nitric oxide to diminish the oxidative damage induced by arsenic in watercress plants (Namdjoyan and Kermanian 2013). An inhibitory effect of arsenic with exogenous sodium nitroprusside supplementation was observed in this study. Likewise, the application of 100μM SNP regulated the weight of the roots and shoots and the chlorophyll content of the leaves remarkably; it also reduced the translocation of arsenic from root to root and slightly increased the content of proteins and proline in the watercress tissues. Also, when applying sodium nitroprusside increased the activities of enzymatic antioxidants, so the results suggest that supplementation with exogenous sodium nitroprusside could alleviate the negative effects of arsenic in watercress plants.

CONCLUSION

Watercress may have some human health benefits. Cardioprotective, antimicrobial, antioxidant, and anticancer activities are some of the functions reported for *Nasturtium officinale*. Despite the important contributions of the biological activities of the cress, some

important aspects still require attention, such as the determination of the mechanisms of action, the effect of environmental factors on the chemical composition and biological activities, the regulation of the genetic expression of its chemical components, among others. Watercress can be implemented in the diet to reduce chronic degenerative diseases. The challenge for the industry is to commit to developing and marketing functional watercress to bring benefits to people's health.

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Chapter 5

CAMELLIA SINENSIS (L.) KUNTZE

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ABSTRACT

Camellia sinensis is an evergreen shrub that has been cultivated for thousands of years in Asia, and from there, it has virtually colonized the world due to English enthusiasts interested in producing tea around the XVIII century. Aside from being one of the most consumed beverages in the world, tea has become the focus of attention due to the immense amount of chemical compounds (polyphenols, theaflavins, thearubigins, caffeine, and mineral content) found in it and the potential health benefits; which are generally associated with its chemical compounds. It has been observed that tea has a beneficial effect against some diseases like cancer, Alzheimer's disease, Parkinson's disease, cardiovascular diseases, gastrointestinal diseases, diabetes, etc.

Keywords: tea plant, theaflavins, thearubigins, caffeine, medicinal properties

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INTRODUCTION

Camellia sinensis or tea plant is a species of evergreen shrub that has been cultivated for thousands of years in Asia (Figure 1). It belongs to the genus Camellia of flowering plants, which is indigenous to India, specifically to the highlands of the south-east region (Sealy 1958). Based on morphological studies carried out in *Camellia sinensis* (var. assamica) and *Camellia sinensis* (var. sinensis), botanists have concluded that there is a dual origin for the tea plant. The first would be in the area that goes from Yunnan province in China to the northern region of Myanmar and the state of Assam in India (*Camellia sinensis* var. assamica); while the second place of origin would be the eastern and southeastern regions of China (Mohan Rao and Ramalakshmi 2011a).

Historically, tea plant has had immense economic importance since their leaves are used to prepare tea, and the custom of drinking tea can be traced at least until 1000 B. C. in the Shang dynasty (territory of present-day China) (Sigley 2013). However, some historians believe that the drink of tea can be traced to 2737 BC (almost 5000 years ago). They support their claim due to the existence of legends like the one from emperor Shen Nong, a skilled ruler, creative scientist, and patron of the arts, who ordered that, as a precaution, all drinking water be boiled. One day, while visiting a region of his realm, he and the court stopped to rest, and dried leaves from a nearby bush fell into the boiling water that he was going to drink, and a brown liquid was infused into the water. As curious as the emperor was, he took a sip of the brew and was pleasantly surprised and delighted with the flavor. This legend has several variations; some even mentioned that the emperor had consumed poisonous herbs, and the tea had worked as an antidote (Yukihiko 2001). Due to the practical narrative found in the legend, many mythologists believe it is related to actual events. Other legends where the tea plant is mentioned have mythological characters such as Bodhidharma or Gautama Buddha as protagonists. Whether these legends have any trace of true or not, we can say that the tea plant has had a very important role in the Asian culture as a status symbol. Its importance is so big that it led to the development of major trade routes like the Tea Horse Road (also known as the Southern Silk Road) (Sigley 2013), so it is not surprising that its discovery is ascribed to religious or royal origins.

Tea drinking started in China and later gained so much popularity that Buddhist monks spread the custom from country to country all along Asia. It was so well received that it even reached Japan in the twelfth century. Its introduction into Europe began some 300 years ago when some enthusiasts brought it by sea from the East and tried to grow tea plants. Although this project failed miserably and was never successful as a commercial crop, as an ornamental plant, it was well-received (Vieitez 1995). It arrived first in England, and from there, it passed to France, Belgium, Italy, Portugal, and Spain. Unexpectedly, the genus flourished all over Europe due to the acid soil and temperate humid climate (Samartín and Samartin 1998).

Later, the tea plant was introduced to the USA at the beginning of the eighteenth century. Surprisingly, and despite its proximity to its natural area of distribution, it only appeared in Australia during the 19th century, and it came from England (the reason why it never reached Australia before the English colonization is still unknown). The last continent to be reached by the genus was Africa (specifically the eastern region), and this happened at the beginning of the 20th century (Vieitez 1995).

Figure 1. Drawing of a *Camellia sinensis* made by Franz Eugen Köhler (1897).

Table 1. Countries contribution to the production of tea, as of 2012

Country	**Tea production (%)**
China	30
India	28
Kenya	10
Sri Lanka	9
Vietnam	4
Indonesia	3.5
Japan	2.5

*With information taken from: https://www.biotea.es/los-principales-paises-productores-de-te.

Table 2. Countries by annual per capita consumption of tea, as of 2016

Country	**Tea consumption**
Turkey	3.16 kg
Ireland	2.19 kg
United Kingdom	1.94 kg
Iran	1.50 kg
Russia	1.38 kg
Morocco	1.22 kg.
New Zealand	1.19 kg
Egypt	1.01 kg
Poland	0.97 kg
Japan	0.97 kg

China is accounted for 42.6 percent of world tea production, with an output of 2.44 million tons; the second-largest producer is India, with an output of 1.27 million tons. However, these two are not the tea largest exporting countries since most of their production is consumed

internally. The major exporters' country title belongs to Kenya and Sri Lanka reaching an output of 475 300 tons and 295 300 tons, respectively (Table 1) (FAO 2018).

Depending on the manufacturing process, tea can be classified into three major types (Willson and Clifford 1992, Rietveld and Wiseman 2003):

- *Non-fermented* or better known as green tea (that is produced by drying and steaming the fresh leaves to inactivate the polyphenol oxidase, and thus, non-oxidation occurs)
- *Semi-fermented*, also known as oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying)
- *Fermented*, also known in the market as black and red teas (they undergo a post-harvest fermentation stage before drying and steaming, although the fermentation of black tea is due to oxidation catalyzed by polyphenol oxidase).

According to McKay and Blumberg (2002), the mean consumption of tea in the world is around 120 ml per day. Approximately 76–78% of the tea produced and consumed is black tea, and it is mainly consumed in Europe, North America, and North Africa. 20–22% is green tea and it is mainly consumed in China, Japan, Korea, and Morocco. Less than 2% is oolong tea, and it is most prevalent in China and Taiwan (Table 2). (Wu and Wei 2002, Zuo, Chen, and Deng 2002).

As previously mentioned, *Camellia sinensis* is a species of evergreen shrub that belongs to the Theaceae family. The Genus *Camellia* has over 200 species, which are mostly indigenous to the highlands of South-East Asia (Sealy 1958) . Also, Linnaeus named the genus in honor of Georg Joseph Kamel, a Jesuit missionary, pharmacist and naturalist known for producing the first comprehensive accounts of Philippine flora and fauna and for introducing Philippine nature to the European learned world (Kroupa 2015).

Sealy, in 1958, classified the genus into 12 subgeneric sections (Sealy 1958) . However, Chang et al. in 1984, based on a monograph of the genus *Camellia*, created new taxa and moved many species treated by Sealy to different sections and divided thee genus into four subgenera *(Protocamellia, Camellia, Thea,* and *Metacamellia*) and twenty sections (Chang, Zhang, and Bartholomew 1984) .

Taxonomy of the genus has been complicated due to the hybridization between species, and this has made the genetic relationships and taxonomy controversial, in fact, even now new species and revision of taxonomic relationships are being made. Surprisingly and despite its economic importance, tea taxonomy was never a priority and those who have worked on it, found a terrible mess due to hybridization events involving different species, this is confirmed by the vast array of hybrids present in some plantations (Wachira and Muoki 1997).

Linnaeus described the genus based on a drawing by Kaempfer of tea collected from Indonesia (1712). Later, he recognized and described several other species based only on the number of petals. The classification remained that way for a long time until, due to its commercial value, *Thea sinensis* was planted extensively and with it, two different taxa were identified: *Thea sinensis* (small-leaved plant) and *Thea assamica* (large-leaved plant) (Banerjee 1992).

At the beginning of the 20th century, some botanists like Sealy (1958), started to reclassify the tea plant in the *Camellia* genus but others, like Roberts, Wight, and Wood (1958) claimed that *Camellia* was just part of a section of the genus *Thea*.

A huge debate started over the classification of *Camellia*. Still, many believed that *Thea* and *Camellia* were separate genera mainly due to the presence of eugenol glycoside in *Camellia* but not in *Thea* (Fujita, Fujita, and H 1973). Aside from the presence of eugenol glycoside, morphological, anatomical, and biochemically speaking, *Camellia* and *Thea* are so much alike that there is no realistic basis for the distinction. Due to this, many authors considered Thea synonymous with *Camellia* and the name *Camellia* prevailed at the end.

In the new classification, three distinct tea varieties have been identified based on leaf features like size, pose, and growth habit. These are the China variety, (*Camellia sinensis* var. sinensis); the Assam variety, (*Camellia sinensis* var. assamica); and the Cambodian variety (*Camellia assamica* sp. lasiocalyx).

PLANT DESCRIPTION

Due to the unrestricted intercrossing in the genus, vegetative characteristics are not very useful in the taxonomy of the *Camellia sinensis*, and it is preferred to use the reproductive structures for this task. However, some botanists still use characteristics of the leaf, floral morphology, and growth habit as a preliminary differentiation of the taxa (Wight 1959).

Camellia sinensis bloom from October to April and the flowers are bisexual, actinomorphic, entomophilous, conspicuous, and fragrant. They present 2 to 3 bracteoles ovate, rounded and finely ciliate, and 2 mm long. They have 5 to 6 sepals, broadly ovate or suborbicular, glabrous or villous, finely ciliate, and 3 to 5 mm long. They also have 7 to 8 white petals with 1 to 3 sepaloid; the inner side is obovate, rounded at apex, and connate at base. They present numerous stamens (1 cm long), glabrous, outer connate for 2 mm into the column, adnate to base of corolla; the filaments are white and the anthers yellow. It only has one - carpellate pistil that is gynoecium and syncarpous; the ovary is 3-locular, globose, villous, and it has 4 to 6 ovules per locule; the style 1 cm long and trifid (Idžojtić 2019).

C. sinensis also present inflorescences with flowers axillary; it can be solitary or in 2 to 3-flowered fascicles; the pedicels are 8 to 10 mm long. The fruit is a 3-locular, flattened-globose, light brown, smooth and woody capsule; it can reach 1 to 1.5 cm long and 2 to 3 cm in diameter. It presents persistent sepal and columella. Also, it usually matures between August and October. The seeds are subglobose, brown, hard, and they can be 1 to 1.8 cm in diameter (Idžojtić 2019).

Also, as previously mentioned, in present-day there are four basic varieties, and each one has some distinctive features, as is mentioned in the next lines (except form the hybrid of China and Assam types) (Rival 2009)

- *China Type*: 1–3 m tall shrub with stems arising from the base. Relatively small, thick, and leathery leaves that have stomata and appear to be sunken in the lamina. Short and stout petioles give leaf an erect pose and are 3–7 in number. Flowers are borne singly or in pairs in the axils, have 6–10 mm long pedicels with 2–3 sub-opposite scars. They are characterized by 7–8 cup-shaped, 1.5–2.0 cm long and broad oval to sub-orbicular petals with about 3–5 styles free. The capsules have up to 3 locules containing 1–3 nearly spherical seeds of 10–15 mm diameter.

- *Assam type*: Trees of 10–15 m high with a trunk and robust branching system. Large, thin, glossy leaves with acuminate apex have distinct marginal veins and broadly elliptic leaf blades that are usually 8–20 cm long and 3.5–7.5 cm wide. Single or paired pedicellate flowers have smooth and green scars of 3 bracteoles, numerous stamens, 5–6 persistent sepals with 7–8 white petals.
- *Cambodia type*: 6–10 m tall tree, with several, almost equally developed branches and erect, glossy, light green to coppery-yellow or pinkish-red leaves, the size of which is intermediate between *C. sinensis* and *C. assamica*. Although the flowers are like the Assam type yet, they have 4 or more bracteoles, 3–4 ovules with five locules, and 3–5 styles that are free up to half the length.

Biochemical Traits

Molecular techniques have proven to be a rapid and efficient mean of examining genetic differences in the tea gene pool without the interference of the environment. Molecular markers have provided an excellent set of descriptors to differentiate between varieties and even different species of the genus *Camellia* that cannot be distinguished using morphological traits. Surprisingly enough, phylogenetic relationships in *Camelli*a, which have been determined using molecular means, are like those obtained using morphological data (Wachira and Muoki 1997). Based on these studies, it was determined that *C. sinensis* is diploid, which suggests a monophyletic evolutionary origin. Still, due to hybridization, it has also been suggested that it could have a polyphyletic origin. Also, polyploidy can occur naturally in tea (up to 2n = 90 chromosomes) or be induced by the alkaloid colchicines and mutagens. It has been observed that increased ploidy in plants means a higher vigor, hardiness, and productivity; in other words, rooting, leaf size, and dry weight benefits from it (Nair 2010).

PLANTATIONS REQUIREMENTS

C. sinensis can grow in a wide latitude (from 42° north to 42° south) and altitude (from mean sea level to 2600 AMSL). Regarding the soil, it can be adapted to different soil types, but its performance is better in well-drained acidic soils with pH 4.5–5.5. Also, it has been observed that the plant can grow at a temperature ranging from -8°C to 35°C and with an annual rainfall that can go from <700 mm to >5000 mm. (Varnam and Sutherland 1994). In addition to that, it has been observed that seasonal stress conditions can give a desirable flavor and aroma to manufactured tea (Nair 2010).

Still, environmental and climatic conditions will affect the roots and growth and, thus, the productivity of the tea plantation. Among the components that can interfere with the productivity in the plantation, light, CO_2, temperature, and water availability are the most important (Hajiboland 2017).

Effect of Light

Reported values for saturating light intensities range from 600-800 μmol m^{-2} s^{-1} up to 1200-1500 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), which means that the net photosynthetic rate in leaves of tea resembles an asymptotic line in the face of increased light intensity (Mohotti 2004). The genotype-environment interaction, the nitrogen supply, and the pruning cycle can affect the PAR, as observed in Mohotti assay, in which was reported that Pmax declines during the latter part of the pruning cycle in parallel with an increase in dark respiration rate (Mohotti 2004).

Since the tea plant evolved as an understory plant, its photosynthetic apparatus is adapted to work at maximum capacity under shade (De Costa, Mohotti, and Wijeratne 2007). It has been observed that tea leaf suffers from photoinhibition when light intensity increases beyond 1400-1500 μmol m^{-2} s^{-1} (Mohotti and Lawlor 2002), but surprisingly, if there is enough Nitrogen, tea plant can still photosynthesize with higher ranges of light intensities (Hajiboland 2017). So due to this, other researchers have studied the beneficial effect of the shading and have determined that the optimal shade level for tea plant is 30 to 40% (Gamage, Wijeratne, and De Costa 2007). However, it has also been observed that shading is necessary only in warm, tropical conditions, but in other parts of the world like cool highlands, shade is not needed (Smith *et al.* 1993).

Effect of CO_2 Concentration and Water Availability

Variation in the atmospheric CO_2 concentration is partly responsible for the variations in the net photosynthetic rate of the leaf. A positive, linear correlation has been observed between net photosynthetic rate and CO_2 concentration (Smith *et al.* 1993). Photosynthesis can be broadly increased when the CO_2 level is up to 1500 μmol mol-1. Consequently, the CO_2 available for assimilation will be determined by leaf water status, and thus, the whole plant photosynthetic capacity is interrelated with water availability (Hajiboland 2017).

Due to this, irrigation is an absolute necessity, especially when the plantation is in a place where annual rainfall may be less than 700 mm. Also, irrigation should be considered where potential soil water deficits are around 300 mm annually (Zhen 2002, Zhen 2003). This is very important since drought is responsible for about 20% reduction in yield and up to 9% mortality of tea plants (Cheruiyot *et al.* 2008).

Stomatal closure is a response to a water deficit that will limit CO_2 diffusion, photosynthesis, and this will reduce the dry matter production (Hajiboland 2017). In tea, there have been observed genotypic differences in the stomatal conductance response to water availability. Some tea genotypes are known to be able to keep their stomata open for a more extended period during a drought, which in turn, allows greater uptake of CO_2 and a higher net photosynthetic rate (De Costa, Mohotti, and Wijeratne 2007).

Since it is such an important factor, at least three indexes are being used to quantify the relationship between biomass production and water loss through transpiration: “Water use efficiency” (WUE), “transpiration efficiency” (TE) and “Intrinsic Water use efficiency” (iWUE). WUE can be estimated with the following formula: “WUE = total biomass/water received or evapotranspiration” (this one is mostly used at plot level); while TE can be estimated with the following formula: “TE = biomass/water transpired” (this one is mostly used

at plant level). IWUE can be estimated with the following formula: iWUE = the instantaneous CO_2 assimilation to transpiration (Vadez *et al.* 2014). Stephens and Carr (1993) observed that when averaged across seasons and irrigation treatments, WUE of tea increased with increasing N fertilizer application. It has also been observed by De Costa, Mohotti, and Wijeratne (2007) that in a cool dry season, WUE responds more to N fertilization, whereas, under warm, dry conditions, water availability becomes a greater limitation than N and, consequently, WUE responds more to irrigation than fertilizer application.

The rooting depth and the water available within the root zone mainly determine water uptake and as mentioned by Carr (2000) the tea plant is considered a shallow-rooting plant.

Although seedling tea has a tap root penetrating as deep as 3.0-5.5 m (Carr 2000). De Costa, Mohotti, and Wijeratne (2007) consider that the major portion of the root system in clonal tea is located within the first 30 cm of the soil profile. Interestingly, it has been reported that clonal tea develops water stress earlier than seedling tea, which some researchers partially attribute to the difference in the root system. Also, significant variation in sensitivity to drought has been observed among the genotypes of tea, and the drought-resistant genotypes can maintain a greater crop canopy cover during the dry period than the susceptible genotypes (Hajiboland 2017).

The level of soil water deficit (SWD) at which all processes begin to be significantly affected is termed as "critical SWD." Accordingly, to Wijeratne and Lalith (2013), shoot extension rate (SER), harvestable shoot density (Nsh) and shoot weight (Wsh) are significantly reduced when SWD exceeded 30-40 mm. In addition to SWD and according to Carr (2000) and Vadez et al. (2014), high ambient vapor pressure deficit (VPD) also reduces shoot growth in tea even when the soil is irrigated. The critical VPD affecting the growth of tea shoots has been reported to be about 1.2-2.3 kPa (Zhen 2002, Cheruiyot et al. 2008).

According to Carr (2000) and Vadez et al. (2014), the ambient vapor pressure deficit exerts influence on the transpiration rate by controlling the water vapor pressure gradient between the leaf sub-stomatal chamber and the outside air which means that a greater leaf-air vapor pressure deficit will equal a higher transpiration rate. This is especially important because it does not matter if the soil is wet since the excessive transpiration rates can lead to a water deficit within the plant.

It is common knowledge that mature plants can withstand drought and water stress better than younger plants; the tea plant is no exception to this. During the first 3 or 4 years after plantation, tea bushes are prone to suffer the drought effects mentioned above; establish a system of irrigation during dry periods and shade has proven to be a reliable solution.

Nonetheless, the lack of water resources and the high cost of irrigation in some parts of the world is a considerable limitation. So, it is imperative to optimize the use of irrigation water, harvesting, improve agricultural practices and identify (and use) the drought-resistant genotypes; with that, we will be able to reduce crop losses and minimize plants death as well as avoiding over-exploitation of water resources (essential for all human activities).

One of the major challenges that agriculture must face come from the climate change that has made the global weather unpredictable and creating uncertainty in all the agricultural products (tea is no exception) (FAO 2018). All the known effects of climate change (drought, changes in temperature, high-intensity rainfall, etc.) have adversely affected yield and production of tea, both in quantity and quality, in recent decades (Duncan *et al*. 2016). According to Bhagat *et al.* (2016), Asia expects a 10% extension of the dry and wet seasons in the main tea plantation area by 2070, together with an increased frequency and severity of

extreme weather events. Temperature increases of 0.4 to 3.0°C are also predicted, while rainfall is expected to increase with an uneven pattern of distribution. This effect most likely will vary across the tea production regions; still, several tea-growing regions have been identified as the most vulnerable to climate change (Wijeratne and Lalith 2013).

Due to the relationships between tea yield and the atmospheric variables, long-term climate change is likely to cause significant impacts, both positive and negative, on the key physiological and developmental processes that determine the production and components of tea (Hajiboland 2017). Among the effects that could be observed, we could find that the increased atmospheric CO_2 will positively affect the photosynthetic rates and yields, although this can fluctuate since there have been reports of genotypic variation regarding this variable (Poorter and Navas 2003).

In addition to atmospheric CO_2, Wijeratne and Lalith (2013) have identified several other variables like temperature, reduction of rainfall, yield projections, and used them in simulation models to predict the impacts of climate change on tea yield at different altitudes. Other researchers like De Costa, Mohotti, and Wijeratne (2007) have also worked in this field and concluded that by the year 2050, under the climate change scenarios, there will be an increased yield at higher altitudes, but yields at lower altitudes will decrease.

NUTRITIONAL REQUIREMENTS

Like all plants, tea needs an adequate amount of nutrients for optimum growth and productivity; therefore, the use of fertilizers is important for increasing yields. With the harvest, we also remove nutrients from the soil, and, in the case of tea, we remove large quantities of the macronutrients N, P, and K (although it will depend on the duration of plucking rounds and their intensity). So it is necessary to replenish these nutrients to the soil (Tabu, Moroamoche Kekana, and David 2015). Also, as well as the nutrients mentioned above, with the harvesting, we will be removing the calcium, sulfur, magnesium, and zinc (Sultana *et al*. 2014). Due to this, we have to put special attention to soil fertilization, because each of the nutrients mentioned above plays a special role in the plant (as we shall see below).

Nitrogen

Nitrogen (N) plays a key role in the growth of plants, and it is the most deficient mineral element in the soil; according to Owuor (1997), nitrogen enhances the yield in tea under favorable conditions of temperature, relative humidity, rainfall, and evaporation. On the other hand, its deficiency leads to the shortening of the internodes and the leaves are lighter than the normal. It is considered that Nitrogen is deficient in the tea plant when the leaf N content is less than 3%, mildly deficient when it is 3.0-3.5%, and sufficiently supplied when it is more than 3.5%. De Costa, Mohotti, and Wijeratne (2007) reported that, in the tea grown in a nutrient solution, the photosynthetic rate and photochemical efficiency increased with increasing N supply up to 105 ppm N, but interestingly, the response in photochemical efficiency was only observed after one month. According to Ruan *et al*. (2016), *C. sinensis* is well adapted to NH4

-rich environments and thus prefers NH_4^+ instead of NO_3 (that produce a poor plant growth) which is largely associated with inefficient absorption of this N source.

Phosphorus

Phosphorus (P) is an essential macronutrient that plays a key role in the cellular structure and energy metabolism in higher plants. It is a major component of new wood and roots in *C. sinensis*. Its deficiency manifests as the absence of brightness, and dieback of young and old woody stems. P availability will depend on the pH of the soil; it is recommended a pH of about 5.5 to 7.0 to increase the P availability (since a pH less than 5.5 or greater than 7.0 will decrease it) (Hamid 2009).

Up until now, no one has been able to determine the optimum requirements of a phosphate fertilizer; in fact, it has been reported a relatively low increase in yield, or even no response to a P fertilizer in tea (Hajiboland 2017). It also has been observed that the tea plant is extremely tolerant of P deficiency and that the P requirement for optimum growth is as low as 50 µM (much less than the P requirement for optimum growth for many other species) (Hajiboland and Salehi 2014). This surprising behavior can be attributed to the high internal use efficiency that comes from the high rate of re-translocation of P from mature to young growing leaves.

Potassium

Potassium (K) is almost as important as Nitrogen. And just like that one, large quantities of it are removed through harvesting and since tea plant has a moderate to the high requirement for K, it's necessary to put attention to this nutrient (Sultana *et al*. 2014). Its deficiency manifests as tip and margin burn-in leaves, degeneration of feeder roots. Also, branches are thin and weak, the recovery from pruning is longer, and dieback may occur in extreme cases.

Micronutrients

Most fertilizers always emphasized on macronutrients and neglect to some extent the micronutrients. That is a problem because they can also affect the growth of the plant negatively, and their availability is affected by different factors such as soil and rhizosphere pH. Soils with high pH will tend to retain the micronutrients and limit the plant uptake (Rengel 2015). Therefore, in this section, we will talk about three of the most important micronutrients for the tea plant: Zinc, Boron, and Aluminum.

Zinc

Zinc plays a significant role in tea growth; in fact, its deficiency will lead to stunted growth and death. One of the main reasons for its shortage is the excessive application of phosphate fertilizers. The simplest way to detect the lack of Zn in the soil is when the leaves are narrow,

erect, and form a rosette at the apex of the stem (Nelson 2006). It is necessary to use a foliar fertilizer to prevent the nastiest effect of lack of Zn (soil fertilizer has proven to be ineffective). However, acid soils don't have this kind of problems (but may suffer from other deficiencies) (Sultana *et al*. 2014).

Boron

Excessive boron can cause off-colors, stunting of growth, mature leaves exhibit leaf-edge burning and necrosis (Bhupen *et al.* 2019); in the other hand, a deficiency of this element, will lead to an impaired root development, reduced shoot height, smaller leaves, a dark green color and, in extreme cases, curling of the leaf lamina. In addition, plants lacking B, are susceptible to drought stress due to the impaired root development (Hajiboland and Salehi 2014). The most problematic thing about this nutrient is that must of the field studies show that there is no considerable improvement in tea yield by B-fertilizer application to either the soil or leaves. The application of the fertilizers is discouraged and not routinely recommended for many of the tea growing areas as it can cause toxicity symptoms to appear (Hajiboland 2017).

Aluminum

Although this is the third most abundant element in the world, the plants can only get it when the pH is under 5.5, but if the soil is too acid. Al can become increasingly available and create severe toxicity that in the worst scenario can lead to death because it can decrease the uptake and utilization of P by fixing it through an absorption-precipitation reaction and also causes a Ca deficiency in plants due to the disruption of its absorption (Poschenrieder *et al*. 2015).

An optimal supply of this element, which is 300 μM Al, will promote the growth of tea bushes and will increase dramatically root biomass (more than threefold). This will also make that tea plants have a greater root surface area that will improve water and nutrient uptake (Hajiboland and Salehi 2014). Interestingly, lack of Al will make that tea roots accumulate Fe up to toxic levels (three times as much as in other species).

PEST AND DISEASES

Although mismanagement of nutrition can cause losses in tea production, diseases and pests are somewhat more worrying as they can lead to greater economic losses. But, fortunately, the effect of diseases and pests on tea production depends greatly on the control strategy decided by the farmer and also by the agro-ecological environment and the altitude of the plantation (Hazarika, Bhuyan, and Hazarika 2009).

The control of diseases and pests is a very interesting topic in itself and sometimes conflict with recommended crop management mentions above (especially with the shading management). For example, two of the most famous and deadliest diseases in the tea world production: "blister blight" (caused by the fungus "*Exobasidium vexans*") and the tea mosquito

bug (*Helopeltis sp.*) can be prevented or greatly controlled by cultivating the tea plant in full sunlight or with reduced shade. Eliminating shade trees (a rather controversial measure), have proved to be an excellent way to mitigate the weed problem, the need for synthetic fertilizers and the incidence of pests and diseases (Hajiboland *et al.* 2013).

Roughly, around 1031 arthropod species are associated with tea. Among all of them, only about 3% are common pests throughout the world. This behavior, accordingly to Banerjee (1981), can be attributed to several different factors such as the influence of climate, altitude, nature of cultivation, and age of plantation; still, it has been observed, that each geographic region may have its distinctive pest complex (Bhupen *et al.* 2019, Poschenrieder *et al.* 2015). Due to this, in the next lines, we will proceed to talk about some of the most important pests that affect the tea plant.

Mirids

The Miridae are a large and diverse insect family. It is the largest family of true bugs belonging to the suborder Heteroptera; it includes over 10,000 known species, and new ones are being described constantly (Cassis and Schuh 2012). Among all this diversity, two species of the genus *Helopeltis* (*H. schoutedeni* and *H. theivora*) have become the major concern of tea planters in Africa and Asia. They are responsible for causing from 11% to 100% crop loss (Rattan 1992, Sundararaju and Sundara Babu 1999, Flamen 1989).

The nymphs and adults of *H. theivora* suck cell sap from the stems, leaves, and buds, forming reddish-brown circular feeding punctures (they can be 0.29 to 2.51 mm in size). In severe infestations, damaged leaves curl upward and desiccate. Sprout of new shoots is prevented due to the death of the stem and in extreme cases, the infestation may lead to a total loss of the crop (Hazarika, Bhuyan, and Hazarika 2009); *H. schoutedeni* on top of the previously mentioned effect, will also cause dieback and stem canker (Rattan 1992).

Tea Tortricids

The Tortricidae are a family of moths, commonly known as tortrix moths or leaf roller moths in the order Lepidoptera. The family has over 10,350 species described, and many of these are economically important pests (Baixeras 2005). 19 species of tortricids (belonging to the genera *Homona* and *Adoxophyes*) are important pests of tea in Asia (especially in Japan, China, India, Sri Lanka, Taiwan, Turkey, Republic of Georgia, Azerbaijan and Bangladesh). Not all 19 species are present in all the countries, but instead, they are distributed by regions, for example, *Adoxophyes honmai* is a major pest of tea in central and southern Japan, while *Homona magnanima* can only be found in southern Japan. *Homona coffearia* is present in India, Indonesia, and Sri Lanka (Nabeta, Nakai, and Kunimi 2005).

Shothole Borer

Scolytinae or bark beetle is one of the 220 genera belonging to the Curculionidae family; some time ago. *Scolytinae* was considered a distinct family “Scolytidae” but recent studies

proved that it is just a very specialized member of the "true weevil" family (Curculionidae) (Lawrence and Newton 1995). It has around 6000 species, and many of them attack and kill the live tree, while others just live in dead, weakened, or dying hosts (Franceschi *et al*. 2005). Nine species of bark beetles are a pest to tea; among them, *Xyleborus fornicatus* is considered the most serious pest (at least in Sri Lanka) because it can cause up to 100% infestation in the wet and dry zones of the country (Walgama and Pallemulla 2005). Females of this species construct galleries in the stems and cultivate the ambrosia fungus (*Monacrosporium ambrosium*) to feed on it. The infestations tend to happen in young stems (older ones, have a harder wood). It is still unknown if the death of the stem occurs due to the wood rot or due to the extensive growth of the fungus (Hazarika, Bhuyan, and Hazarika 2009).

Mites

Mites are small arthropods belonging to the class Arachnida and the subclass *Acari* (also known as Acarina). The term "mite" refers to the members of several groups in *Acari* but it is not a clade as it spans two different groups of arachnids; it also excludes the ticks (they belong to the order Ixodida) (Dhooria 2016). They are considered as the most persistent and serious pests of tea in almost all tea producing countries (Das, Roy, and Mukhopadhyay 2010). *Tetranychus kanzawai* is a huge problem in Japan, China, Taiwan, and the Philippines (Jin *et al*. 2018); while *Brevipalpus phoenicis, Acaphylla theae,* and *Calcarus carinatus* can be found in almost all of the tea-growing countries from Asia and Africa (Hajiboland *et al*. 2013, Nabeta, Nakai, and Kunimi 2005, Poschenrieder et al. 2015).

Among all of them, arguably, the most serious pest is the red spider mite (*Oligonychus coffeae*); it can be found all over Asia and Africa (Lawrence and Newton 1995). This mite lacerates cells, producing reddish-brown marks on the upper surface of mature leaves (they can turn red in severe cases); and infestation of this species can lead to losses up to 46% of the crops (Hazarika, Bhuyan, and Hazarika 2009).

CHEMICAL COMPOSITION

As previously mentioned, tea is one of the oldest and most consumed beverages in the world. The health benefits of tea have been known to human civilization for centuries, but it was only possible until the contemporary age to corroborate this ancestral knowledge.

The chemical composition of tea plant is extremely complex, since we can found a vast array of products such as polyphenols, fluoride, vitamin K, caffeine, minerals (sodium, potassium, and calcium) alkaloids, amino acids, glycerides, volatile compounds, and trace elements like aluminum, chromium, selenium, manganese, and iron (Reto et al. 2007).

Some compounds are as unique as the plant itself. These substances tend to undergo dynamic changes during the manufacturing process, and they can undergo rapid changes due to the agricultural practices, sunlight intensity, plant location, and the genotypic variance itself (Varnam and Sutherland 1994). Also, it is believed that the interactions between these chemical constituents (together or individually) affect both the quality and the medicinal attributes of tea (Krishnamoorthy 1991). The chemical composition is so complex that an entire book could be

written just describing every one of these compounds (and all the changes that they can undergo). Since this is not the objective of this chapter, we decided to group (and describe) them for an easy lecture as polyphenols, caffeine, carbohydrates, amino acids, vitamins, and minerals. It is important to know that, from the medical point of view, polyphenols and caffeine are the most important compounds.

Polyphenols

Tea is reported to contain nearly 4000 bioactive compounds, of which one third is contributed by polyphenols (Mahmood, Akhtar, and Khan 2010). They are responsible for the health benefits that have traditionally been attributed to tea, especially green tea (Cabrera, Artacho, and Giménez 2006). Polyphenols, in general, possess high antioxidant properties and can protect human cells from the adverse effect of the ROS (reactive oxygen species).

They can act as scavengers to ROS and prevent damages to cellular macromolecules (Dufresne and Farnworth 2001) . The reactive oxygen species tend to react with other molecules to trap electron away from them. Thus, these molecules become radicals and start harmful chain reactions. Various researchers showed that all types of organs and tissues are subjected to radical damages with varying intensity (Gramza *et al*. 2005). Tea contains about 30 to 42% polyphenols on a dry weight basis, among these compounds, the most important are catechins (Figure 2), and the most abundant are: (−)-Epigallocatechin3-gallate (EGCG); (−)-Epigallocatechin (EGC); (−)-Epicatechin-3-gallate (ECG); and (−)-Epicatechin (EC). The highest concentration is that of EGCG, followed by EGC, ECG, and EC in decreasing order (Sharangi 2009, Mohan Rao and Ramalakshmi 2011b).

A normal cup of tea may contain between 300 and 400 mg of polyphenols in total and them, EGCG is the major polyphenolic constituent (about 25 to 40%) of the total catechin load of the tea. It is important to note that the catechin concentration depends on the plant variety; for example, Assam variety generally accumulates more catechin than the Chinese variety. In the former, catechin accounts for 30% of the dried matter against 20% in other varieties (Sharangi 2009, Lorenzo and Munekata 2016).

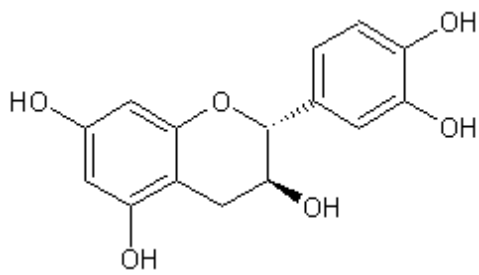

Figure 2. General chemical formula of catechins.

Catechins mixed with polyphenol oxidase create bioflavonols and theaflavins. Thearubigins are formed by the polymerization of catechins and constitute 10 to 20% in black tea that is 10–20 times greater than the dry weight of theaflavins. It is important to know that higher theaflavin content usually implies good manufacturing practices (Brückner *et al*. 2012, Sharangi 2009, Mohan Rao, and Ramalakshmi 2011b).

Caffeine

Caffeine is a purine alkaloid (Figure 3), it constitutes 2.5 to 5.5% of the total chemical constituent present in tea and can be found along with with small quantities of monomethylxanthine and dimethylxanthine (Wood et al. 1964). Caffeine is pharmacologically classified as a central nervous system stimulant and a diuretic. It possesses the ability to improve the wall elasticity of blood vessels, promoting blood circulation, increasing the efficient diameter of the vessel, and stimulating urination and auto-oxidative activity (Shi, Dalal, and Jain 1991). Besides its direct use in pharmaceuticals, caffeine is a key component in stimulant beverages and forms the starting material for the manufacture of Theophylline, Aminophylline, and Bramanine (basically, it has direct use in pharmaceutical and food industry) (Mohan Rao and Ramalakshmi 2011c).

Caffeine was first isolated from tea in 1820 by Runge, and he named “theine” (he thought that it was a new compound), but later it was found that it was the same as the caffeine from coffee so the name was abolished (Bedigian 2005). It was believed that caffeine was the byproduct of the degradation of ribonucleic acid, but recently this was proved wrong; in fact, a gene in the tea leaf encoding caffeine synthase (N-methyltrasferase) was found, cloned and the recombinant enzyme was produced in *E. coli* (Ashihara and Crozier 2001).

In the case of the tea plant, the variety and type of tea (black, green, oolong, etc.) could affect the caffeine content; but it is not significantly reduced during tea processing, although it may decrease during the brewing process. The caffeine contained in the leaves is higher in younger stages and gradually decreased while they grow. The caffeine contents in 1st and 2nd leaf (3.4% in dry weight) are higher than that in the mature leaf (around 1.5% in dry weight) (Zhen 2002).

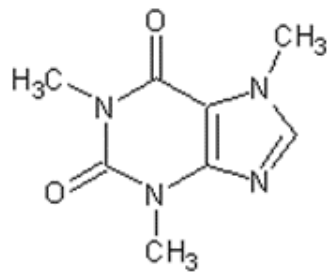

Figure 3. Chemical formula of caffeine.

Carbohydrates

Carbohydrates constitute about 4–5% of solids extracted in tea infusion. Though concentrated mostly in roots, carbohydrates are not uncommon in leaves. The contents of carbohydrates in tea flush change under natural and shaded conditions; sucrose is the major primary product of photosynthesis; it increases with the growth of tea shoots and may occupy more than 50% of the totally free sugar content (Anan, Takayanagi, and Ikegaya 1985).

The polysaccharides present in the tea were determined to be hemicellulose, cellulose (6–8% dry weight basis), and other extractable polysaccharide fraction (1–3%) composed of different sugar residues like glucose, galactose, mannose, arabinose, xylose, ribose, and

rhamnose. The cellulose and hemicellulose contents are negatively correlated with the tenderness of the tea shoot; the higher their content, the less tender is the raw material, and also the quality of the product is lesser (Zhen 2002). According to several researchers, the composition of tea polysaccharides differs from the tenderness of the raw material and season.

Amino Acids

Amino acids constitute around 4% in tea flush. These include theanine, aspartic acid, threonine, glutamic acid, glycine, α-alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, glutamine, asparagines and tryptophan (Mohan Rao and Ramalakshmi 2011b).

The most abundant amino acid is theanine (5-N-ethylglutamine), which is unique to tea and can reach up to 50% of the free amino acid fraction (Figure 4); its biosynthesis site is in the root, and from there it is transferred to younger leaves (Zhen 2002).

Other major amino acids present in the tea bush are aspartic acid, threonine, glutamic acid, glycine, α-alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, glutamine, asparagine and tryptophan and most of them are usually associated with proteins (Mohan Rao and Ramalakshmi 2011b).

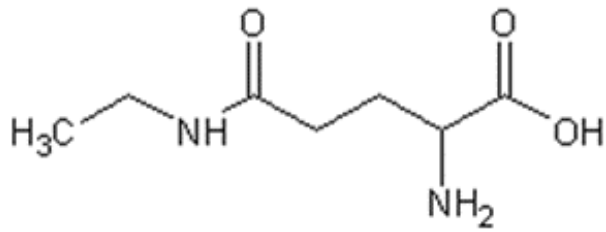

Figure 4. Chemical formula of theanine.

Vitamins

Tea is especially rich in vitamin C. In fact, it is reported that the vitamin C contents in 100 g of green tea are as high as 150–300 mg. However, this concentration is not homogeneous among all tea varieties; for example, vitamin C content in oolong tea and black tea is less than that in green tea due to decomposition in the fermentation process. Also, the vitamin B group content in green tea and black tea are similar, around 1–2 mg per 100 g made tea. Vitamin E mainly exists in the lipid fraction of tea, and the content is about 24–80 mg per 100 g of tea, and vitamin K content is around 300–500 i.u. per gram of tea (Zhen 2002).

Minerals

The average total mineral content of tea is around 5% of the dry matter. There are around 28 elements reported in tea flush. Besides molybdenum, iodine and lead are situated in the V–VI period in the periodic table; the remaining 25 elements contained in tea flush are situated in

the I–IV period. The elements contained in tea leaves can be classified according to their concentration (Zhen 2002).

Interestingly, fluorine, potassium, aluminum, iodine, selenium, nickel, arsenic, and manganese are present in higher than average levels compared to other plants. For most of these minerals, the reason for the high concentrations is still unknown, except for the fluorine and aluminum, since the tea plant is known as a fluorine and aluminum concentrating plant. The role of fluorine in the plant is not known (while the aluminum role was mentioned above); however, the fluorine in tea is beneficial to human health, especially in the prevention of dental caries (Zhen 2002). Regarding aluminum content in the tea plant, we can say that it is much higher than those in other crops, and it can range from 200 to 1500 mg/k (Pennington 1988).

Most of the Al species found in tea leaves are in the catechin complex while in other parts of the tea plant, Al can be found as two distinctive forms: catechin and fluorine complexes; interestingly, the fluorine forms were not found in the leaves. These could mean that the Al-F complex is then translocated form, which is rapidly converted into another form in the leaves (Horie *et al*. 1994). It is recommended adding Al to commercial tea for improving the infusion color; however, it is not acceptable from the viewpoint of human health. Overdose of Al from food may induce neurotoxicity and is possibly related to Alzheimer's disorder (Tomljenovic 2011).

Copper and Manganese deserves close attention due to their importance. Cu is of particular importance in tea biochemistry because the polyphenol oxidase enzyme (PPO) contains this mineral. The Cu content in tea leaves amounted to 12–18 mg/kg, which is average compared to other plants. However, the Cu contents may be increased due to the Cu residue caused by the application of copper fungicides for controlling blister blight disease of the tea plant (Chen *et al*. 2002). Manganese participates and catalyzes the activity of many enzymes, such as DNAase, choline esterase, phosphatase, phosphohexokinase, adenosine kinase, pectin kinase, trans-glutaminase, polymerase, etc. in humans. The Mn content in fresh tea leaves ranges from 200–1200 mg/kg. However, the content in old leaves is higher than that in tender leaves (Chen *et al.* 2002).

NUTRACEUTICAL PROPERTIES

As we mentioned before, tea is one of the most popular beverages consumed all over the world, and its consumption has increased in recent years due to its preventive effects against certain human diseases (Costa, Gouveia, and Nobrega 2002, Rietveld and Wiseman 2003). The increase in consumption has been so significant, that today is the most consumed drink in the world after water; well ahead of coffee, beer, wine and carbonated soft drinks (Cabrera, Artacho, and Giménez 2006).

Interestingly, although health benefits have been attributed to green tea since its discovery just as one of the variants of the legend of Emperor Shen Nong indicates, scientific investigations on this beverage and its constituents have been underway for less than three decades (McKay and Blumberg 2002).

The studies carried out have been very varied, and have used different methods such as *In vitro* and animal studies, clinical trials employing putative intermediary indicators of disease, particularly biomarkers of oxidative stress status, etc. All of them have provided good and very

reliable evidence that green tea compounds (especially polyphenols or GTP) may play a role in decreasing the risk and pathogenesis of several chronic diseases, especially cardiovascular disease and cancer, and related pathologies. On the other hand, it is also suggested that its consumption has a beneficial impact on bone density, cognitive function, dental caries, and kidney stones, among other effects (McKay and Blumberg 2002).

Due to this, even more, numerous epidemiological and clinical studies have been made over the last years and have revealed several physiological responses to green tea that may be relevant to the promotion of health and the prevention or treatment of some chronic diseases. However, some of the results are mixed. But this could be due to ignoring socioeconomic and lifestyle factors or due to inadequate methodology to define tea preparation and intake (McKay and Blumberg 2002, Rietveld and Wiseman 2003, Wu and Wei 2002, Zuo, Chen, and Deng 2002). Due to the mentioned above, this section deals with the preventive activity of tea in some diseases

Effect on the Cardiovascular System

Epidemiological observations have established an indisputable inverse correlation between tea consumption and the incidence of cardiovascular diseases. The chemical compounds that have been identified as responsible for this correlation are the polyphenolic compounds. However, the mechanisms of how polyphenols help prevent cardiovascular diseases are not very well understood (Yamada and Watanabe 2007). Still, some people believe that the protective effect of green tea on cardiovascular diseases is due to antioxidant activity. Oral intake of green tea extract by human volunteers showed an increased resistance of plasma LDL to oxidation in vivo, an effect that is believed might lower the risk of atherogenesis (Miura *et al.* 2000).

In line with these results, Negishi *et al.* (2004) observed that both black and green tea polyphenols attenuate the blood pressure increment through their antioxidant properties, in stroke-prone spontaneously hypertensive rats. Also, some epidemiological studies like the ones of Hodgson et al. (2003) and Yang et al. (2004) show that the consumption of green tea or oolong tea significantly reduces the risk of developing hypertension.

It has been documented that tea intake has a cholesterol-lowering effect in mice, and green tea consumption has been shown to reduce the development of aortic atherosclerosis (hardening, thickening, and elasticity-loss of arteries) in rabbits (Hodgson and Croft 2010, Riemersma *et al.* 2001, Samman *et al.* 2001). Several studies have demonstrated that green tea may affect the mechanisms of action related to LDL-cholesterol oxidation. The oxidation of LDL-cholesterol, which is associated with a risk for atherosclerosis and heart disease, is inhibited by green tea due to epicatechin (EC) and epigallocatechin-3-gallate (EGCG) antioxidant activity, in fact, it was observed that the antioxidant activity of EGCG on LDL oxidation was stronger than that of EC (Williamson and Manach 2005, Khan and Mukhtar 2013). Also, Trevisanato and Kim (2000) claimed that GTP might slow atherogenesis by reducing the oxidative modification of LDL-cholesterol and other associated events.

The most significant flaw of these studies is that the effects in the cardiovascular system are observed with high doses of these polyphenolic compounds (doses that are not compatible with tea intake in daily life). Because of that, some researchers have proposed that the effect is due to some other component or the interaction of several compounds present in the tea.

However, due to the immense quantity of compounds present in the tea, it has not been possible to determine which of them is responsible yet. Still, many believed that the beneficial effects of tea are due to (−)-epigallocatechin-3-gallate (one of the major catechins) or due to theaflavins (Mohan Rao and Ramalakshmi 2011b). So the possibility that dietary tea intake reduces the risk of cardiovascular events remains open to the need for further clinical trials to clarify the mechanisms and the chemical compounds that are responsible for the effects of tea humans (Yamada and Watanabe 2007).

Cancer

The possible cancer-preventive activity of tea intake and tea polyphenols has been studied extensively. Many studies in animal models, cell lines have been done and the cancer-preventive activity of tea constituents have been demonstrated in many animal models including cancer of the skin, lung, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, bladder, prostate, and mammary gland (Yang *et al*. 2007). In some studies, the inhibition correlated with an increase in tumor cell apoptosis and a decrease in cell proliferation (Mittal *et al.* 2004). The major active constituents are polyphenols and caffeine, of which epigallocatechin-3-gallate (EGCG) is the most abundant, most active, and most studied. However, the mechanisms of the cancer-preventive action are just beginning to be understood. Many believe that the chemopreventive effects of green tea depend on one of the following processes: the antioxidant activity, the induction of detoxifying enzymes, the molecular regulatory functions on cellular growth, development and apoptosis, and a selective improvement in the function of the intestinal microbiota (Cabrera, Artacho, and Giménez 2006).

In 1987, Yoshizawa *et al.* (1987) published the inhibitory effects of topically applied EGCG against skin tumor promotion in mice , and from there, many studies had been conducted, demonstrating the inhibitory activities of tea and preparations of tea constituents against tumorigenesis. Although the majority of the studies demonstrated cancer-protective effects, some studies indicated otherwise (Yang *et al*. 2007).

Inhibition of lung tumorigenesis by consuming the constituents of green tea and black tea has been demonstrated by Yang *et al*. (2005) and Clark and You (2006) in different models. Including those induced by tobacco smoke-related chemical carcinogens such as 4-(methylnitrosamino)-1-(3- pyridyl)-1-butanone (NNK), benzo[a]pyrene, and N-nitrosodimethylamine as well as spontaneously developed lung tumors in A/J mice. It was also observed that the intake of green tea, black tea, EGCG, or theaflavins during the initial stages of an NNK-induced lung tumor significantly decrease its volume (Yang *et al*. 2007).

Green tea catechins have also shown to be chemopreventive compounds; the aromatic nature of polyphenols makes them potential targets of hypochlorous acid and peroxynitrite, and these reactions may create novel pharmacophores at the site of inflammation. Also, a major mechanism of the anticarcinogenic activity of green tea in animals is the impairment of the interaction of carcinogens with DNA leading to mutations. However, this mechanism is still not fully understood, so it will probably be reviewed in futures studies alongside the role of GTP and catechins.

Also, caffeine must be evaluated as a potential chemopreventive compound (Lin and Lin 1997). Another constituent that shows a promising anticancer potential due to its antioxidant,

antimutagenic and chemopreventive properties is EGCG, so it would not be strange that shortly, many studies were carried out with this compound (de Mejia, Ramirez-Mares, and Puangpraphant 2009, Dai and Mumper 2010).

Although most of the studies about the cancer-preventive activity of tea have been made using animal models, several human studies supporting a positive effect of tea consumption and cancer prevention (mainly about breast cancer). Nakachi *et al*. (1998) found that stages I and II breast cancer patients (n = 472) showed a lower recurrence rate (16.7%) and a longer disease-free period (3.6 years) when consuming more than five cups of green tea per day, compared to those drinking fewer than four cups per day. While Zhang *et al*. (2007) also associated green tea consumption with a reduced risk of developing breast cancer.

Tea polyphenols have chemo-preventive properties, they can protect all stages of carcinogenesis due to its antioxidant properties against free radicals, and thus it initiates apoptosis and cell cycle arrest (Lee et al. 2013). Also, there is enough evidence to claim that black tea polyphenols protect against different types of cancer through their ability to inhibit carcinogenic activating enzymes, activate antioxidant and detoxifying enzymes, modulate xenobiotic-metabolizing enzymes, scavenge free radicals, induce apoptosis and inhibit angiogenesis, cell proliferation and metastasis (Kumar, Pillare, and Maru 2010, Murugan et al. 2009).

Also, green tea polyphenols protect against different types of cancer thanks to several different mechanisms such as inhibition of anti-apoptotic protein expression, induction of pro-apoptotic protein expression, inhibition of cell proliferation, metastasis and angiogenesis and induction of cell cycle arrest and thus it inhibits uncontrolled cell proliferation (Sanlier, Gokcen, and Altuğ 2018).

Gastrointestinal System

Gastric injury can cause damage to the stomach organs. Ethanol promotes the rapid formation of injuries in the stomach, which occurs mainly due to an inflammatory reaction (Szabo *et al.* 1985). Also, as previously mentioned, tea chemical components are known to have an anti-inflammatory reaction, and several studies prove that tea can help in these cases too.

It was reported that black tea extract given to rats for seven days inhibited the development of both aspirin and gastric ulcer. It was also found that tea extract reduces acid and peptic activity of gastric secretion induced by aspirin and cold-restrained stress (Maity, Vedasiromoni, and Ganguly 1995). It has also been observed that tea stimulates the overall production of gastric mucosa because with its consumption the levels of gastric glutathione peroxidase, hexosamine, and sialic acid contents are increased (Maity *et al*. 2003). Ingestion of black tea infusion inhibited aspirin, alcohol, and serotonin-induced ulcer in rats. It was also observed that there was a significant restoration of superoxide dismutase (SOD) and catalase (CAT) enzyme activity in gastric and liver tissue following ethanol-induced oxidative stress (Chaudhuri *et al*. 2005).

Recent studies have shown that consuming large quantities of yellow tea, (around 1000 mg/kg) can reduce the levels of the serum pro-inflammatory cytokines interleukin, the tumor necrosis factor and the gastric injury (Wang *et al*. 2013). Also, yellow tea can contribute to the protection against liver injury (Hashimoto *et al*. 2007).

Many studies have reported the antioxidant effect of catechins, can diminish oxidant stress-induced experimental liver injury (Kim, Yang, and Cho 2009). Another research suggests that the drinking of green tea may help to prevent and attenuate the development of fibrosis in hepatitis (Abe *et al*. 2007). The abilities of EGCG to suppress matrix metalloproteinase MMP-2 activation and hepatic stellate cells (HSC) invasiveness suggest that EGCG can be used hepatic fibrosis (Zhen *et al*. 2006). Some reports show that EGCG inhibited collagen production regardless of enhanced collagen transcription and suppressed collagenase activity, which indicates that it may have a therapeutic potential in liver fibrosis (Nakamuta *et al.* 2005). It is reported that EGCG is also able to induce phosphorylation of mitogen-activated protein kinases, reaffirming that EGCG has therapeutic potential in the setting of liver fibrosis (Higashi *et al*. 2005).

There are a large number of studies that suggest that flavonoids present in tea are effective against a wide variety of experimental, synthetic, and natural materials that can cause liver associated injuries (Maiti *et al.* 2019). The whole green tea extract has also been shown to reduce D-galactosamine-induced acute liver injury. While searching the mechanism, it was found that green tea augments the inhibition of apoptotic and pro-inflammatory signaling (Lin *et al.* 2009).

Neurological Effects

Among the deadliest and most common causes of death worldwide are neurological diseases (especially Alzheimer's disease and Parkinson's disease) (Firoz *et al.* 2015). It is believed that lifestyle, diet, stress, and other factors can affect the brain's ability to fight against neurological disease and its ability to protect itself against cognitive decline and dementia (Gomez-Pinilla and Kostenkova 2008). On the other hand, tea has been considered as a natural cognitive enhancer, and recent studies have shown that regular tea intake is related to better verbal fluency scores, cognitive function and lower the risk of cognitive decline (Noguchi-Shinohara *et al*. 2014, Shen *et al.* 2015).

Due to this, studies have been carried out and have found that aside from the anti-inflammatory and antioxidant activity tea intake can induce iron-chelating effect, modulate cell survival, and cell signaling pathways. It can also regulate the secretion of stress hormone and the production of catecholamine, reduce oxidative stress associated with an age-related brain disorder (Singh, Mandal, and Khan 2016, Schmidt *et al*. 2014, Feng *et al*. 2015, Almajano, Vila, and Gines 2011). Tea polyphenols have been proposed as a potential neuroprotective agent. Also, caffeine has shown to have protective effects against neurological diseases such as Alzheimer's and Parkinson's disease by stimulant effects on the central nervous system (Prediger 2010, Cappelletti *et al*. 2015).

Tea polyphenols reduce the risk of Alzheimer's disease by reducing the levels of Amyloid β peptide (Alzheimer's disease trigger protein) and its production (Ho, Hiu-Ling Hung, and Chang 2013, Ide *et al*. 2014). In the case of Parkinson disease, tea has also shown to have a neuroprotective effect against it due to the anti-aggregation, anti-chelating, anti-inflammatory, and anti-oxidant properties along with its inhibitory activity on α-synuclein aggregation and modulation of intracellular signaling pathways (Caruana *et al*. 2011, Li, Ji, and Shen 2012). Also, tea intake has shown to be associated with a lower risk of Parkinson's disease and can delay the age of this disease onset (Hu *et al*. 2007, Dutta and Mohanakumar 2015).

Diabetes Mellitus

Tea contains caffeine and polyphenols, compounds that are known for possessing the anti-diabetic effect (Fu *et al.* 2017). Also, it has been observed that tea polyphenols affect glucose metabolism and insulin signalization, decrease serum glucose, total cholesterol, and triglycerides. It is also known to be able to inhibit the activity of amylase and glucosidase, modulates insulin secretion, sensitivity and glucose tolerance, mimics the cellular effect of insulin and attenuate high glucose-induced adverse effects (Fu *et al.* 2017, Khan and Mukhtar 2013, Roghani and Baluchnejadmojarad 2010, Mousavi *et al.* 2013, Suzuki, Miyoshi, and Isemura 2012).

Tea polyphenols (both green and black) have also been shown to have the potential to prevent SGLT1 and GLUT2-mediated glucose uptake by inhibiting carbohydrate hydrolyzing enzymes such as alpha-glycosidase and alpha-amylase (Yang *et al.* 2014, Satoh *et al.* 2015).

In a study carried out in Poland, tea consumption was negatively associated with metabolic syndromes such as central obesity and fasting plasma glucose; in other words, tea intake can be associated with a reduced risk of diabetes (Grosso *et al.* 2015). These results were corroborated by van Dieren *et al.* (2009), who found, in a 10-year follow-up study, that the consumption of at least 3 cups of tea reduced the hazard of type 2 diabetes mellitus in 42%. Interestingly, aside from these examples, several follow-up studies have been carried out in different countries such as United Kingdom, Japan, and Pakistan and in all of them, the same conclusion was reached: there is an undeniable association between tea consumption and reduced risk of type 2 diabetes mellitus (Iso *et al.* 2006, Hamer *et al.* 2008, Siddiqui *et al.* 2015). To support these studies, data were adjusted for age, gender, ethnicity, and social status. Also, a survey carried out in Denmark showed that tea intake might have a protective effect against gestational diabetes (Hinkle *et al.* 2015). In China, it has been reported that tea intake can alleviate the decrease of fasting blood insulin and also may help in reducing waist circumference (Li *et al.* 2016).

Surprisingly, the different breeds of tea have shown different effects and mechanisms of action. Green tea and oolong tea have shown to protect against the development of type 2 diabetes mellitus, but the first was associated with a lower risk of impaired fasting glucose, while the second was associated with a lower risk of impaired glucose tolerance (Huang *et al.* 2013). This divergence in the mechanism is mainly attributed to the variation in the chemical composition due to fermentation since the major bioactive component in green tea is epigallocatechin gallate; while in oolong tea are theaflavins and thearubigins (Liang *et al.* 2008, Liang *et al.* 2003).

Oolong tea, has shown to be an effective hypoglycemic agent since it was able to lower plasma glucose (from 229 to 162 mg/dL) and fructosamine (from 410 to 323 mol/L) concentrations in type 2 diabetes patients (Hosoda *et al.* 2003).

Another study showed that theasinensin A (a polyphenol flavonoid created during tea fermentation) significantly reduced the serum glucose levels, while increased fecal fat excretion and lowered hepatic triacylglycerol (Miyata *et al.* 2013).

Oolong tea has also shown to increase cholesterol excretion in subjects with a high-lipid diet (Hsu *et al.* 2006). Also, oolong tea has proved to be able to increase fat oxidation and energy expenditure; surprisingly, the effect was higher that of green tea, suggesting that oolong tea can be a weight loss aid (Rumpler *et al.* 2001, Komatsu *et al.* 2003). In this field, black tea polyphenols have shown the ability to interfere with lipid and saccharide uptake to reduce

calorie intake; they also can activate AMP-activated protein kinase to attenuate lipogenesis and lipid accumulation which in turn leads to faster lipid metabolism (Pan, Gao, and Tu 2016) . Another study showed that black tea polysaccharides could also regulate fat metabolism in a high-fat diet obese rat model (Wu *et al*. 2016). Also, black teas flavonoids can prevent hyperlipidemia in high cholesterol or high glucose diet-induced rat models (Imran *et al.* 2018).

Due to all the evidence, all the brews of tea are considered as a new strategy and supplementary food for the prevention or treatment of obesity and diabetes.

CONCLUSION

Camellia sinensis is a plant with a long and deep history in human societies. Due to that, it has immense economic importance, to the point that we can even say that, without it, human history would have never been the same. In the present day, besides its economic and cultural value and thanks to the numerous studies that have been carried out, we know that consumption of green, oolong, and black tea help to have a good health since there is enough evidence to claim that tea has many nutraceutical compounds that show anticancer, anti-cardiovascular, antidiabetes, and anti-obesity activity. However, the mechanism of action of many of the compounds still needs to be determined.

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Chapter 6

THE NUTRACEUTICAL POTENTIAL OF CINNAMON

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ABSTRACT

Cinnamon is a common species well appreciated due to its unique flavor and taste, it is consumed in tea preparations from harvested sticks or as specified in pulverized. It is largely accepted worldwide for many centuries, its flavor is mainly attributed to its chemical composition where cinnamaldehyde plays an important role not only as a flavor source, it also has a remarkable antioxidant effect that scavenges Radical Oxygen Species (ROS) responsible of cancer. Other compounds as trans-cinnamic acid and polyphenols as proanthocyanidins confer to cinnamon important biological activities such as antifungal (against *Aspergillus flavus*), antibacterial (against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*), antiosteoporotic, emmenagogue, reductor agent of blood glucose, total cholesterol and triglycerides levels enhancing agent of HDL cholesterol levels, anti-inflammatory agent and other interesting activities. Cinnamon also has been used in the treatment of nausea, diarrhea, diabetes, frigidity, cough, rheumatism, neuralgia, vaginitis. Nevertheless, secondary effects may be observed if it is consumed in larges amounts due to the presence of coumarins, although there are more benefits than secondary effects. Also, emerging technologies are using microencapsulation techniques to preserve cinnamon characteristics because many bioactive compounds are volatile.

Keywords: antioxidant, cinnamaldehyde, antibacterial, antifungal, species, harvested sticks, antidiabetes, polyphenolic, nutraceutical, anti-inflammatory

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INTRODUCTION

Cinnamon is a common species, its unique flavor and taste make it an excellent option in cuisine. Four of the 250 species of the genus *Cinnamomum* are the most used in gastronomy; the most common species are *Cinnamomum cassia*, *C. aromaticum* (also known as Chinese cinnamon), *C. loureiri* (Saigon or Vietnamese cinnamon) *and C. burmannii* (Indonesian cinnamon). Another cinnamon species less known are used as infusions, for example, *Cinnamomun zeylanicum*, *Cinnamomun loureirii* y *Cinnamomun bejolghota* (Adisakwattana et al. 2011).

Cinnamon is available in markets and stores; it is sold in products as harvested sticks or powdered. Teas and infusions are prepared from cinnamon; they are aqueous extracts that possess a wide variety of different bioavailable compounds. Cinnamon powder is used as a food condiment with a lower amount of bioavailable compounds in comparison with sticks. Bioavailability of bioactive compounds is affected by several factors, among them the methodology of preparation of the aqueous extract, manipulation of cinnamon, storage, and weather conditions. Many components present in cinnamon are volatile, and high temperatures may affect them.

Herbal medicines are a rich source of natural products, many of them with several interesting and remarkable bioactive properties. People have explored herbal medicines since ancient times in the treatment and/or prevention of different illnesses. Millenary civilizations around the world have used cinnamon in the treatment of nausea, diarrhea, and diabetes. Various medical applications of cinnamon have been reported as a traditional option in the treatment of some illness in oriental cultures as Chinese, Indian, Persian, and others. Its use has been extended for several conditions as flatulence, amenorrhea, diarrhea, toothache, cold, headache, and leukorrhea. Also, it has been used as a food supplement traditionally recommended for the treatment of frigidity, cough, rheumatism, neuralgia, vaginitis, eye inflammation (Hajimonfarednejad et al. 2019).

Cinnamon is a spice that several ancient cultures have used in different applications. For example, Mexican natives spiced their meals in 7000 BC using cinnamon.

On the other hand, the use of cinnamon was extended to western cultures from India and others as Chinese, Persian, Mesopotamic, and Egyptian. There is evidence found in pyramids showing people used it as gifts for their gods. Papyrus Ebers" compiled the importance of spices, describing how spices were used as burial gifts. During different centuries spices were introduced from China and Egypt to India and Europe, and cinnamon was widely spread (Hajimonfarednejad et al. 2019).

Cinnamon is a term derived from a Greek word that means "sweet wood," this is attributed to the flavor and to the source where it is obtained. In present-day, it is widely consumed in and produced in different countries, for example between 2010 and 2014 Indonesia was the major producer of this spice (83176.79 tons), China (53,176.79 tons), Sri Lanka (13,938.21 tons), Vietnam (13984.43 tons) and Madagascar (1797.36 tons). The leading producers of cinnamon are Asian countries. This could be due to the broad ancient knowledge of folk medicine of these cultures and also due to the endemic feature of cinnamon in Asian countries as Sri Lanka (Ribeiro-Santos et al. 2017).

As already mentioned, cinnamon is considered a medicinal plant since ancient times. Also, cinnamon is widely used in cuisine. Not only "Ebers Papyrus" but also other ancient reports

describe and talk about cinnamon's benefits. Nowadays, there is vast scientific evidence that supports the human health benefits of cinnamon consumption (Zare et al. 2019).

CINNAMON TAXONOMY

Cinnamomun genus belongs to the Lauraceae family. Two hundred fifty species have been identified, but only four of them are used to obtain the spice cinnamon. It has a considerable variability according to the region, and due to this, endemic species from Mexico, Sri Lanka, China, and Indonesia have been described as Ceylon cinnamon or Mexican cinnamon *Cinnamomun zeylanicum*, *C. verum*, Chinese cinnamon (*C. aromaticum* or *C. cassia*), Indonesian cassia (*C. burmannii*). It is important to mention that environmental factors such as altitude and latitude influence the chemical content of the plants. Also, species such as *C. loureiroi* and *C. tamala* from India and Vietnam have been described. Commercial cinnamon is obtained from the Brown inner bark of several evergreen trees and shrubs of this genus and is widely used as condiment and spice (Ribeiro-Santos et al. 2017).

HARVESTING AND PRODUCTION OF CINNAMON

A cinnamon tree lasts 2-3 years growing to be useful for removing the bark, after that time the tree is harvested, branches are separated and processed by scraping off the outer bark, and after that, with a hammer, the branch is beat to lose the inner bark. The inner bark is more useful than the outer due this last is thick and brownish. Cutting of the tree must be done in a maturation stage that ensures the optimal characteristics of the inner bark. Harvesting is typically done in the wet season. The optimal quality of bark is obtained from shoots with uniform brown color, and a thin bark 1.0-1.25 m length and 1.25 cm diameter are gotten. One technique to know the ideal time for cutting the stem is when the red flush of the young leaves turns to green and is the indication of the free flow of sap between the bark and the wood. The shoots ready for peeling are removed from the stems, and the terminal ends of shoots are also removed (Thomas and Kuruvilla 2012).

Still wet, bark needs to be processed to avoid the loose of properties while it is wet. Processing represents 60% of the cost of production of cinnamon. Usually peeling of bark from the stems is a work-intensive done by hand because the quality of cinnamons is in the function of the process of the bark; it depends on how well bark is removed from the stems. Once all the bark is removed, a drying process is done. This process is quite important due to it contributes to the final quality of the product. The drying process lasts 4 -5 days at subdued sunlight, but in humid climates or during the rainy season it is important to use a mechanical dryer to complete the drying process. Also, it is important to take care of humidity because pests may proliferate in the bark and the uses of pesticides lower the quality of barks (Thomas and Kuruvilla 2012).

CHEMICAL COMPOSITION OF CINNAMON

As mentioned above, cinnamon has a unique taste, which, together with its nutraceutical action on humans, is mainly attributed to its chemical composition. It has been observed that cinnamon contains a vast range of organic and volatile compounds; most of them are considered as secondary metabolites. Cinnamaldehyde is the compound responsible for the taste and aroma of cinnamon as it is the main constituent of its essential oil. Its concentration is directly proportional to the degree of maturity of the stem. It has also been observed that its concentration is related to the place of origin of the plant. The younger the bark, the higher the concentration of ester cinnamyl acetate; to release this compound as a commercial preparation a fermentation process is needed (Dewick 2009).

Cinnamaldehyde belongs to a family of secondary metabolites named polyphenols that include "secondary metabolites" (a group of molecules bio-synthetized by plants and are not essential for the survival of it in contrast to primary metabolites, although they serve as defensive compounds against competitor organisms) derived from the shikimate-phenylpropanoids and polyketide pathways. Also, cinnamaldehyde is known as phenylpropene, and it is synthesized from phenylalanine as a precursor. The resulting molecules have one or more phenolic rings and a lack of any nitrogen-based functional group. The resulting phenolic rings exhibit interesting properties as antioxidants; red fruits, some vegetables, grains, tea, and wine possess polyphenols with some differences in their chemical structure but with the same skeleton of the phenolic ring. These differences give them particular antioxidant activity. In this way, for example, flavan-3-ols, gallic acids, flavonoids, flavanols differ between them in the position and number of hydroxy groups in the phenolic ring and these products are present in different amounts according to the source and as consequence the different human diet including some of these products they have a different effect on the anti-inflammatory and antioxidant activity (Gunawardena, Govindaraghavan, and Münch 2014).

A wide group of phenolic molecules, including cinnamaldehyde are present in cinnamon. Cinnamon is mainly consumed in tea; in this aqueous extraction, many of these phenolic molecules are bioavailable to humans as a function of the preparation technique and cinnamon species used. The analytical method of High-Performance Liquid Chromatography (HPLC) has allowed the detection of different compounds. In Table 1 there are summarized the compounds detected by aqueous extraction for species *C. cassia*, *C. zeylanicum*, *C. burmannii*. This last species shows four times more compounds detected in comparison with *C. cassia* (Ribeiro-Santos et al. 2017, Santos and da Silva 2018).

Table 1. Major Compounds found in three Cinnamon Species detected by HPLC

Species	Major Compounds Identified
C. cassia	Protocatechuic acid, (-) epicatequin cinnamic acid, cinnamaldehyde and eugenol
C. zeylanicum	Cinamyl alcohol and cinnamaldehyde
C. burmannii	Procyanidin Type-A oligomers; chlorogenic acid, ferulic acid, t-cinnamic acid, guiacol, cinnamic acid methyl ester, homovainillic acid, cinnamide, isovainillic acid cinnam 2-mehtoxy cinnamaldehyde, cinnamyl alcohol, 3-methoxytyrosine, clove oil, 4-oxo-4h-1-benzopyan- carboxylic acid, p-coumaric acid, resveratrol, o-coumaric acid, vainillic acid, curcumin, vainillin azine and eugenol.

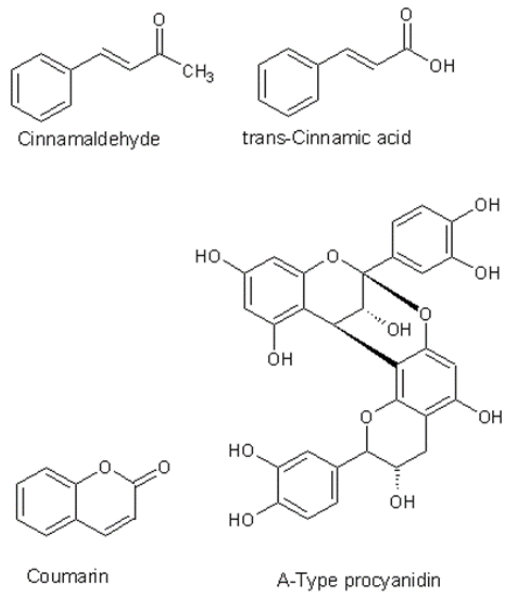

Figure 1. Major constituents of cinnamon water extract.

From Table 1, *C. burmannii* showed a high number of phenolic compounds in comparison to the other species. Phenolic compounds are reported having high activity against oxidative stress by inhibiting lipid peroxidation level is not healthy patients. One of the most important phenolic compounds present in cinnamon is gallic acid, which is associated with antioxidant activity. A study showed that the addition of 3 g of cinnamon to mouse diet increased 3.5 times their phenolic content especially when the mouse consumed *C. burmannii* (Silva et al. 2019).

Cinnamon water extract (Jiao et al. 2013) contain proanthocyanidins, t-cinnamic acid, coumarin, and cinnamaldehyde, respectively, as the major constituents (Figure 1).

Trans-cinnamic acid is derived from the oxidation of t-cinnamaldehyde, an unsaturated aldehyde unstable to air and easily oxidized to cinnamic acid responsible for antibacterial activity.

Other bioactive molecules of larger sizes are Proanthocyanidins (PA), this group of molecules is condensed tannin oligomers of flavan 3-ols polymers. These compounds exhibit important antioxidant, antimicrobial, anti-obesity, anti-cancer, anti-inflammatory activities, and neuroprotective effects. Inhibition of Aβ fibrillization explains their ability as neuroprotective components. They also have been described as promising agents for favorable treatment of Alzheimer's disease as a consequence of their productive activity as scavengers of active oxygen species improving a cognitive performance via increasing blood flow to the brain. Some structural and functional modifications and their corresponding inflammatory responses and oxidative stresses (Li et al. 2011, Momtaz et al. 2018).

Phenolic groups present in large molecules as PAs confer them biological activity due to its ability to bind to protein by their hydroxy groups as the O-dihydroxy and O-trihydroxy positions in the phenyl ring. PAS are biological active molecules called tannins, a group of secondary metabolites divided into two groups, hydrolyzable and condensed tannins. Hydrolyzable tannins are small phenolic compounds esterified to a carbohydrate while

condensed tannins as PAs, occur as polymers with 50 or more repeating units and a mean molecular weight of 5,000 Da (Zuiter 2014).

A remarkable biological action of PAs is the key role that they exhibit against Alzheimer's disease. This may be attributed to the active binding proteins and deactivation of oxygen radicals species. Those important properties point them as efficient cinnamon water extract components; due this, there is a large amount of research focused in cinnamon since it has been observed that these species have the highest PA's content in comparison with of particular interest due it has the highest PAs contents in comparison with spices and berries (Zuiter 2014).

Since cinnamon is a rich source of organic bioactive compounds, the vast majority of them are volatile organic compounds, when aqueous extracts are prepared for consumption, as a consequence of heating, volatile organic compounds vaporize and therefore are lost. These volatile organic compounds are mainly present in the essential oil of cinnamon, although different cinnamon species have different proportions and organic compounds. For instance, *C. osmophloeum* revealed that essential oil of this species possess similar chemical constituents are to those of commercial *C. cassia* (commonly used in food and beverages), such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, b-caryophyllene, E-nerolidol, and cinnamyl acetate. When some of these compounds were tested, they showed excellent anti-inflammatory activity by suppression of NO synthase and cytotoxic activity against HepG2 hepatocellular carcinoma cells (Tung et al. 2008).

The great variety of different organic compounds in cinnamon confers its special features in the treatment and prevention of various illnesses. In this sense, some authors have described the cinnamon bark (Thomas and Kuruvilla 2012) components as rich in volatile oils, 0.4-2.8% tannins formed by polymeric 5,7,3,'4'-tetrahydroxy flavan 3-4-diol units, catechins, and proanthocyanidins, resins mucilage gum, sugars, calcium oxalate. Also, there are present two insecticidal compounds (cinnezalin and cinnezylanol), coumarins, and others. The bark oil consists of cinnamaldehyde (80-90%), eugenol, eugenol acetate, cinnamyl acetate, cinnamyl alcohol, methyl eugenol, benzaldehyde, cinnamaldehyde, benzyl benzoate, linalool, monoterpene, hydrocarbon, caryophyllene, safrol, and others such as pinene, phyllandrene, cymene, and cineol. The bark oil is a pale yellow to a dark yellow liquid with a strong, warm, sweet, and spicy taste. When a root bark oil is obtained by steam distillation a colorless to pale yellowish-brown oil is produced, the leaf oil has a significant presence of eugenol (65-92%), and more than 47 compounds have been identified. Cinnamaldehyde and *trans*-cinnamic acid are not present because these compounds appear in more advanced maturation and as a consequence, the flavor and aroma of this oil are not sweet and spicy (Thomas and Kuruvilla 2012).

In a proximal study, cinnamon was described with an ash content (2.4%), crude protein (3.5%), crude fat (4.0%), crude fiber (33.0%), moisture (5.1%) and nitrogen (52%). Cinnamon is mainly composed of carbohydrates, low in fat and protein contents. Also cinnamon is rich in essential minerals as potassium and calcium, it was described with iron (7.0 mg/g), zinc (2.6 mg/g), calcium (83.3 mg/g), chromium (0.4 mg/g), manganese (20.1 mg/g), magnesium (85.5 mg/g), sodium (0 mg/g), potassium (134.7 mg/g) and phosphorous (42.4 mg/g). Data are summarized in Tables 2 and 3 (Gul and Safdar 2009).

Table 2. Proximal composition of cinnamon

Nutrient	% Composition
Moisture	5.1
Ash	2.4
Crude Protein	3.5
Crude Fat	4.0
Crude Fiber	33
Nitrogen Free Extract	52
Energy	258 Kcal /100 g

Table 3. Mineral composition of the cinnamon

Minerals	Amount in mg/g
Iron	7.0
Zinc	2.6
Calcium	83.8
Chromium	0.4
Manganese	20.1
Magnesium	85.5
Potassium	134.7
Sodium	0.0

Cinnamon does not possess toxic minerals such as arsenic, nickel, and lead, but it may absorb them from a polluted environment. For example, lead has been reported in cinnamon content of 1.49 mg/kg, which means 0.49 mg/kg above the maximum permissible levels established by the Polish National Ministry of Health (Krejpcio, Krol, and Sionkowski 2007). On the other hand, cinnamon also has been reported with low contents of arsenic, cadmium, nickel, and lead, 0.45, 2.43, 1.00 and 8.00 mg/L respectively; the reported contents do not represent any risk to human health (Baig et al. 2019).

CINNAMON BIOACTIVE COMPOUNDS

As previously mentioned, cinnamon is a common spice known mainly by its unique and interesting benefits to human health as a consequence of its large amount of different bioactive compounds. These compounds show significant therapeutic efficiency in cancer, oxidative stress, cardiovascular disease, wound healing, inflammatory syndromes, cholesterol levels, and immunomodulatory disorders. Also, cinnamon has potent compounds with important biological activities as a neurostimulator, antibacterial, antifungal, and others (Momtaz et al. 2018).

Phenolics Compounds in Cinnamon

Polyphenolic and phenolic compounds are the principal responsible molecules of the high biological activity described for cinnamon (Bonilla and do Amaral Soral 2017, Durak, Gawlik-Dziki, and Pecio 2014).

Polyphenols are present in cinnamon and are available for humans in aqueous extractions; they tend to display antioxidant activity and can inhibit the production of reactive oxygen species, many of them associated with cancer illness (Sarmiento-Salinas et al. 2019). On the other hand, the toxic components of cinnamon are lipid-soluble. They include terpenes, aldehydes, eugenol, and fortunately, they will not be bioavailable in a tea infusion. The antioxidant effects of polyphenolic compounds of cinnamon present synergistic benefits, enhancing insulin production and activity and, as a consequence, have significant advantages for diabetes treatment as described before (Anderson et al. 2004).

A comparison between cinnamon and other plants, well known to be the rich sources of polyphenol compounds, has done by evaluating the antioxidant activity of cinnamon ethanolic extracts against ethanolic extracts of plants as guarana, rosemary and boldo leaves. Guarana is a native Brazilian plant, is the most abundant vegetable source of caffeine and catechins with antioxidant and antimicrobial properties to the food and pharmaceutical industries. On the other hand, boldo leaf is an endemic Chilean plant, the phenolic constituents are mainly proanthocyanidins, and flavanol glycosides, alkaloids, and essential oils are the main bioactive compounds of the boldo leaf extracts. It was demonstrated that cinnamon and guarana presented high content of phenolic acids (Bonilla and do Amaral Soral 2017).

Interesting therapeutic applications of cinnamon against chronical diseases diminishing free radicals that may be involved in cancer are related not only to cinnamaldehyde but also to the presence of other phenolic compounds that confers to cinnamon these therapeutics applications. Phenolic compounds detected in cinnamon are protocatechuic acid, urolignoside, quercetin, rutin, kaempferol, isorhammetin, cinnamaldehyde, 2-hidroxycinnamaldehyde, and eugenol. Sri Lankan cinnamon and Chinese cinnamon *(C. zeylanicum and C. cassia* respectively) barks showed tannins levels between 0.65-2.18%; these levels are above other plants sources. Secondary metabolites catechin and isorhamnetin showed no significant differences between barks of the species *C. zeylanicum* and *C. cassia*. While rutin, quercetin, and kaempferol were significantly higher in Sri Lanka cinnamon than that in Chinese cinnamon (Gunawardena, Govindaraghavan, and Münch 2014). Also high levels of phenolics have been reported in water extracts of cinnamon fruits, i.e., 3,4-dihydroxybenzoic acid (protocatechuic acid), epicatechin (2β→O-7, 4β→8)-epicatechin-(4β→8)-epicatechin(cinnamtanninB-1),4-[2,3-dihydro-3-(hydroxymethyl)-5-(3- hydroxypropyl)-7-(methoxy)benzofuranyl]-2-methoxy-phenyl-β-D-glucopyranoside (urolignoside), quercetin-3-*O*-α -L-rhamnopyranoside.

Three types of oil are obtained from *Cinnamomum verum* from the leaf, stem, bark, and root bark. Eugenol is one of the major components of leaf oil. On the other hand, cinnamaldehyde is the main component of the oil obtained from the stem bark. While *Cinnamomun cassia* produces only one type of oil, bark oil, obtained by distillation of the leaves and bark, almost 95% of the oil obtained is cinnamaldehyde (Gunawardena, Govindaraghavan, and Münch 2014).

Several techniques are available today for extracting and studying phenolic compounds in cinnamon. Recently it has been reported an ultrasound-assisted-cinnamon extraction process using fuzzy and response surface models. A maximum concentration of phenolics was achieved with this methodology corresponding to a total phenolic content (TPC) of 149.3 mg GAE/g dry weight; two components were mainly identified, *trans*-cinnamic acid (41mg/g) and *p*-coumaric acid (2mg/g) (Cebi et al. 2019).

Coumarins from Cinnamon

Coumarin (benzo-α-pyrene) is a common naturally occurring substance present in a wide variety of plants, such as citrus fruits, green tea, but especially in cinnamon (Blahová and Svobodová 2012). They are secondary metabolites of higher plants, few microorganisms (bacteria and fungi) and sponges. The function of this type of end product of secondary metabolism is related to defense mechanisms against consumers (herbivores and microorganisms). These compounds are biosynthesized from phenylalanine via the shikimic acid. Natural coumarins are generally unsaturated lactones and comprise another class of compounds C_6C_3. Almost all the natural coumarins have an oxygenated substituent at position 7, either free as in hydroxylated umbelliferone, or combined in other derivatives. Structurally they are considered derivatives of the ortho-hydroxy-cinnamic acid (Matos et al. 2015).

Cinnamon contains essential amounts of coumarins; the structural similarities between ortho-hydroxy-cinnamic acid and cinnamaldehyde could explain the high presence in cinnamon. Nevertheless, coumarins may hurt human health. *C. cassia* contains up to 1% coumarin and *C. zeylanicum* contains only a trace of about 0.004% (Blahová and Svobodová 2012). Coumarins are known for their adverse effects; they cause liver and kidney damage in rats, mice, and probably humans (Lungarini, Aureli, and Coni 2008).

Coumarin as a food additive has been banned since the 1950s by the US Food and Drug Agency due to the hepatotoxicity observed in laboratory animals that consumed it daily, in this sense, it is important to preserve human health to avoid consumption of cinnamon with high levels of coumarin (Khan et al. 2003, Senthil Kumar et al. 2019). The European Commission specified a maximum level of 2 mg/kg of coumarin in food and beverages, except for special caramels, alcoholic drinks, and chewing gum (10, 10, and 50 mg/kg respectively) (Blahová and Svobodová 2012).

Polyphenoloxidase is an enzyme that catalyzes enzymatic browning in fruits and vegetables, coumarins can delay this enzymatic browning, in spite of they can be used as additives in food, their toxicity may represent a potential hazard for humans although it is possible to use an aqueous cinnamon extract as a natural source of coumarins instead of synthetic molecules and preserve fruits and vegetables in a natural form (Thada et al. 2013).

Compounds in Leaves and Fruits of Cinnamon

Leaves of cinnamon are quite different than bark when they are crushed they emit a spicy odor and a hot taste; cinnamon leaf oil has a warmer spicy and rather harsh odor, it lacks the body of the bark oil. In spite of having a fragrant odor, it has a very pungent taste. More than 53 components have been identified from cinnamon leaf oil. On the other hand, thirty-four compounds have been identified in cinnamon fruit oil those compounds are (*E*)-cinnamyl acetate (42-54%) and (*E*)-caryophyllene (9-14%) the major components. It was found that 1,8-cineole (62.4%) and trans-cinnamaldehyde (41.3%) are the major compounds in *C.camphora* and *C. zeylanicum*, respectively. Through chiral gas chromatography the enantiomeric distributions of linalool and terpinen-4-ol were determined to be (3*R*)-(-)-linalool (95%), (3S)-(+)-linalool (5%) and (4*R*)-(-)-terpinen-4-ol (69%), (4*S*)-(+)-terpinen-4-ol (31%), respectively. Twenty-six compounds constitute 97% of the volatile oil from cinnamon flowers and it was

found to contain (*E*)-cinnamyl acetate (42%), (*E*)-R-bergamotene (8%), and caryophyllene oxide (7%) as the principal compounds

This great variety of different compounds in distinct proportions in leaf, root, and barks and according to not only to the species but also to the maturating stage of cinnamon the resulting odor and flavor could change. Due to its sweet flavor, the bark is more preferred than leaves or fruits, also by its chemical composition richest in phenolic compounds with remarkable antioxidant effects.

CINNAMON AS A PROMISE IN THE TREATMENT OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative and irreversible brain disorder; it is a progressive age-related and is one of the most prevalent types of dementia. One possible origin and development of Alzheimer's disease comes from the accumulation of soluble oligomeric assemblies of β-amyloid polypeptides [amyloid beta (Aβ)] (Frydman-Marom et al. 2011). In AD patients, cinnamon has reduced the neurotoxicity of Aβ generation and accumulation, cinnamon polyphenolics may improve dementia through their hypotensive and vasorelaxant potentials and by attenuating vascular cell adhesion molecules expression within the endothelial cells. Cinnamon displays remarkable neuroprotective effects in AD patients (Momtaz et al. 2018).

CINNAMON BIOLOGICAL COMPOUNDS THAT HELP IN THE TREATMENT OF DIABETES

Obesity is considered a chronical cardiovascular disease that affects the population of all ages around the world. Some studies have revealed that consumption of cinnamon as a food supplement improves glycemic, lipids levels (cholesterol total LDL-c and HDL-c) (Zare et al. 2019) and assist in decreasing hepatic glucose production. Experiments with induced obese hyperglycemic mice have shown an acutely lowering fasting blood glucose levels. When the mice consumed a phytochemically-enhanced functional food ingredient enriched with water-soluble polyphenols from aqueous extract of cinnamon, there was observed inhibition of hepatic glucose. The possible mechanism associated could be related to a decreasing of the gene expression of phosphoenolpyruvate carboxylase and glucose 6-phosphatase, two major regulators of hepatic gluconeogenesis. Due to this those results suggest that an alternative treatment of T2DM could be food enriched with cinnamon extracts (Cheng et al. 2012).

Cinnamaldehyde is the major constituent of cinnamon bark essential oil (92%), recently it has been described cinnamon as a potential and promising agent in the treatment of patients T2DM with obesity and diabetes, those results were linked to cinnamaldehyde in cinnamon. Reduction of anthropometric indices, body mass index, and visceral fat decreasing levels were observed in patients with T2DM, besides the improvement of glycemic and lipids outcomes and reduction of insulin resistance. Those effects were observed when diabetic patients were treated with cinnamon bark powder in 500 mg capsules twice daily for three months (Zare et al. 2019).

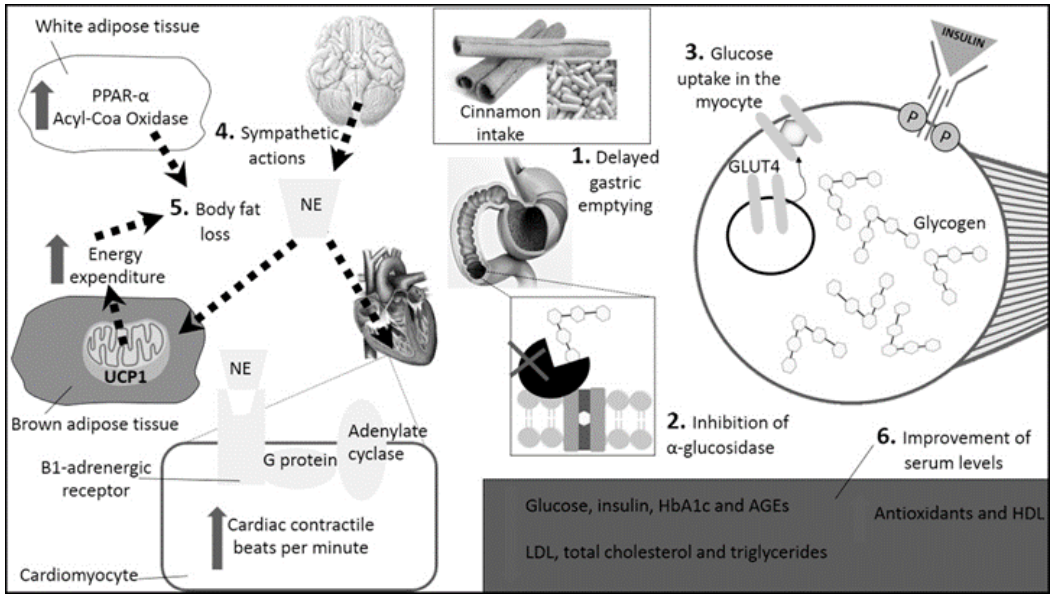

Figure 2. Cinnamon intake and proposed pathways that improve serum parameters and decrease fat. AGEs, advanced glycation end products; GLUT4, glucose transporter type 4; NE: noradrenaline; PPAR-α peroxisome proliferator-activated receptor alpha; UCP1: Mitochondrial uncoupling protein 1. Image recompiled from (Santos and da Silva 2018).

Cinnamon hypoglycemic effects may be attributed to the activation of the insulin receptor kinase and inhibition of the insulin receptor, also inhibition of glycogen synthase kinase 3 is associated to cinnamon extracts inducing an increase of glucose uptake. In spite of some author's point that cinnamon extracts do not reduce serum levels of glucose and other glycemic parameters and anthropometric indices, a large evidence supports the benefits of cinnamon at lowering glucose levels (Namazi et al. 2019).

A recent compilation of data about cinnamon and diabetes (2013-2018) revealed that the treatment of individuals with T2DM with cinnamon decrease fasting blood glucose from 12.9 to 52.2 mg/dL and HbA1c from 0.27 to 0.83%, whereas a decrease in serum insulin in few studies. Research papers ranged from 6 to 17 weeks in duration. These results allow us to conclude that about 1-6 g of cinnamon species, mainly in powder, could be efficient adjunct drug treatment for T2DM nevertheless, more controlled clinical trials are needed. Six biochemical pathways have been proposed as possible mechanisms of action and repercussion of biochemical parameters after administration of cinnamon extracts (Figure 2) (Santos and da Silva 2018).

A mechanism of improvement of glycemic lipids has been proposed. Cinnamon contains dietary fiber from 50 to 75%. At first, when cinnamon is consumed, dietary fiber produces a decrease in gastric emptying. In a second step, eugenol from cinnamon acts as α-glucosidase inhibitor; nevertheless, when larger doses of eugenol are ingested, adrenergic side effects may be present as a consequence of liver damage. In a third step, in the myocyte, there is an improvement of the translocation of GLUT-4 to glucose uptake and consequently an increase of glycogen. At step 4, sympathetic actions are promoted by the cinnamaldehyde; the heart rate is increased, and also a thermogenic effect on the brown adipose tissue is produced. At step 5, body fat loss occurs from the UCP-1 activation in mitochondria, this raises PPAR-α expression

in the white adipose tissue and also β-oxidation using enzymatic action of acyl-CoA-oxidase is produced. And finally at step 6, an expected improvement of glycemic lipid, serum levels, and antioxidant parameters are reached (Santos and da Silva 2018).

From different cinnamon species studied, *Cinnamomum burmannii* has shown an improvement in the glycemic response of healthy and type 2 diabetic patients. The tea of this species can significantly lower postprandial maximum glucose concentration (glucose levels between meals) on non-diabetic adults, this could be a useful therapeutic application for individuals with prediabetes. Watson, Singh, and Takahashi (2018) described the decreasing of fasting blood glucose due to an administration for individuals with diabetes with cinnamon tea three times per day over four months showed, this effect was mainly attributed to cinnamaldehyde.

Different concentrations of cinnamaldehyde are bioavailable according to cinnamon species, maturation age, preparation of teas, etc. Indeed more studies are needed in this regard to gain information about the best cinnamon species for the treatment of diabetes and also to avoid secondary effects.

ANTIBACTERIAL ACTIVITY OF CINNAMON

Is well known that bacterial infections are a severe problem that is increasing, the unrestricted use of drugs against pathogens produces a common phenom called multidrug resistance, in this sense, bacteria, virus, and fungi get more resistant to drugs because they develop different mechanisms to avoid the effect of the drug. It has been demonstrated that cinnamaldehyde is not able to produce bacterial lysis against *Listeria innocua*, although *Cinnamomum cassia* essential oil posses different compounds besides cinnamaldehyde that can produce the bacterial lysis (Trinh et al. 2015).

Cinnamon essential oils have shown effective activity against Gram-negative bacteria as *Pseudomona aureginosa* mainly attributed to cinnamaldehyde and eugenol, and current research is looking for powerful synergistic effect among cinnamon bark oil and current antibiotics (Utchariyakiat et al. 2016).

In vitro studies of cinnamon oil and extract have shown that they were active against 27 strains of *Vibrio cholerae* and also against *Shigella* and Gram-positive bacteria such as *Bacillus cereus, Micrococcus luteus,* and *Staphylococcus aureus* and Gram-negative bacteria such as *Alcaligenes faecalis, Enterobacter cloacae, Escherichia coli,* and *Pseudomona aureginosa*. Also, there was observed activity against fungi like *Aspergillus niger* and *Rhizopus oliosporus* and the yeast *Candida albicans.*

Cinnamomum burmannii (cinnamon stick) from Indonesia showed significant antibacterial activity against five common pathogenic bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella anatum*). When this species was analyzed by Gas Chromatography coupled with Masses (GC-MS) and liquid chromatography (LC-MS) a predominant volatile component (E)-cinnamaldehyde and proanthocyanidins were detected and are quite probable that the antibiotic activity could be done by cinnamaldehyde and proanthocyanidins (Shan et al. 2007).

Several mechanisms have been proposed to explain the interaction of cinnamon components and inhibition of bacterial activity; for example damaging the bacterial cell

membrane, altering the lipid profile, inhibiting ATP-ases, cell division, membrane porins, motility and biofilm formation via anti-quorum sensing effects (Vasconcelos, Croda, and Simionatto 2018). Also, it has been demonstrated that cinnamaldehyde destroys the cell membrane of *Pseudomona gingivalis* increasing the cell membrane permeability. In addition to that, it has been described the synergic effect when cinnamaldehyde is mixed with other plant extracts like eugenol, thymol, and carvacrol, increasing its potency against bacterial this constitutes a valuable tool in therapeutic medicine (Wang et al. 2018).

It has been observed that a combination of cinnamon and clove and thyme oil can be used against *B. subtilis*, *B. cereus, S. aureus*, while against *E. coli, S. typhimurium* the results have not been as good. Nevertheless, more studies are needed to explain the interaction mechanism between cinnamaldehyde and the compounds present in clove and thyme oil (thymol, carvacrol, p-cymene, and eugenol) (Fei et al. 2011).

Cinnamon essential oils are rich in phenolic compounds, they are able to cross the phospholipid membrane of bacterial cells and then to bind to proteins where they may alter normal functions of cell producing an alteration in the membrane permeability and the loss of functional proteins transporting molecules that causes cytoplasmic coagulation, denaturation of enzymes and loss of metabolites and ions altering the cytoplasmic membrane weakening it and finally the lysis is produced with the bacterial cell dead. Of course, these effects are not observed in all bacteria; different species react in distinct manners, and resistance may be possible. There is also described a synergistic effect between cinnamon essential oil from *C. zeylanicum* and different antibiotics, that synergy is based on the principle that the formulation may enhance efficacy decreasing toxicity, adverse side effects and the dose required while there will be an increasing at the bioavailability. Some antibiotics that have shown effective synergistic effects are amikacin, gentamicin, and piperacillin (Vasconcelos, Croda, and Simionatto 2018).

In spite of cinnamon has demonstrated a certain antibiotic effect, its medicinal use doesn't supply medicines, although cinnamon may aid to prevent some bacterial diseases. In this sense, it is important to consider cinnamon as a food supplement instead of medicine.

ANTI-INFLAMMATORY ACTIVITY OF CINNAMON

Arthritis is a chronic inflammation that includes over 100 forms of arthritis, e.g., rheumatoid arthritis, osteoarthritis between the most common. Cartilage damage conduces to several degenerations with painful situations. Arthritis may be attributed to several factors like genetic disposition, obesity, heavy physical work, trauma, sex, and age, a loss of cartilage is produced with also loss of synovial liquid (Miraghajani and Ghiasvand 2019).

An inflammatory process is a normal defense mechanism of the organism against tissue injury and microbial invasion, it is caused by physical damage, UV irradiation, microbial invasion, and obesity reactions. At the site of injury or alteration, an increase in blood vessel wall permeability followed by migration of immune cells can lead to edema formation during inflammation (Lu et al. 2011, Tung et al. 2008).

It is necessary to understand the biological mechanism of the inflammation process to understand the action pathway of the bioactive components of cinnamon. Different enzymes and signaling proteins are active during the inflammation process, for example, nitric oxide

synthase (iNOS), a member of the NOS protein catalyzes the formation of nitric oxide (NO) from L-arginine. According to the concentration of NO released by iNOS, it is likely to contribute to the antimicrobial activity of the macrophages against certain bacterial pathogens. An endotoxin and constituent of the cell membrane of gram-negative bacteria is lipopolysaccharide (LPS) that activates macrophages by binding to toll-like receptor 4 and stimulates the production of inflammatory cytokines, like NO, TNF-α, and Interleukin-1β, Interleukin-6-geenan (Carr). Acute inflammation is induced by Carr with infiltration of phagocytes, the production of free radicals as well as the release of inflammatory mediators. A way to avoid the inflammatory effect is to involve antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in tissues.

Cinnamomun osmophloeum has been reported due to its effective anti-inflammatory potential when administered as essential oil; its mechanism of action involves inhibition of IL-1Band IL-6 protein production (Chao et al. 2005).

Phenolic compounds from cinnamon act as antioxidants and may stop a series of the inflammatory process, by scavenging free radicals. In this way, today several natural products are investigated to gain information about their phenolic contents and their ability to reduce or suppress inflammatory activity by their antioxidant or free radical scavenging activity that also may. Due inflammation process is a natural biological response to an injury or damage in tissues, but when it is uncontrolled, the resulting inflammation is associated with a number of chronic diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and Alzheimer's disease, and also has a role in various human cancers (Chao et al. 2005, Gunawardena, Govindaraghavan, and Münch 2014).

It has been demonstrated anti-inflammatory activities of *Cinnamomun cassia* constituents (cinnamic aldehyde, cinnamic alcohol, cinnamic acid, and coumarin) in vitro and in vivo against iNOS, COX-2 and NK-B protein levels suggesting an adequate anti-inflammatory power of cinnamaldehyde while cinnamic alcohol and coumarin presented a minimum anti-inflammatory effect. Cinnamaldehyde is a major component of cinnamon, chemically the structure is simple and is constituted of an unsaturated carbonyl moiety exerted a suppressive influence on toll-like receptor-4- (TLR4) mediated signaling. The mechanism is associated with the increase in the activities of antioxidant enzymes (CAT, SOD, and GPx). Cinnamaldehyde may be used as a pharmacological agent in the prevention or treatment of diseases where the free radical formation is involved (Gunawardena, Govindaraghavan, and Münch 2014).

Cinnamon also may be a possible alternative in the treatment of arthritis rheumatic and osteoarthritis. Nevertheless, self-medication, especially in the treatment of chronical diseases, is not recommended. Medical guidance and observance should be taken, and more research is needed to confirm the optimum dosage of cinnamon, and also different cinnamaldehyde contents and the presence of some components may cause adverse side effects (Miraghajani and Ghiasvand 2019).

It is also well known that arachidonic acid metabolites play an important role in inflammation. Since the lipoxygenase pathway of the arachidonic acid and metabolism produces ROS catalyzed by lipoxygenase enzyme, the reaction undergoes to the oxygenation of polyunsaturated fatty acids containing a cis-cis-1,4-pentadiene system to hydroperoxides (Durak, Gawlik-Dziki, and Pecio 2014). Cinnamon contains several antioxidants that may be useful scavenging ROS species and deactivating the production of arachidonic acid metabolites and reducing inflammatory effects as a consequence.

CINNAMON AND DYSMENORRHEA

Dysmenorrhea is defined as cramping and intermittent spasmodic pain in the lower abdomen occurring just before or during menstruation; it is one of the most common gynecological disorders among women of reproductive age. Although the high prevalence of dysmenorrhea, it is not well treated by health professionals because many women consider painful periods as normal conditions (Alghamdi, Al-Zahrani, and Alabdulaziz 2019, Chikhalekar and Bansode 2019).

Since 1947 it is estimated that approximately 140 million hours are lost annually due to dysmenorrhea; this loss of time represents an entire year of work by approximately 58,000 women (Torpin, Woodbury, and Child 1947).

Dysmenorrhea treatment is multifaceted, some other practices different to medication have been used, and among most popular methods to treat dysmenorrhea, there is the use of medicinal herbs as fennel, ginger, cinnamon, and rose, all of them are available in the kitchen of all people. Many women use herbs to calm dysmenorrhea symptoms as for example the main herbs used are thyme (*Thymus vulgaris*), chamomille tea (*Matricaria chamomilla*), St. John's wort (*Hypericum perforatum*), fennel (*Foeniculum vulgare*), cinnamon (*Cinnamomum*), dill (*Anethum graveolens*), saffron (Colchicum), celery (*Apium graveolens*), anise (*Pimpinella anisum*), balm (*Balsamum*), valerian (*Valeriana officinalis*), mint extract (*Menthe longifolia*) and organic honey were effective in alleviating primary dysmenorrhea (Aksu and Özsoy 2016).

Excessive secretion of uterine prostaglandins (PGs) is today an accepted cause for the Primary Dysmenorrhea. Prostaglandins are potent regulatory substances synthesized by every tissue in the body; excessive production of them is the cause of several symptoms as inflammatory, ulcerative, autoimmune, and degenerative bone diseases. When polyunsaturated fatty acids, such as arachidonic, react with oxygen endoperoxides (cyclooxygenase), endoperoxides by enzymatic cleavage reactions are converted into prostaglandins. High levels of prostaglandin F_2 (PGF_2) are present in the menstrual fluid of women suffering painful periods. PGF_2 stimulates myometrial contractions, ischemia, and enhanced sensitization of nerve endings. A sex-hormone disorder has also been associated with primary dysmenorrhea accompanied by a decrease of progesterone before menstruation and by a series of different responses of PGs and leukotrienes, which subsequently causes hyperalgesia, inflammatory pain, vasoconstriction, ischemia, and myometrial contraction. Also the level of vasopressin and oxytocin may play similar roles. Primary dysmenorrhea symptoms are better associated with multiple factors in the neuro-endocrine-immune (N-E-I) network (Su et al. 2013).

Since 1979 two drugs were approved for menstrual pain, Ibuprofen, 2-(4-isobutylphenyl) propionic acid, and Mefenamic acid, *N*-(2,3-xylyl)-anthranilic acid, they relieve pain by inhibiting biosynthesis and/or action of prostaglandins without side effects of other drugs Ibuprofen has also been used in treatment of rheumatoid arthritis and osteoarthritis, is a nonsteroidal anti-inflammatory agent. The absence of Ibuprofen side effects was reported in 1979 when these drugs were released, although synthetic drugs, especially in long-term administration have side effects as nausea, stomach irritation, ulcers, renal papillary necrosis and decreased renal blood flow (Jaafarpour et al. 2015).

It is necessary to study natural medicinal herbs that may diminish the effects of dysmenorrhea. Cinnamon, specially *Cinnamomun cassia* has been used since ancient times as an effective emmenagogue, an herb that stimulates blood flow in the pelvic area and uterus,

and also stimulates menstruation in India, Mexico, and Europe. Camphor is a uterine stimulant, while eugenol and myristicin are potentially active in reducing dysmenorrhea by inhibiting the biosynthesis of prostaglandins. Also cinnamon is included as an abortifacient ingredient in Chinese literature by inhibition of Monoamine Oxidase (De Montellano and Browner 1985). The main components of cinnamon essential oil are cinnamaldehyde (55-57%) and eugenol (5-18%). Cinnamaldehyde has an antispasmodic effect while eugenol can prevent the biosynthesis of prostaglandins and reduce inflammation. Cinnamon not also posses cinnamaldehyde and eugenol, it is a rich source of vitamins such as vitamin A, thiamin, riboflavin and ascorbic acid, adverse side effects as nausea, vomiting and spasmodic contractions due the dysmenorrhea have been reported to decrease with a total dose of 2.52 g of dried bark cinnamon (Aksu and Özsoy 2016).

In spite of cinnamon efficacy in decreasing pain due to dysmenorrhea, when compared to ibuprofen, its effect is weaker, although it could be a reliable and effective treatment for primary dysmenorrhea since it does not produce side-effects such as excessive bleeding, nausea or vomiting (Aksu and Özsoy 2016).

LIPID-LOWERING EFFECT OF CINNAMON

Cinnamon, has been used in traditional medicine to relieve gastrointestinal distress, arthritis, high blood pressure, dermatitis, toothache, colds and for improving menstrual irregularities and for wound healing. There are four species of cinnamon widely used, *C. cassia*, *C. burmannii*, *C. zeylanicum* and *C. loureiroi* for human consumption and for their use as supplements in treating high blood glucose and lipid levels and other symptoms of the metabolic syndrome (Singletary 2019). It has been demonstrated that cinnamon possesses the ability to reduce blood glucose, total cholesterol, and triglycerides levels and to raise HDL cholesterol levels. The first clinical report of the use of cinnamon to lower triglycerides and cholesterol levels was in Pakistan. It showed that cinnamon powder *Cinnamomum cassia* took over 40 day period with doses ranging from 1-6 g daily reduced mean fasting serum glucose (18-29%), triglyceride (23-30%), LDL cholesterol (7-27%) and total cholesterol (12-26%) levels (Anderson et al. 2004).

It is known that consumption of aqueous extracts of cinnamon, helps lowering fat contents in an organism. The anti-obesity effects have been related to weight loss by increasing energy expenditure. Song et al. (2017) examined the effect of *Cinnamomun cassia* Cortex extract on obese mice showing that cinnamon extract at 100 and 300 mg/Kg prevented weight gain and an increase of blood glucose levels. Also, administration of the cinnamon extract to high-fat-diet-induced obese mice remarkably suppressed hepatic steatosis in liver and tissues and decreased adipocyte size in adipose tissues and total serum cholesterol levels. And also it was observed that cinnamon extracts elicit its anti-obesity effect by suppressing lipid accumulation in the liver and increasing skeletal muscle mass. CC extract enhances the energy of metabolism via the regulation of the energy-sensing network, increasing the mRNA expressions of mitochondrial biogenesis -related factors, such as PGC1α, NRF-1, Tfam, AMPK and ACC signaling cascade in skeletal muscle (Song et al. 2017).

Cinnamon contains trans-cinnamic acid (*t*CA), this acid show a strong bodyweight reduction of obese rats by improving insulin sensitivity and blood lipids. According to Kang,

Mukherjee, and Yun (2019) *t*CA present in bark of the *Cinnamomum cassia* reduced expression of key adipogenic transcription factors in white adipocytes, but enhanced their expression in brown adipocytes, also upregulates lipid catabolism and can induce browning, increase fat oxidation, reduce adipogenesis and lipogenesis suggesting its potential to treat obesity.

ANTIOSTEOPOROTIC EFFECT OF CINNAMON

Postmenopausal osteoporosis may occur when bone resorption exceeds bone formation, and the integrity of the skeleton usually controlled by calcitropic hormones and cytokines are not in a dynamic balance. Decreasing serum estrogen after the menopause is associated with bone loss and osteoporosis. Therefore estrogen replacement therapy has been considered as an effective alternative in preventing bone loss as an alternative, dietary natural plant estrogens (phytoestrogens) are possible candidates to replace estrogens. Phytoestrogens as daidzein, genistein, and coumestrol have a chemical structure similar to 17 β-estradiol and exert estrogenic activity through estrogen receptors. Phytoestrogens are effective in preventing bone loss; they are present in plants, and they may provide a potential therapeutic alternative for bone loss. Cinnamon is well known as a particular medicine herbal, and its antiosteoporotic response is well accepted. For instance, *Cinnamon cassia* extract showed an estrogenic activity and competed more strongly with estrogens receptors; this activity could be a useful key to designing more studies *in vitro* and *in vivo* for applying cinnamon extracts as a substitute of phytoestrogens (Lee and Choi 2006).

A recent study has demonstrated that ethanolic extract of the C. *burmannii* can normalize serum bone turnover markers, due to an increase in bone turnover conduces to aging with pathological states such as osteoporosis. This represents a possibility to diminish the effects that conduce to osteoporosis (Kania et al. 2018).

C. zeylanicum exhibited potent inhibitory effects on osteoclastogenesis. Osteoclastogenesis is the maturation process of the osteoclasts, specialized cells that maintain homeostasis in bone tissue. Inhibitory effects on osteoclastogenesis are presented, through a mechanism of action that involves the suppression of NFATc1-mediated signal transduction, inhibiting, or delaying osteoporosis (Tsuji-Naito 2008).

HEPATOPROTECTIVE EFFECT OF CINNAMON

The liver is the organ that realizes metabolism and detoxification. Hepatotoxicity produces free radicals like reactive oxygen species (ROS). Free radicals exist in organisms in the form of reactive oxygen and nitrogen species. Also, animals and humans produce free radicals as part of their normal physiology but an imbalance of the body antioxidant defense system and free radical formation brings oxidative stress, which leads to overproduction of the reactive species. Xenobiotics cause an ionic imbalance in tissues and thus lead to health hazards. Therefore, carbon tetrachloride has been studied in detail for its hepatotoxic properties to induce liver injury in laboratory animals (Rasool et al. 2019).

The effect of *C. verum* essential oil (CvEO) on hepatotoxicity-induced in mice by administration of carbon tetrachloride was studied. Pretreatment with CvEO (100 mg/kg)

protected from the oxidative damage. Antioxidants are present in CvEO, in literature antioxidants have been described by its ability to scavenge free radicals and to inhibit ROS accumulation. It was demonstrated that CvEO exhibit a robust protective effect against CCl_4 induced hepatotoxicity. As a preliminary study diet supplements based on cinnamon may be suggested to patients with chronical liver diseases, but there is still needed more research about the efficacy in humans (Bellassoued et al. 2019).

Cinnamon may also act with glycyrrhizin to enhance hepatoprotective effects. An antioxidative, anti-inflammatory and hepatoprotective activity of standardized extracts of cinnamon and glycyrrhizin under carbon tetrachloride-induced hepatic injury in mice has been described. Combination of glycyrrhizin and carbon tetrachloride in extracts possess an antioxidative property with significant effect at lowering the lipid peroxidation and also capable of reinstalling the altered serum biomarkers (ALT, AST, ALP, TP) level to normal indicating the recovery of liver injury of hepatocytes induced by CCl_4 in mice (Rasool et al. 2019). Nevertheless, it is important to continue the studies to confirm the same hepatoprotective effects in humans.

ANTIFUNGAL ACTIVITY OF CINNAMON EXTRACTS

Fungal infections are one of the most critical health issues concerning people around the world since ancient times. Especially fungal infections of the skin, hair, and nails are a common public health problem.

Cinnamon is rich in (E)-cinnamaldehyde (with a concentration between 6,000–30,000 ppm) a compound known for its antifungal activity. An extract of *Cinnamomum zeylanicum* showed effective antifungal activity against *Aspergillus flavus*, comparable to the standard reference antifungal drug amphotericin B and fluconazole (Hameed, Altameme, and Mohammed 2016). Besides, it has been reported the antifungal activity of blended cinnamon oil and usnic acid nanoemulsion using animal models of candidiasis and dermatophytosis. In this study, it was observed that the active antifungal components were cinnamaldehyde, cinnmyl cinnamate, and benzyl cinnamate. It showed efficacy both *in vitro* and *in vivo* (Meghani et al. 2018). These last results may be applied in the next pharmaceutical design as antifungal medicaments.

It is interesting that a cinnamon essential oil isolated from a Taiwan species named "indigenous cinnamon tree" (*C. osmopholeum)* posses significant antifungal activity against brown and white rot mushrooms. The application of cinnamon essential oil with a MIC (minimum inhibitory concentration) of 50 and 75 ppm of cinnamaldehyde inhibited the growth of these fungi by 100%. It is important to note that this oil comes from natural sources, and due to that, the negative effect on the environment would be null (Wang, Chen, and Chang 2005).

Cinnamon contains essential oils composed of volatile compounds, many of them have remarkable antifungal activities associated with its nature; in this sense, and interesting applications of essential oils of cinnamon are reported. For example, antifungal films based on a starch-gelatin blend, containing essential oils have been studied and despite several organic volatile compounds are lost during a drying process, the films prepared showed significant antifungal activities against *Colletotrichum gloesporoides*, and *Fusarium oxysporum*. In spite of the high volatility of essential oils, their presence in films improved their barrier properties

(to water and to oxygen) and their transparency, leading to films with better functional properties. The films prepared to contain cinnamon-essential oils are more recommendable to be used in food products susceptible to *Fusarium oxyspor* (Acosta et al. 2016).

Other studies made with cinnamon essential oils showed effective against pathogens as *Colleotrichum musae* and *C. Goesporoides* on films based on Arabic gum (Maqbool et al. 2011). When compared to ginger, cinnamon has antifungal properties. Films containing cinnamon essential oil displayed higher antifungal activity *in vitro* against *Aspergillus niger* than those containing ginger essential oil (Noshirvani et al. 2017).

New potential applications of cinnamon in cinnamon-containing polycaprolactone (PCL) bandages has been suggested as a result of the anti-fungal activities observed against *Candida albicans*. When preparing and spinning polymer solutions of cinnamon with PCL, fibers capable of inhibiting fungal growth could be produced. Also when it was compared with raw cinnamon powder, the novel cinnamon loaded fibers had outstanding long-term activity, new applications in biomedical technology may be interesting, especially in wound healing. Crude and unpurified extracts of natural materials serve in many cases against the fear of using synthetic products, also with the secondary adverse effects that synthetic products may present in organisms. Interesting applications may be useful in designing of the fibrous patch without needing purification methods (Ahmed et al. 2019).

ANTIOXIDANT ACTIVITY OF CINNAMON

All the living organisms are at every time performing millions of biochemical reactions to establish a homeostatic equilibrium, but unfortunately not all products generated are necessary for them, some products are toxic, or present chemical reactions that unbalance the homeostatic equilibrium producing illness. Free radicals are some chemical species generated as radical oxygen species (ROS) which are produced in the form of free radicals during normal cell metabolism, both in animals and plants. Although an excess of ROS may produce oxidative stress, resulting in oxidative DNA damage. As a consequence, numerous disorders, e.g., cardiovascular atherosclerosis, reperfusion injury, rheumatoid arthritis, inflammatory disorders, and cancer can be developed (Vaz et al. 2011). When free radicals are produced they are neutralized by the cellular antioxidant defenses (enzymes and non-enzymatic molecules). Organisms that have a normal equilibrium between free radicals and production and antioxidant defenses possess a normal healthy condition, but when non controlled production of free radicals occurs, hundred of different diseases may appear as a consequence of this instability, these diseases include several kinds of cancer, diabetes, among others previously cited.

Organisms have adapted and developed defense techniques against free radicals, they are equipped with antioxidant defense and repair systems that have evolved to protect them against free radicals and oxidative damage. These systems not always are efficient in preventing oxidative stress-induced damage. In this sense, it is recommended in human diet to include products containing bioactive compounds or antioxidant supplements to reduce oxidative damage and also to prevent chronical diseases linked to reactive oxygen species (Reis et al. 2012). Many of these bioactive antioxidant compounds are phenolic compounds, they not only act as antioxidants, they exhibit antioxidant, antibacterial, antiviral, anticarcinogenic and anti-inflammatory activities, they play a useful role in the prevention of cardiovascular problems,

reduction of blood cholesterol and treatment of illness such as cancer and diabetes (López-Vázquez et al. 2017).

There are many compounds associated with antioxidant capacity, high molecular with compounds as polyphenolics and flavonoids whose formula weight is over 400 Da. Other molecules with smaller formula weight, lower than 400 Da, are volatile compounds. Antioxidant activity is associated with the concentration of phenolic compounds in a sample. This antioxidant activity has been explained by different mechanistic methodologies proposed. Phenolic compounds act as free radical scavengers, transition-metal-chelating agents, and singlet oxygen quenching agents. Phenolics compounds are also able to stabilize lipid peroxidation and to inhibit various types of oxidizing enzymes. Total equivalent antioxidant capacity has a positive linear relationship with total phenolic content. Phenolic compounds contribute directly to antioxidant capacity (Shan et al. 2007).

The chemical structure of phenolic compounds is related to its antioxidant power. The phenolic ring in many cases with conjugated double bonds is an excellent site for electronic delocalization; this delocalization gives chemical species (resonance) some of them more stable than others.

An exciting application of essential oil of cinnamon (*Cinnamomun zeylanicum*) has been explored in a synergistic combination with strawberry fruits (*Fragaria ananassa*), resulted in the increase of antioxidant levels and a synergetic effect observed (Silva-Espinoza et al. 2013). More studies may be done with other red fruits famous by their high content of antioxidant levels.

Different interesting aspects concerning to antioxidant capability of cinnamon mainly due to cinnamaldehyde, and other phenolic compounds have been described. The main reason for using cinnamon during different centuries for treatment, prevention of various diseases is mainly attributed to its cinnamaldehyde content, although in comparison with another herb species cinnamon does not possess a significant high antioxidant activity. Less of a 10% of antioxidant activity has been reported in comparison with herb species as pine needles, angelica, lavender, chamomile, rosemary, basil, thyme, clove bud, eucalyptus, eucalyptus, and aloe (Kim and Lee 2008).

In spite of the many compounds present in a natural product, some of them may experiment with synergistic or antagonistic relationships between them. Some studies describe the synergistic effects of infusions and preparations of mixed herbs used in folk medicine, some of them known antioxidants such as total phenolics, flavonoids, ascorbic acid and reducing sugars. Many antioxidants are present in some herbs as *Aloysia citrodora, Foeniculum vulgare,* and *Mentha spicata.* A combination of these herbs achieved a high synergistic effect (Guimarães et al. 2011). Many people use to consume a combination of herbs in teas, and as consequence different synergistic or antagonistic effects may be present. For example when cinnamon is combined with black or peppermint teas, it showed a synergistic effect on the total antioxidant activities of both drinks (Büyükbalci and El 2008).

In literature, several synergistic effects enhancing antioxidant properties of different herbs are described. For example, combination of green tea *Camelia* species with grape seed (*Vitis vinifera*), Amla (*Phyllanthus emblica*), Anar (*Punica granatum*), Chinese cinnamon (*Cinnamomun cassia*) and Maiden hair tree (*Ginkgo biloba*) in the ratio 5:3:3:3:3 resulted in a synergistic enhancement of antioxidant effects in this tea blend. The combination of lemon (*Citrus limon*), bergamot (*Citrus bergamia*), clove (*Syzgium aromaticum*), cinnamon sticks and ground cinnamon (*Cinnamomun verum*) the antioxidant activity is increased in comparison to

individual herbs although antagonistic effects were described if there were added milk and sweeteners to the blended tea (Maqbool et al. 2011).

In Mexico, coffee is prepared with an aqueous cinnamon extract obtained by boiling cinnamon bark in water and then adding coffee. The main components in coffee are ferulic, caffeic and chlorogenic acids; these compounds can neutralize free radicals. Chlorogenic acids are a family of esters formed between trans-cinnamic acids and quinic acid, one of the most common is the formed between caffeic acid and quinic acid. When coffee is consumed, phenolic compounds are bioavailable as a consequence of the hydrolysis of these esters compounds in the stomach at a low pH and are less available in thin intestine at pH levels around 7.5. On the other hand, cinnamon extracts are attributable to the scavenging ability of their components (cinnamaldehyde and cinnamic acid) against reactive oxygen species including superoxide anions and hydroxyl radicals as well as other free radicals. Cinnamon extracts are weak metal chelators, but their scavenging activity against free radicals is compared to synthetic antioxidants as BHA.

In cinnamon aqueous extract there are more than 80 compounds observed in the gas chromatograms when combined with other plants; different synergistic interactions may occur. Nevertheless, it is hard to know a precise model of interactions to realize it the effects will be positive or adverse to individuals. Humans need to complement diet by the intake of food products that contain different antioxidants that may serve to scavenge oxygen species and to inhibit lipid peroxidation, associated with many diseases, like cancer, arteriosclerosis, diabetes and immune deficiency (Malongane, McGaw, and Mudau 2017).

Natural antioxidants are preferred rather than synthetic to avoid secondary effects. Natural antioxidants are promising alternatives for the prevention of several diseases such as cancer, cardiovascular disease, cataract, atherosclerosis, diabetes, arthritis, immune deficiency diseases, aging, and brain dysfunction. Some plants, especially cinnamon, are an excellent source of natural antioxidants.

Natural antioxidants are not only for medical usages; they also possess important characteristics that allow them to be used in the food industry to maintain food freshness, flavor, taste, and color by preventing oxidation deterioration. In past years aroma extracts isolated from plants primarily were considered only as flavors and fragrances, but now they are considered as natural antioxidants. Different modern aromatherapies use chemicals from plants widely used in folk medicine (Thomas and Kuruvilla 2012).

Comparison between Antioxidants of Cinnamon and Spices versus Common Food Additives

Different organic volatile compounds confer to herbs and species, particular aroma and flavors. Moreover, their organoleptic properties, chemical composition (flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, and curcumins) are responsible for their useful biological activities for treatment and prevention of several chronical illnesses.

The antioxidant properties of seven dessert species (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) were compared with typical food antioxidant additives as butylated hydroxyanisole (BHA E-320), butylated hydroxytoluene (BHT E-321) and propyl gallate (E-310). An assay that allows relating antioxidant activity with a synthetic antioxidant is the Trolox

equivalent antioxidant capacity. The decreasing order of antioxidant capacity was as follows cinnamon ~ propyl gallate > mint > anise > BHA > licorice ~ vanilla > ginger > nutmeg > BHT. Also mint and cinnamon showed a higher percent of inhibition of oxidation than the other species analyzed and the food antioxidants, as tested by the lipid peroxidation assay, these results allow to suggest cinnamon as a useful source of antioxidants with a high percent of inhibition of free radicals (Murcia et al. 2004).

ADVERSE SIDE EFFECTS OF CINNAMON

Cinnamon has been used mainly as an astringent, antiseptic, antispasmodic; also it is known to help in the treatment of chronic bronchitis, impotence, frigidity, dyspnea, inflammation, leukorrhea, rheumatism, headache, and toothache. Nevertheless, it not always is safe to use cinnamon since there can be an adverse side effect due to the dose level.

There are many cases reporting toxicity linked to allergic reactions to cinnamon oil when people have dermal contact with products containing cinnamon oil as toilet soaps, mouthwash, toothpaste, perfumes, mud and baths, beverages (colas, vermouth, bitters), or baking products. Allergic reactions presented by some people in contact with cinnamon products include contact dermatitis, perioral dermatitis, cheilitis, stomatitis, gingivitis, glossitis, chronic lichenoid mucositis, contact urticaria and rarely immediate hypersensitivity reactions as urticaria and asthma (Barceloux 2009).

Nevertheless, from a compilation of thirty-eight clinical trials, there were found only five studies that showed an adverse effect. Yes, this is not a high percentage (13%). Moreover, in the compilation of 141 people data, only 17 of them experienced adverse effects like gastrointestinal problems (stomachache, nausea), constipation, heartburn, headache, dermatological problems (hives and rash) and menstrual cramps, people who presented adverse effects represent 12%. Cinnamon administration to patients of the analyzed groups was in a short period lasting four weeks and the longest six months, with a minimum daily dose of 200 µg in the form of nasal spray and the maximum treatment orally consumed was 80 mg and 1.5 g. In these studies, *Cinnamomun cassia* and *Cinnamomun zylanicum* were used. People who presented adverse effects to cinnamon and that were exposed by methods as supplements or flavoring agents showed dermatitis and stomatitis, other reported adverse reactions in case reports were a worsening of rosacea, erythema multiform-like sensitivity reaction, cheilitis, mucositis, squamous cell carcinoma, and acute hepatitis. This last one could be due to a hepatotoxic substance present in cinnamon, coumarin; which is present in different levels from 0.01 g/kg to 3.6 g/kg in *C. verum* and *C. cassia* respectively. Adverse effects disappeared when exposition was suspended (Hajimonfarednejad et al. 2019).

From 1973 to June 2016, the World Health Organization has registered 44 spontaneous adverse reports to mono-preparations of cinnamon; most of the cases were reported in the United States. However, cinnamon has more benefits related to its intake than adverse effects, although attention must be paid in the use of cinnamon as an herbal medicine being careful of the dose or allergenic issues (Hajimonfarednejad et al. 2019).

According to the European Food Safety Authority (EFSA), a teaspoon of *Cinnamomum cassia* powder contents between 5.8−2.1 mg of coumarin; since the tolerable daily intake for humans is 0.1 mg/kg body weight, EFSA suggests that cinnamon must not more than one

teaspoon be consumed at day, because of the adverse side effects previously described. Also, it is not recommended the intake of 60 mL of cinnamon essential oil because of a burning sensation in the gastrointestinal system, lethargy, double vision, vomiting, lightheadedness and irritation of the skin and urinary tract may be presented. Although cinnamon consumption in large quantities has no toxic effects for the cinnamaldehyde or the coumarin components, safrol, and styrene should be considered. Safrol is a genotoxic carcinogen, according to EFSA the maximum level recommended is 1 mg/Kg, while styrene might be carcinogenic to humans, the tolerable daily intake of styrene established by the WHO is 7.7 mg/kg body weight (Miraghajani and Ghiasvand 2019).

A large list of potential uses in therapeutic are described, and just a little number of adverse reactions are described for cinnamon, a spicy and peculiar medicinal herbal. If it is used as a therapeutic supplement, medical observance must be taken because not all individuals respond in the same way to its components.

MICROENCAPSULATION TO ENSURE SUSTAINED BIOACTIVITY

Many biological properties of cinnamon as for example, antibiotics, hypolipidemic, anti-diabetes, anti-inflammatory activity are attributed to organic volatile compounds as polyphenols which are extremely sensitive to environmental conditions (e.g., UV radiation, temperature, oxygen, digestion), furthermore distinct handling of cinnamon when infusions are prepared, packaging and storing of cinnamon barks affect polyphenols. The microencapsulation process has proved to be an efficient technology for protecting this sort of compound. It turns suspensions into powdered particles, comprised of wall material and a core made principally by carbohydrates as maltodextrins. Flavors and aromas are protected by cores for long periods and help to preserve polyphenols and as a consequence to maintain biological activity (Santiago-Adame et al. 2015). Once microencapsulation is achieved it requires a natural biopolymer and several tests for microencapsulation characterization as gas chromatography analysis, moisture sorption-desorption isotherms, infrared spectroscopy (IR), antibiotic and antifungal activity against pathogens to ensure the optimal preservation of the organic volatile compounds.

Sodium alginate has been tested as an effective microencapsulation agent, cinnamon oil was microencapsulated by simple coacervation, the size distribution was described narrow and the mean diameter of 53.79 μm, the morphology of the surface of the resultant microcapsules characterized by scanning electron microscopy (SEM) showed an adequate surface and a round morphology. Finally, the rates of cinnamon oil released were not only affected by relative humidity in the microenvironment around the microencapsulated powder, but it was also affected by the temperature (Li et al. 2011). Sodium alginate is adequate to protect cinnamon components from relative humidity and is safe for consumers.

Another technique of microencapsulation described in literature consists of microencapsulation with β-cyclodextrins for cinnamon leaf oils and garlic oils. This technique has been described and characterized by gas chromatography analysis, moisture sorption-desorption isotherms, infrared spectroscopy (IR). Antibiotic and antifungal activity against *Alternaria alternate* were evaluated for microcapsules, and it displayed an effective antifungal activity. By IR, there were detected hydrogen bonds between oil constituents and β-

cyclodextrins, and the stability of essential oils could be affected by water uptake in the β-cyclodextrins surface. Indeed the exclusive features of microencapsulated cinnamon leaf and garlic oils can have important applications in the food industries, because of an improvement of stability, and bioavailability of the guest molecules in the inclusion complex

Maltodextrins are the main component of cell wall materials; these carbohydrates are obtained from starch hydrolysis, they are cheap and highly water-soluble, they have commonly low viscosity, all of these features allow to use maltodextrins as affordable, accessible and practical microencapsulating agents. Microencapsulation of organic compounds of cinnamon is done with spray drying at a feed rate of 10 mL/min and with temperatures of 160 and 180°C were both ideals for microencapsulating cinnamon infusions and it resulted in protection of labile polyphenols, increasing their stability during long-term storage while preserving their biological activity (Santiago-Adame et al. 2015).

Also a binary and ternary blends of gum arabic, maltodextrin, and modified starch as wall materials in proportion 4:1:1 respectively have been studied. Microencapsulation protects oleoresin against damages and converts it into a free-flowing powder; it also protects flavor from undesirable interactions with food and flavor to flavor interaction. Microcapsules prepared by Vaidya, Bhosale, and Singhal (2006) were evaluated for the content and stability of volatiles and entrapped and total cinnamaldehyde. Structural characteristics were determined by scanning electron microscopy resulting in spherical particles with a smooth surface, showing that the blend was suitable for the encapsulation of oleoresin and the antioxidant characteristics were preserved in 100%.

USES AND APPLICATIONS OF CINNAMON IN THE FLAVOR INDUSTRY

Due to its characteristic odor and taste, a little spicy and pungent, cinnamon oil is an excellent source for the aroma in the cosmetic industry. A great number of cinnamon species are harvested and from the bark and leaf are used as spices in the production of essential oils. Different remarkable characteristics of aroma are present in cinnamon that makes it an exciting product. A great variety of species of cinnamon are spread around the world; each species has a distinct flavor; the most used species are *C. zeylanicum* and *C. camphora* and *C. cassia*. When cinnamon is distilled it produces a volatile oil with its particular aroma; many of the oils obtained are locally sold or exported.

CONCLUSION

Cinnamon is a peculiar spice; its unique flavor, aroma, and taste make it an excellent product as a condiment of food and desserts. The aqueous preparations as infusions or teas have a large number of polyphenolic compounds which exhibit a great spectra of biological activities that may be useful as food complement to maintain human health. Nevertheless, it is important that one not consume excess cinnamon because the presence of coumarins may cause secondary undesirable effects. The benefits of natural resources are well known not only in health or in the human diet, but also in industry. Various fragrances may be obtained from the organoleptic features of cinnamon oil, and also remarkable applications in the food industry as an additive

to prevent browning of fruits and vegetables. Cinnamon has been used for thousands of years in culinary and medical applications. Further exploration will undoubtedly reveal new uses for this unique plant.

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Chapter 7

HIBISCUS SABDARIFFA L.: A FLOWER WITH HEALTH BENEFITS

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ABSTRACT

The Roselle (*Hibiscus sabdariffa* L.), also is known as jamaica rose, Abyssinian rose, roselle flower, sarent, and alleluia, is a hibiscus of the family of malvaceae. Hibiscus is successfully cultivated in Mexico, Central America and Southeast Asia, including South China. *Hibiscus* is known for its use as a traditional drink in Mexico. This plant is rich in a variety of phytochemicals or nutraceutical compounds such as phenolic compounds such as anthocyanins and procyanidins, strong antioxidants that are the cause of intense red color and which represent a potential alternative for the replacement of synthetic dyes in foodstuffs. Additionally, it has a significant content of vitamins A and C, a large number of minerals, citric, and malic acid. The antioxidants found in *Hibiscus* makes it a food that can help to combat various diseases. It could eliminate alcohol discomfort by stimulating the action of the liver and blood vessels, the absorption of certain minerals, lowers blood pressure, and because of this, it is used as a cardiac tonic, diuretic, antiseptic, analgesic, anti-inflammatory, antimicrobial, astringent, healing, digestive, depurative, emollient, sedative, mild laxative, weight reducer, detoxifier, antioxidant, toner, stimulant, aphrodisiac, and vasodilator.

Keywords: roselle, hibiscus, biological properties

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GENERALITIES

The *roselle* flower (*Hibiscus sabdariffa* L.) is a shrub native to India and Malaysia, while other records mention that its origin is in tropical Africa, from Egypt and Sudan to Senegal, although for its medicinal properties, is cultivated in other countries such as Mexico, Central America and South, and Southeast Asia, including southern China (Vasavi et al. 2019). It also is known as Abyssinian rose or hibiscus flower (Da-Costa-Rocha et al. 2014). Table 1 shows the taxonomy of hibiscus.

The scientific name of roselle flower is *Hibiscus sabdariffa*, or also known as Saril, vinuela, roselle Sorrel, cabitutu, Malva morada (Singh, Khan, and Hailemariam 2017). Currently, there are more than 150 varieties of this plant worldwide, among them, there are six outstanding varieties which are 1) Sudan variety, 2) Chinese or purple variety, 3) red variety (long and short/America), 4) giant black variety (Nigerian), 5) giant purple variety (Thai), and 6) non-acid variety (Vietnam) (Ariza-Flores et al. 2014).

Roselle is an annual malvaceous family plant that grows extensively in the tropics and subtropics of both hemispheres at a height above sea level of zero to one thousand four hundred meters. The growth temperature is 22 to 25°C; annual rainfall is 500 to 1000 mm in heavy or clay soils with permanent humidity, although it has been naturalized in many areas of America with dry climates (Vasavi et al. 2019). Harvesting takes place when the plant begins to mature, and its cycle is 6 to 7 months. This plant grows as a shrub and reaches two meters or up to three meters in height. It reproduces by self-fertilization, and its roots are superficial, and it is a very demanding plant as far as light hours are concerned (photoperiod > 11 to 12 h/light). Its flowers are fleshy, reaching a diameter of 10 cm, and the corolla is white (Ramírez-Cortés et al. 2011). The calyx when ripe, turns red with four or five petals and long thorns surrounding the flower, and has a conical shape, resembling a small poppy and stem. Once dry, the calyx has a shelf life of one year; it should be stored dry, shaded, and aerated to avoid pests. It is generally red and violet. Its leaves have three to five lobes of fifteen centimeters in length alternating on the stem. The most remarkable thing about the plant is the fleshy calyx with intense color, rich in malic, citric, and tartaric acids (Bobadilla-Carrillo et al. 2016, Ramírez-Cortés et al. 2011).

Table 1. Taxonomy of the roselle flower (*Hibiscus sabdariffa* L.)

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Dicotiledonea
Order	Malvales
Tribe	Hibisceae
Familia	Malvaceae
Genus	Hibiscus
Species	sabdariffa L.

PHYSICAL, CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF ROSELLE FLOWER

Table 2. Nutrimental composition of *Hibiscus sabdariffa* L.

***Hibiscus sabdariffa* L.**	**Chemical and nutritional composition**	**Analytical methods**	**Reference**
Calyx	Carbohydrates (arabinans, xylose, arabinogalactans, rhamnogalacturans, mannose, sucrose, pectin, mucilage)	Methylation analysis, partial hydrolysis, and nuclear magnetic resonance, thin-layer chromatography	(Kassakul et al. 2014, Shimizu et al. 1993)
	Dietary fiber	Enzimatic treatment	(Mercado-Mercado et al. 2015)
	Vitamins (thiamine, niacin, riboflavin)	Iodometric titration, HPLC	(Kane et al. 2018, Peter et al. 2014)
	Proteins	Kjeldhal method (NF V03-050	(AFNOR 1982)
	Fats	Soxhlet method	(AOAC 1990)
	Phytosterols	Colomimetric method	(Ismail et al. 2008)
	Organic acids (citric acid, ascorbic acid, maleic acid, hibisc acid, oxalic acid, tartaric acid, (+)-acid allooxycitronic-lactone, allohydroxycitric-acid, glycolic acid, utalonic acid, protocatechic acid)	Chemical process	(Abaza, Blake, and Fisher 1968, Adanlawo and Ajibade 2006, AOAC 1990)
	Chemical non-nutritional (cyanidine-3-glycoside, cyanidine-3-sambubioside, cyanidine-3-xyloglucoside, delphinidine, delphinidine-3-glucoside, delphinidine-3-sambubioside, delphinidine-3-xyloglucoside, delphinin, gosipetine, gosipetine-3-glucoside, hibiscetin, hibiscin, hibiscitrin, sabdaretin, sabdaritrin, gosipetin, phenolic acids, flavonoid, β-carotene)	Colorimetry method, HPLC	(Abou-Arab, Abu-Salem, and Abou-Arab 2011, Mercado-Mercado et al. 2015)
	Resin	Denture acrylic resin	(Okeke, Vahed, and Singh 2018)
	Minerals (iron, phosphorus, calcium, manganese, aluminum, magnesium, sodium, potassium)	Ashes-Chromatography	(Adanlawo and Ajibade 2006, AOAC 2000)
	Ashes	AOAC 1990	(Adanlawo and Ajibade 2006)
Seed	Carbohydrates (starch, cellulose)		(Emmy Hainida et al. 2008, AOAC 2000)
	Dietary fiber	Enzymatic process Chemical process	(AOAC 2000, Emmy Hainida et al. 2008, Tazoho Ghislain 2014)
	Protein	Kjeldahl method	(Emmy Hainida et al. 2008)
	Fat (cholesterol, propionic acid, pentosanes, pelargonic acid, palmitoleic acid, palmitic acid, oleic acid, myristic acid, methanol, linoleic acid, sterulic acid, caprylic acid, formic acid, stearic acid, cis-12,13-epoxycis-9-octadecenoic acid, cyclopropenoid fatty acid)	Chemical process	(Akpinar et al. 2012, Ahmed and Hudson 1982)
	Phytosterols (campesterol, β-sitosterol, ergosterol)	Supercritical carbon dioxide	(Holser, Bost, and Van Boven 2004)

Table 2. (Continued)

***Hibiscus sabdariffa* L.**	**Chemical and nutritional composition**	**Analytical methods**	**Reference**
Seeds	Organic acids (malvalic acid)	Chemical method	(Eggensperger and Wilker 1996)
	Minerals (phosphorus, magnesium, calcium)	Ashes-Chromatography	(Adanlawo and Ajibade 2006, AOAC 2000)
	Amino acids	Chromatography method	(FAO/WHO 1991)
	Others (eugenol, cadalene, isopropyl alcohol, alcohol, isoamyl, ethanol, 3-methyl-1-butanol)	Dynamic headspace sampling; GC/MS	(Inikpi et al. 2014, Juhari and Petersen 2018)
Leaf	Carbohydrates	Molisch's teste, Ruthenium red test, Iodine test, TLC, HPAEC	(Kassakul et al. 2014)
	Dietary fiber	Chemical process	(Mohammed Yusof et al. 2018)
	Vitamins (thiamine, niacin)	Colorimetric method	(Nwibo 2017)
	Protein	Kjeldhal method	(AFNOR 1982)
	Fats	Soxhlet method	(AOAC 1990)
	Organic acids (malic acid, α-terpinyl acetate, anisaldehyde)	Chemical method	(Eggensperger and Wilker 1996)
	Phytosterols (β-sitosterol, β-sitosteryl benzoate)	Colomimetric method	
	Chemical non-nutritional (β-caroteno)	Spectrophotometry method	(Musa 2012)
	Minerals (calcium, phosphorus, iron)	Ashes-Chromatography	(AOAC 2000, Adanlawo and Ajibade 2006)
	Others (isoamyl alcohol, isopropyl alcohol, methanol, 3-methyl-1-butanol, benzyl alcohol, ethanol)	Dynamic headspace sampling; GC/MS	(Inikpi et al. 2014)
	Ashes	Chemical process	(AOAC 1990)

Roselle calyces have substances such as glucose, galactose, xylose, mucopolysaccharides, pectin, water-soluble polysaccharides, high amounts of organic acids (hibiscus acid, malic, tartaric, and citric) and their glucosylated forms, different pigments (hibiscin and gossipitin) and low concentration of anthocyanidins (myrtillin, chrysanthenin, and delphinidin), *β*-carotene, essential oil, protocatechuic acid and significant content of vitamins A and C (Eltayeib and Elaziz 2014, Gomes Maganha et al. 2010, Vizcaino et al. 2007). Table 2 presents the chemical and nutritional composition of different parts of roselle.

The nutritional analysis of the roselle shows it to be rich in protein content and carbohydrates, followed by dietary fiber in flower, leaves, calyces, and seed (Table 2). Roselle is also rich in minerals, especially potassium and magnesium, however, *Hibiscus* calyxes are an important source of calcium and in lower concentrations iron, magnesium, phosphorus, chromium, copper, cobalt, fluorine, iodine, manganese, molybdenum, selenium, vanadium, zinc, nickel, silicon, arsenic and tin. (Adanlawo and Ajibade 2006, Alarcón-Corredor 2009, Ali, Wabel, and Blunden 2005, Carvajal-Zarrabal et al. 2012, Jung, Kim, and Joo 2013, Ojokoh 2006). Nutritional studies of hibiscus have indicated that its consumption is consistently related to a decrease in the incidence of different diseases: bactericidal, antifungal, hypo-cholesterolemic, diuretics, anti-inflammatory, antihypertensive, among others (Ojulari, Lee, and Nam 2019, Riaz and Chopra 2018). On the other hand, it has been seen that calyces such as flowers have compounds that establish a relationship with the prevention of diseases such as anthocyanosides derived from delphinidol, and cyanidol (delfinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3,5-diglucoside, among others), flavonoids (catechins), phenolic

acids (protocatechuic, acid, p-coumaric, chlorogenic acid), in addition to dietary fiber (mucilage, pectin) and phytosterols (Table 2) (Mahadevan and Kamboj 2009, Mercado-Mercado et al. 2015).

BIOACTIVE COMPOUNDS OF *HIBISCUS SABDARIFFA*

Hibiscus sabdariffa plant contains mucilaginous polysaccharides and pectins; which also form part of its composition and many types of phenolic compounds. Furthermore, *Hibiscus* seeds contain a wide range of less polar compounds, such as sterols (sitosterol, ergosterol, campesterol). Although the composition of this plant has been thoroughly studied, one important group of compounds present in this plant are phenolic compounds, which recently have attracted a great deal of attention due to their beneficial effects in the promotion of human health and well-being (Ali, Wabel, and Blunden 2005). The main constituents of *Hibiscus sabdariffa* relevant in the context of its pharmacological properties are organic acids, anthocyanins, polysaccharides, and flavonoids (Sukkhaeng, Promdang, and Doung-ngern 2018, Zheoat et al. 2019).

Phenolic Compounds

Phenolic compounds are products of the secondary metabolism of plants that originate from two main synthetic pathways: the shikimate pathway and the acetate pathway (Cutrim and Cortez 2018). These compounds can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins (Adebooye, Alashi, and Aluko 2018). They occur mainly in conjugated forms, with one or more sugar residues (monosaccharides, disaccharides, or oligosaccharides) linked to hydroxyl groups, although direct linkages to aromatic ring can occur. They can also be associated with carboxylic and organic acids, amines, lipids, and other phenolic compounds (Cutrim and Cortez 2018).

Calyxes contain phenolic acids (protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid), anthocyanins and flavonoids (hibiscetin and its aglycone hibiscetin, gossipitrin, gossitrin, gossipetin, quercitin, and sabdaritrin) (Figure 1, designed in ADC/ChemSketch) (Table 3) (Mercado-Mercado et al. 2015).

Anthocyanins

Anthocyanins, detected in high amounts in the calyces, are responsible for the bright red color. The most frequent anthocyanins of hibiscus flowers are delphinidin-3-sambubioside, cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-sambubioside or delphinidin-3-xylosylglucoside, and cyanidin-3-ylosylglucoside or gossypicyanin (Figure 1) (Ali, Wabel, and Blunden 2005).

The stability of hibiscus anthocyanins has been studied in model systems testing the effect of different chemical compounds (ascorbic acid, BHA, propyl gallate, disodium EDTA, sodium sulfite, cyclodextrins) (Aramwit et al., 2010) and temperature (Sinela et al., 2017). Some studies

have shown that the thermal degradation of hibiscus anthocyanins follows first-order reaction kinetics (Pragalyaashree, Tiroutchelvame, and Sashikumar 2018). Thermal stability of hibiscus anthocyanins in the temperature range of 60-90°C in aqueous solutions (Sinela et al. 2017).

Flavonoids

Hibiscus contains phenolic compounds, mainly flavonol and flavanol (Maciel et al. 2018). The following flavonoids have been described in *Hibiscus* extracts: hibiscitrin (hibiscetin-3-glucoside), sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides, quercetin, and luteolin (McKay et al. 2010). Earlier, the flowers were recorded to contain 3-monoglucoside of hibiscetin (hibiscitrin) (Maciel et al. 2018).

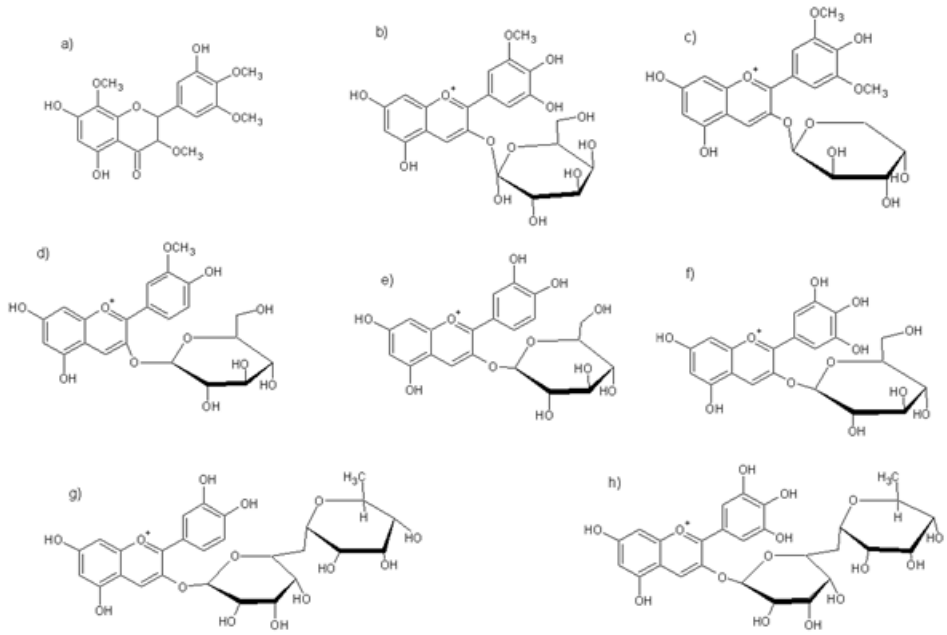

Figure 1. Bioactive compounds identified in *Hibiscus sabdariffa*.a) Hibiscetin, b) Petunidin 3-O-galactoside, c) Malvidin 3-O-arabinoside, d) Peonidin 3-O-glucoside, e) Cyanidin 3-glucoside, f) Delphinidin 3-glucoside, g) Cianidin 3-sambubioside, h) Delphinidin 3-sambubioside.

Table 3. Profile of phenolic compounds of calyx *Hibiscus sabdariffa* L.

Phenolic acid	**Flavonoids**	**Anthocyanins**
Gallic acid, chlorogenic acid isomer I, chlorogenic acid, 3-cafeoilquinic acid, protocatechic acid, glycoside protocatechic acid, coumaroylquinic acid, 4-cafeoilquinic acid, caffeic acid	Miricetin 3-arabinogalactoside, quercetin, quercetin-3-sambioside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-*O*-rutinoside, feruloil derivative, myricetin, kaempferol-3-*p*-cumaryl glucoside, astragaline, quercetin derivative	Definidine-Sanbubioside, Delphinidine-3,5-diglycoside, Delphinidine-3-glucoside, Petunidine-3,5-diglycoside, Petunidine-3-diglycoside, Cyanidine-3-sambioside, cyanidine-3,5-diglucoside, cyanidine-3-glucoside, malvidine-3,5-diglucoside, malvidine-3-glucoside, peonidine-3,5-diglucoside, peonidine-3-glucoside

THERAPEUTIC EFFECTS OF INFUSIONS OF ROSELLE

The dehydrated calyces of the roselle are used to obtain extracts whose most common use is the production of beverages. "*Roselle water*" is most often homemade. It is prepared by boiling the dried calyces in water for 8 to 10 minutes (or until the water turns red) and served cold. Also, roselle infusion contains compounds responsible for the taste, aroma, color, and antioxidant properties reported in beverages and extracts obtained from this plant (Puro et al. 2017). The acids and pigments contained in calyces are used for the production of tea, wine, beer, beverages, juice, sausage coloring, jellies, syrups, sweets, jams, compotes, desserts, cakes, sauces, decorative edible (soups, salads), and culinary use (Caro, Machuca Sánchez, and Flores Berrios 2010, Galicia-Flores et al. 2008).

Roselle infusion is a beverage of crimson or magenta-deep (sepals) made by the calyces, has a bitter taste, and can be consumed both cold and hot. This beverage is commonly consumed throughout the world, mainly in western Sudan, the Caribbean, Central America, and Mexico (Fasoyiro et al. 2005, Mohammed Yusof et al. 2018, Singh, Khan, and Hailemariam 2017, Abdallah 2016). The antioxidants of roselle, mainly phenolic compounds, make it a food to prevent various diseases, mainly cardiovascular disease, liver disease, diabetes mellitus, blood pressure, and hypertension, hyperlipidemia, and obesity (Table 4) (Bolade and Ojo 2009, Mohammed Yusof et al. 2018, Abdallah 2016).

Table 4 shows that roselle has been used in the prevention of hypertension and hyperlipidemia (Guardiola and Mach 2014, Hopkins et al. 2013, Sudan et al. 2014). *In vitro* and *in vivo* studies have supported the ability of hibiscus extracts to inhibit carbohydrate-related enzymes such as α-amylase and α-glucosidase, and starch absorption, which helps reduce body weight and obesity (Gondokesumo, Kusuma, and Widowati 2017, Hansawasdi, Kawabata, and Kasai 2000, Shadhan and Bohari 2017). Epidemiological studies support the positive effect of roselle on weight control in people by administering 5% to 15% roselle infusion (Carvajal-Zarrabal et al. 2005). Morales-Luna et al. (2019) reported that 22.5 mg of aqueous extract of white Roselle variety might prevent body weight gain in rats fed a diet high in fructose. Also, roselle phenolic compounds help to lower blood levels of lipids, cholesterol, and triglycerides, as well as platelet aggregation and increased excretion of bile acids, pointing to the possibility of using hibiscus as an anti-obesity agent and preventing cardiovascular disease (Yang et al. 2010). Animal models studies have allowed verifying the activity on the lipid metabolism of hibiscus extracts. Likewise, aqueous hibiscus extracts inhibit LDL oxidation in a dose-dependent manner, lower total cholesterol, LDL-c, VLDL-c, triglycerides, and lipid peroxidation, and increase HDL-c levels. The administration of 0.5 and 1.0% of the aqueous extract of roselle in the diet of rabbits of the New Zealand strain decreases the levels of lipids in blood and an antiatherosclerotic activity. Similarly, hibiscus extracts regulate lipid homeostasis through inhibition of SREBP-1c and PPARγ by blocking the increase of IL-1, TNF-α mRNA and lipoperoxidation; to counteract liver damage (Villalpando-Arteaga et al. 2013). Flavonoids also decrease oxysterols (a cholesterol derivative) in the metabolism of bile acids and block the accumulation of lipids in the liver (Huang et al. 2015, Villalpando-Arteaga et al. 2013).

Table 4. Biological effects of *Hibiscus sabdariffa* L extract

Biological effects	Mechanisms	Reference
Diuretic	Non-electrolytic type, Uricosuric	(Wansi et al. 2014, Mea et al. 2018)
Liver disease	Cadmium and tertiary-butyl hydroperoxide induced Lipo-peroxidation	(Adeyemi et al. 2014, Huang et al. 2015)
Diabetes mellitus	Decrese blood secretion by enhancing insulin secretion	(Zainalabidin et al. 2018, Mohammed Yusof et al. 2018)
Blood pressure and hypertension	Inhibition of ACE I	(Abubakar et al. 2019, Aliyu et al. 2014, McKay et al. 2010, Mohammed Yusof et al. 2018)
Hyperlipidemia	Inhibition and oxidation of LDL, Reduction of the serum lipid level (LDL, Triacilglycerides, cholesterol)	(Hajifaraji et al. 2018, Ochani and D'Mello 2009, Zainalabidin et al. 2018)
Obesity	Inhibition and oxidation of LDL and atherosclerosis, Reduction of lipid seric levels (LDL, triacylglycerides, cholesterol), Inhibition in the adipocyte difference Cholesterol Absorption	(Alarcon-Aguilar et al. 2007, Carvajal-Zarrabal et al. 2005, Yang et al. 2010)
Cellular apoptosis	Cytotoxicity and apoptosis in gastric carcinoma cells Inhibition of skin tumors Apoptosis in human leukemia cells	(Gheller et al. 2017)

In patients with fatty liver (18-65 years) it has been seen that after the administration of two capsules of roselle extract (1 g) after meals, three times a day, the level of free fatty acids in serum is significantly reduced, regulating hepatic steatosis. Thus, the effect of the capsule is dose-dependent on triglyceride levels, concentrations of fatty acids, and cholesterol of plasma lipids and hepatic lipids (Huang et al. 2015, Yang et al. 2010). Type 2 diabetic patients showing hyperlipidemias demonstrated a significant improvement in lipid profile, with reduction of total cholesterol, LDL-c, TGs and APO-B100, and a substantial increase in HDL-c by administering an infusion of hibiscus (2 g) twice a day for one month (Sudan et al. 2014). The aqueous extract of hibiscus decreases the differentiation of preadipocytes, reduces the expression of leptin during adipocyte differentiation. Mechanisms involved in the regulation of adipogenesis could include inhibition, through the PI3/Akt and ERK pathways, in the expression of transcription factors such as PPAR-γ and SREBP-1c (Huang et al. 2015, Villalpando-Arteaga et al. 2013).

Recent studies have demonstrated the potent inhibitory activity of pancreatic lipase (IC50: 5.8 ± 0.8 µg/mL) by hibiscus methanolic extract. Some of these activities may be due to the synergistic action of different phenolic compounds (Ado et al. 2013, Ojulari, Lee, and Nam 2019). Thus, hibiscus calyxes and hibiscus infusion can be used for people with metabolic syndrome, improving glucose and total cholesterol levels, and increasing HDL-c levels (Carvajal-Zarrabal et al. 2009, Huang et al. 2015).

Also, multiple clinical studies have shown that calyx extracts show significantly positive effects on systolic and diastolic pressure (Hopkins et al. 2013, McKay et al. 2010). Calyx extracts are generally used in concentrations ranging from 1.25 to 10 g favoring patients with mild to moderate hypertension or in stages I or II (Hopkins et al. 2013). Studies in hypertensive rats have demonstrated a vasodilator effect with methanolic extracts of calyxes, due to a release of nitric oxide-mediated by cGMP and therefore, would be a relaxation endothelium-dependent or a blockage of calcium channels (Ajay et al. 2007, Zheoat et al. 2019). On the other hand, hibiscus extracts have also revealed an effect on the three components of the renin-angiotensin-aldosterone system in hypertensive people (Sarr et al. 2009, Zheoat et al. 2019). A clinical study evaluated the effect of hibiscus on blood pressure and electrolyte profile in 80 Nigerian patients

with mild to moderate hypertension and compared the results with those obtained with the administration of hydrochlorothiazide (HCTZ), a diuretic used in the treatment of hypertension. The decrease in both systolic and diastolic pressure was higher in the hibiscus group (Nwachukwu et al. 2015).

CONCLUSION

The presence and distribution of phenolic compounds present in *Hibiscus sabdariffa* L. promote beneficial health effects with significant antioxidant activity. Anthocyanins are the main metabolites of the calyxes of *Hibiscus*, constituting useful compounds in the quality control of this species. Delphinidin-3-sambubioside and cyanidin-3-sambubioside are present in all *Hibiscus* species. It is suggested to develop food supplements with *Hibiscus* calyxes or their extracts and to analyze their contribution to the prevention and/or treatment of chronic degenerative diseases.

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Chapter 8

SALVIA AND ITS POTENTIAL TO TREAT METABOLIC DISORDERS

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ABSTRACT

The *Salvia* genus is widely distributed throughout the world, found in temperate, subtropical, and tropical regions. *Salvia* species have been traditionally used in folk medicine since ancient times due to their medicinal properties against some ailments. Currently, there are thousands of bioactive compounds derived from different sage species, such as phenolics, flavonoids, and terpenoids, which have been isolated and studied to be used as potential biopharmaceutical agents against different noncommunicable diseases. Furthermore, *Salvia* species are of commercial interest in the pharmaceutical and food industries due to their antioxidant capacity. This chapter provides relevant information on the *Salvia* genus and some of the species that make up this genus as well as its compounds and its effect related to metabolic dysregulation, including metabolic syndrome, cancer, and neurogenerative diseases.

Keywords: *Salvia*, polyphenols, terpenes, flavonoids, phenolic compounds, bioactive compounds, noncommunicable diseases, antioxidants

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INTRODUCTION

Around the world, in different cultures, and since ancient times plants have been used as healing remedies. Civilizations such as Indus, Arabian, Chinese, Japanese, Tibetan, African, and Mesoamerican provide evidence on the use of different plants as a treatment in various ailments (Venkateshappa & Sreenath, 2013). Currently, 80% of the world population, more than four billion people, uses plants as the main medicinal remedy (R. Yadav & Agarwala, 2011). There is a wide diversity of plants that have been used as herbal remedies; within them, it is the family of Lamiaceae. Plants of this family are highly aromatic, medicinal and rich in secondary metabolites such as polyphenolic compounds (caffeic and its derivates, flavonoids like luteolin, apigenin, hispidulin, kaempferol, and quercetin) and terpenoids (diterpenes and triterpenes mainly) that give high antioxidant capacity (Lopresti, 2017; Rehan, Tahira, Rehan, Bibi, & Naeemullah, 2014). Within this family we can find aromatic plants like basil, mint, rosemary, marjoram, thyme, lavandula, othosiphon, ocimum, leuccas, anisomeles, coleus, hyptis, oreganum, brunella, scutellaria, lamium, teucrium and perilla and sage, the latter belongs to the genus *Salvia* the largest of this family (Venkateshappa & Sreenath, 2013). Genus *Salvia* is distributed throughout the world comprising over 1000 species, and the common species are *Salvia officinalis*, *Salvia miltiorrhiza*, *Salvia lavandulaefolia*, *S. fruticose*, *Salvia sclarea* and *Salvia hispanica* (Lopresti, 2017), they have used as antibacterial, antioxidant, anti-cancer, and against a wide range of diseases like nervous system diseases, cardiovascular, respiratory system, digestive system and metabolic and endocrine diseases (Hamidpour, Hamidpour, Hamidpour, & Shahlari, 2014).

Taxonomy

Lamiaceae is a diverse family consisting of about 252 genera and 7173 species widely distributed throughout the world, belongs to the order Lamiales and is related to the families Verbenaceae, Acanthaceae, Scrophulariaceae, Gesneriaceae, Lentibulariaceae, Buddlejaceae, and Bignoniaceae (Harley et al., 2004; Savolainen et al., 2000). Among the genera of the family Lamiaceae we find the genus *Salvia* (sage) of the subfamily Nepetoideae and the Mentheae tribe, with more than 1000 species, grouped into five subgenera: *Salvia*, *Leonia*, *Sclarea*, *Jungia* and *Audibertia*, the most common are *Salvia officinalis* (common sage), *Salvia miltiorrhiza* (Chinese sage or Danshen), *Salvia lavandulaefolia* (Spanish sage), *Salvia fruticose* (Greek sage), *Salvia sclarea* (clary sage) and *Salvia hispanica* (chia) (Ličina et al., 2013; Lopresti, 2017; Škrovánková, Mišurcová, & Machů, 2012; Walker & Sytsma, 2007; Wu et al., 2012). The plants of the family to which sage belongs, are considered herbs or shrubs, their stems are quadrangular, have opposite leaves, terminal or lateral inflorescences, racey appearance. Their flowers have a regular five-part calyx, bilabiate corolla; the ovary is divided into 4 loci, the stamens are two, its fruit consists of four free nuts (tetranucula) (Cornejo-Tenorio & Ibarra-Manríquez, 2011; Fernández-Alonso & Rivera-Díaz, 2006; Fernández-Alonso, Vega, Filgueira, & Pérez, 2003).

Origin

Genus *Salvia* is a herbaceous, suffruticose, or shrubby perennial plants; its name is derived from the Latin word "salvare" meaning "health," in French means sauge (sage) and sawge in Old English, which refers to the medicinal effects (G. Kamatou, Makunga, Ramogola, & Viljoen, 2008; Llurba-Montesino & Schmidt, 2018). *Salvia* has been used for thousands of years as a folk medicine in different regions of the world, as in ancient Egypt, and by Romans, Greeks, Anglo-Saxons, Mesoamerican people, who believed that it is magic (Ličina et al., 2013; Sharifi-Rad et al., 2018). Despite being located in different parts of the world, some species are endemic to specific regions (Sharifi-Rad et al., 2018), such as *Salvia fruticosa* Mill., which is endemic to the Eastern Mediterranean basin (Karousou, Koureas, & Kokkini, 2005) Iran reported 17 endemic species of 61 (Jamzad, 2013), in Mexico almost the 88% of 312 species are endemic (Cornejo-Tenorio & Ibarra-Manríquez, 2011) such as Chia (*Salvia Hispánica*) (Xingú López et al., 2017), in Turkey 53 of 100 species are endemic (Celep, Dirmenci, & Güner, 2015). Likewise, *Salvia officinalis* is native of southern Europe and Asia (Y. Jiang, Zhang, & Rupasinghe, 2017).

Production

Sage production is distributed in several regions of the world from China to Europe and from Mexico to South Africa (Table 1) (M. B. Bahadori, Dinparast, et al., 2017), from temperate zones to warm zones, mainly in Central and South America (500 spp.) Central Asia/Mediterranean (250 spp.) and Eastern Asia (100 spp.) (Wu et al., 2012, Li et al., 2013). Despite not being located in the North or tropical areas such as Amazona and Central and West Africa, in Mexico, there are a large number of species, around 300 (G. Kamatou et al., 2008; Wu et al., 2012). Depending on the species, they grow at different times of the year, *Salvia palaestina* flowering from April to July (Al-Jaber, Abrouni, Al-Qudah, & Abu Zarga, 2012), *Salvia officinalis* in May-July, and its fruits mature in August, its yield increases with the age of the plant (maximum 5 years) of the plant (Vázquez, 2009) *Salvia hispanica* in May-June to avoid frost, this species in the year 2014 had a production of 367,000 hectares in all the word (Peperkamp, 2015), *Salvia santolinifolia* and *Salvia nemarosa* are collected in April, *Salvia mirzayanii* in March both in Iran (M. B. Bahadori, Asghari, et al., 2017; M. B. Bahadori, Valizadeh, & Farimani, 2016; Ebrahimi et al., 2014). In different Turkey regions different endemic species were collected (*S. adenocaulon, S. aurechi, S. ciclica, S. blepharochlaena, S. absconditiflora, S.divaricata, S. euphratica, S. huberi, S.hypargeia, S. rosifolia, S. sclarea*) in the months June, July and August (Gezek et al., 2019), likewise *S. officinalis, S. verbenaca, S. aegyptiaca* and *S. argentea* were collected at flowering period (March, April, May, June and July) in Tunisia (Farhat, Landoulsi, Chaouch-Hamada, Sotomayor, & Jordán, 2013).

Table 1. Sage species from different regions around the world

Region	*Salvia* species	Reference
Serbia	*Salvia amplexicaulis*	(Mihailo, Velickovic, Andrija, Dragan, & Novica, 2003)
Iran	*Salvia hydrangea*	(M Moridi Farimani et al., 2011; Mahdi Moridi Farimani et al., 2013)
	Salvia hypolueca	(Saeidnia, Ghamarinia, Gohari, & Shakeri, 2012)
	Salvia tebesana	(Eghbaliferiz et al., 2018)
	Salvia spinose	(M. B. Bahadori et al., 2015)
Turkey	*Salvia fruticosa*	(Perfumi, Arnold, & Tacconi, 1991)
	Salvia modesta	(Zengin, Atasagun, et al., 2019)
	Salvia palestina	(Al-Qudah, Al-Jaber, Zarga, & Orabi, 2014)
Egypt	*Salvia triloba*	(Ahmed, Salem, Sabry, Husein, & Kotob, 2013)
	S. multicaulis	(González-Tejero et al., 2008)
Andes	*S. sarmentosa*	(Jenks & Kim, 2013)
Peru	*S. revoluta*	(Jenks & Kim, 2013)
Ecuador	*S. pichincensis*	(Jenks & Kim, 2013)
Spain	*S. microphylla*	(González-Tejero et al., 2008)
Jordan	*Salvia palaestina*	(Al-Qudah et al., 2014)
China	*Salvia yunnanensis*	(G. Xu et al., 2006)
	Salvia przewalskii	(G. Xu et al., 2009)
	Salvia miltiorrhiza	(Guo et al., 2014)
	Salvia plebeia	(Zou et al., 2018)
Eastern Asia	*Salvia miltiorrhiza*	(Nan et al., 2001; Topçu, 2006)
Japan	*Salvia miltiorrhiza*	(Topçu, 2006)
Lebanon	*S. indica*	(Baydoun, Chalak, Dalleh, & Arnold, 2015)
Pakistan	*S. moorcroftiana*	(Saeed Khattak, Nouroz, Ur Rahman, & Noreen, 2015)
Italy	*S. argentea*	(Riccobono et al., 2015)
Germany	*Salvia nemarosa*	(Skała & Wysokińska, 2004)
Mexico	*Salvia hispanica*	(Sharifi-Rad et al., 2018)
	Salvia ibugana	(Valdivia-López & Tecante, 2015)
	Salvia ramirezzi	(Gutiérrez-Grijalva et al., 2018)
	Salvia carreyesii	(Argumedo Delira, Parra-Delgado, Ramírez Apan, Nieto Camacho, & Martínez-Vázquez, 2003)
	Salvia mexicana	(Cornejo-Tenorio & Ibarra-Manríquez, 2011)
Africa	*Salvia reflexa*	(G. P. Kamatou, Viljoen, & Steenkamp, 2010)
	Salvia sclarea	(Van Wyk, 2011)
	Salvia coccinea	(G. P. Kamatou et al., 2010)
	Salvia runcinata	(G. P. P. Kamatou et al., 2005)
	Salvia africana-careulea	
	Salvia repens	
	Salvia stenophylla	
	Salvia thermara	
	Salvia officinalis	
Larkana (Cyprus)	*Salvia lanigera*	(Tenore et al., 2011)
Canary Islands	*Salvia verbenacea*	(Farhat, Sotomayor, & Jordán, 2019)
	Salvia canariensis	(Karousou et al., 2005)

PHYTOCHEMICALS IN SALVIA SPECIES

Phenolic Compounds

Phenolic compounds are a widely diverse and widespread group of metabolites derived from the secondary metabolism in plants. Plant polyphenols have a basic structure of one or more hydroxyl groups (-OH) attached to a benzene ring (Figure 1) (Kutchan, Gershenzon,

Møller, & Gang, 2015; Vermerris & Nicholson, 2006). The presence of the hydrogen in the hydroxyl group makes phenolic compounds weak labile acids. Phenolic compounds are derived from the shikimate phenylpropanoid and/or the polyketide pathways, with one or more phenolic rings and lacking nitrogen functional groups (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011). Once they are biosynthesized, phenolic compounds are accumulated mainly in the vacuoles of plant cells and are usually present as esters or glycosides (Vermerris & Nicholson, 2006).

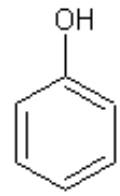

Figure 1. Graphical representation of a phenol conformed of an aromatic ring with a hydroxyl group (-OH) attached.

Phenolic compounds can be classified according to their structural characteristics in flavonoids and phenolic acids:

- Flavonoids are characterized by the presence of three rings (C6-C3-C6) designated as A, B, and C; and depending on the distribution of their -OH radicals, glycosylation, prenylation, and alkalization. Flavonoids can be sub-classified depending on their structural characteristics into flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones, etc. (Gutiérrez-Grijalva et al., 2018).
- On the other hand, phenolic acids can be sub-classified as derivatives of hydroxycinnamic or hydroxybenzoic acid (Gutiérrez-Grijalva et al., 2018). Some commonly hydroxybenzoic acid derivates found in *Salvia* species are vanillic acid, syringic acid, gallic acid, protocatechuic acid, and hydroxybenzoic acid. Furthermore, some hydroxycinnamic acid derivates found in Salvia species are caffeic acid, rosmarinic acid, chlorogenic acid, and coumaric acid.

As previously stated, the genera *Salvia* is one of the most widely studied herbs from the Lamiaceae family and comprises around 1000 species distributed in various regions around the world. As many herbs from the Lamiaceae family, *Salvia* species are rich in phytochemicals of nutraceutical interest such as terpenes and phenolic compounds (Wu et al., 2012). The most commonly identified phenolic compounds in *Salvia* plants belong to the sub-groups flavones, flavonols, hydroxycinnamic acids, hydroxybenzoic acids, phenolic terpenes, and depsides. However, the most abundant are hydroxycinnamic acids and depsides, such as rosmarinic acids and salvianolic acids, respectively (Zengin, Mahomoodally, et al., 2019).

The polyphenolic content in *Salvia* plants varies depending on several factors such as species, geographical localization, weather, daylight, harvesting time, among others (Gutiérrez-Grijalva et al., 2018). Moreover, these compounds are distributed in all organs from the *Salvia* plants, for instance, flavonoids, triterpenoids, and monoterpenes are mostly distributed in the aerial parts of the plants (flowers and leaves), while phenolic acids and diterpenoids are mainly found in the roots (Jianping Xu et al., 2018).

Table 2. Phenolic compounds found in *Salvia* species

***Salvia* spp.**	**Plant organ**	**Extraction Method**	**Analysis Method**	**Constituents**	**Reference**
Salvia viridis L.	Roots	Supercritical fluid extraction	HPLC-MS/MS	Vanillin, ethyl syringate, syringaldehyde, antiarol, indole-4-carbaldehyde, coumarin, coniferyl aldehyde, dimethoxy-trihydroxy(iso)flavone, dihydroxy-dimethoxy(iso)flavone, genkwanin, viroxocin, apigenin-4',7-dimethyl ether	(Zengin et al., 2018)
Salvia viridis L.	Roots	Methanol	HPLC-MS/MS	Quinic acid, gallic acid, caffeoylglucose isomer, kynurenic acid, chlorogenic acid, coumaroylhexose isomer, vanillin, feruloylhexose isomer, ethyl syringate, coumaroylquinic acid, syringaldehyde, 4-coumaric acid, antiarol, caffeoylshikimic acid, luteolin-C-hexoside-C-pentoside isomer, dimethoxy-hydroxybenzoic acid, ferulic acid, coniferyl aldehyde, coumaroylshikimate, sinapyl aldehyde, myricetin-O-hexoside, apigenin-C-hexoside-O-pentoside, verbascoside, Luteolin-O-(pentosyl)hexoside, luteolin-O-glucuronide, luteolin-7-O-glucoside, luteolin-O-(deoxyhexosyl)hexoside, isoquercitrin, rosmarinic acid-O-hexoside, salvianolic acid isomer, lipedoside A isomer, leucosceptoside, cosmosiin, apigenin-O-glucuronide, methoxy-trihydroxyflavone-O-glucuronide, methyl caffeate, rosmarinic acid, ethyl caffeate, 3-O-methylrosmarinic acid	(Zengin, Atasagun, et al., 2019)
Salvia viridis L.	Roots	80% Methanol	UPLC-PDA-ESI-MS	Caffeic acid, prolithospermic acid, salvianolic acid J, rosmarinic acid hexoside, salvianolic acid E, rosmarinic acid, methyl rosmarinate, salvianolic acid F	(Grzegorczyk-Karolak & Kiss, 2018)
Salvia viridis L.	Shoots	80% Ethanol	UPLC-DAD-ESI-MS	Phenylethanoids, verbascoside, forsythoside A, isoverbascoside, lipedoside A, mertynoside, isomartynoside, 6-O-caffeoylglucose, 5-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, caffeoyl-hexoside derivative, dicaffeoylquinic acid, rosmarinic acid, luteolin-O-rutinoside, apigenin-O-hexuronide, methylluteolin-O-hexuronide, luteolin-O-dihexoside	(Grzegorczyk-Karolak & Kiss, 2018)
Salvia miltiorrhiza	Roots	EtOAc, n-BuOH, MeOH	HPLC	Salvianolic acid Y, 9'-methyl-salvianolic acid B, 9'''-methyl-salvianolic acid B, 8'''-epi-9'-methyl-salvianolic acid B	(Jin et al., 2018)
Salvia plebeian R. Br.	Aerial parts	Methanol: water: formic acid (50:45:5)	UPLC-DAD-qTOF/MS	Apigenin, 5,6,7,4'-tetrahydroxyflavone, luteolin, luteolin 5-O-glucoside, luteolin-7-O-glucoside, 6-hydroxhyluteolin, 6-hydroxyluteolin 7-O-glucoside, nepetin, nepetin 7-O-glucoside, cirsimaritin, jaceosidin, eupatilin, eupatorine, neocafhispidulin, scutellarein, sorbifolin, pectolinarigenin, hispidulin, hispidulin 7-O-glucoside, hispidulin 7-O-glucuronide, eriodictyol, 6-methoxynaringenin, 6-methoxynaringenin 7-O-glucoside, naasalvinin A, filifolin, naasanone, quercetin, isorhamnetin, 2'-hydroxy-5'-methoxybiochanin A	(S.-H. Lee et al., 2018)

***Salvia* spp.**	**Plant organ**	**Extraction Method**	**Analysis Method**	**Constituents**	**Reference**
Salvia splendens	Leaves		UV, 1D and 2D NMR and negative ESI-MS spectroscopy	Caffeic acid, rosmarinic acid, methyl rosmarinate, luteolin 7-O-(4’’,6’’-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside, apigenin 7-O-β-D-rutinoside, cosmosiin, cinaroside, luteolin, apigenin, pedalitin, crisiliol, 6,7-dihydroxycoumarin	(Moharram, Marzouk, El-Shenawy, Gaara, & El Kady, 2012)
Salvia eriophora Boiss. & Kotschy	Leaves	Methanol	LC-MS/MS	Quercitrin, gallic acid, epigallocatechin, epicatechin, cyanidin-3-O-glucoside, cyanidin chloride, catechin, apigenin, caffeic acid, ellagic acid, kaempferol, salvigenin, fumaric acid, pyrogallol, t-ferulic acid, luteolin, isorhamnetin, quercatagetin-3,6-di-methylether, rosmarinic acid, luteolin-7-O-glucoside, luteolin-5-O-glucoside, kaempferol-3-O-rutinoside, rutin, curcumin	(Bursal et al., 2019)
Salvia officinalis L.	Leaves	30% Ethanol	HPLC UV-PDA	Vanillic acid, caffeic acid, syringic acid, rosmarinic acid, salvianolic acid K, salvianolic acid I, methyl rosmarinate, 6-hydroxyluteolin-7-glucoside, luteolin-7-glucoside, luteolin-3-glucuronide, apigenin-7-glucuronide, apigenin-7-glucoside	(Dent, Kovacevic, Bosiljkov, & Dragovic-Uzelac, 2017)
Salvia amplexicaulis	Stems, leaves, inflorescences	Dichloromethane	HPLC UV-DAD	Genkwanin 5-O-(6’’-O-malonylglucoside), hyperoside, coumarin	(Alimpić et al., 2017)
		Ethyl acetate		Apigenin, luteolin, genkwanin 4’-O-glucoside, kaempferol 3-O-(6’’-O-malonylglucoside)-7-O-glucoside, kaempferol 3-O-(6’’-O-acetilglucoside)-7-O-rhamnoside, hyperoside, coumarin	
		Methanol		Caffeic acid, rosmarinic acid, apigenin, apigenin 5-O-glucoside, apigenin 4’-O-glucoside, luteolin 5-O-glucoside, genkwanin 5-O-glucoside, genkwanin 5-O-(6’’-O-malonylglucoside), genkwanin 4’-O-glucoside, kaempferol 3-O-(6’’-O-malonylglucoside)-7-O-glucoside, kaempferol 3-O-(6’’-O-acetilglucoside)-7-O-rhamnoside, kaempferol 3-O-glucoside-7-O-rhamnoside, kaempferol 7-O-rhamnoside, rutin	
		Ethanol		Caffeic acid, luteolin 5-O-glucoside, genkwanin 5-O-glucoside, kaempferol 3-O-(6’’-O-acetilglucoside)-7-O-rhamnoside, rutin, hyperoside	
		Water		Caffeic acid, rosmarinic acid, apigenin 4’-O-glucoside, luteolin 5-O-glucoside, kaempferol 3-O-(6’’-O-acetilglucoside)-7-O-rhamnoside, kaempferol 3-O-(6’’-O-malonylglucoside)-7-O-glucoside, kaempferol 7-O-rhamnoside	
Salvia amplexicaulis Lam.	Dried plants	Ethanol	UPLC-MS/MS	Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid	(Šulniūtė, Pukalskas, & Venskutonis, 2017)
		Water		Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosic acid	
Salvia austriaca L.	Dried plants	Ethanol	UPLC-MS/MS	Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid	
		Water		Caffeic acid, apigenin-7-O-β-D-glucuronide	
Salvia forsskaolii L.	Dried plants	Ethanol	UPLC-MS/MS	Caffeic acid, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, rosmarinic acid	

Table 2. (Continued)

***Salvia* spp.**	**Plant organ**	**Extraction Method**	**Analysis Method**	**Constituents**	**Reference**
		Water		Caffeic acid, quercetin 3-glucuronide, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide	
Salvia glutinosa L.	Dried plants	Ethanol	UPLC-MS/MS	Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid	
		Water		Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid	
Salvia nemorosa L.	Dried plants	Ethanol	UPLC-MS/MS	Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid	
		Water		Caffeic acid, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, carnosic acid	
Salvia officinalis L.	Dried plants	Supercritical CO_2	UPLC-MS/MS	Carnosol, carnosic acid, methyl carnosate	(Šulniūtė et al., 2017)
		Ethanol		Caffeic acid, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosol, carnosic acid, methyl carnosate	
		Water		Caffeic acid, quercetin-3-glucuronide, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosic acid	
Salvia officinalis	Leaves	80% Methanol	UPLC-ESI-MS	Syringic acid, rosmarinic acid, hesperidin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, quercetin-3-glucoside, quercetin-3-galactoside, rutin, carnosol	(Sarrou et al., 2017)
Salvia pratensis L.		Ethanol		Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosic acid	(Šulniūtė et al., 2017)
		Water		Caffeic acid, apigenin-7-O-β-D-glucuronide	
Salvia officinalis L.	Dried plants	Supercritical CO_2	UPLC-MS/MS	Carnosol, carnosic acid, methyl carnosate	(Šulniūtė et al., 2017)
		Ethanol		Caffeic acid, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosol, carnosic acid, methyl carnosate	
		Water		Caffeic acid, quercetin-3-glucuronide, luteolin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosic acid	
Salvia officinalis	Leaves	80% Methanol	UPLC-ESI-MS	Syringic acid, rosmarinic acid, hesperidin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, quercetin-3-glucoside, quercetin-3-galactoside, rutin, carnosol	(Sarrou et al., 2017)
Salvia pratensis L.		Ethanol		Caffeic acid, apigenin-7-O-β-D-glucuronide, rosmarinic acid, carnosic acid	(Šulniūtė et al., 2017)
		Water		Caffeic acid, apigenin-7-O-β-D-glucuronide	

***Salvia* spp.**	**Plant organ**	**Extraction Method**	**Analysis Method**	**Constituents**	**Reference**
Salvia officinalis L.	Leaves	Water (infusion), Water (decoction), 80% Methanol	HPLC-DAD	6-O-caffeoyl-fructosyl-glucoside, caffeic acid hexoside, apigenin O-pentoside, caffeic acid, salvianolic acid I, luteolin diglucuronide, 6-hydroxyluteolin 7-O-glucuronide, sagecoumarin, rosmarinic acid hexoside, luteolin 7-O-rutinoside, luteolin 7-O-glucuronide, luteolin 7-O-glucoside, sagerinic acid, salvianolic acid B, cis rosmarinic acid, trans rosmarinic acid, apigenin 7-O-glucoside, luteolin acetylglucoside, hispidulin glucuronide, apigenin acetylglucoside, hispidulin	(Martins et al., 2015)
Salvia officinalis	Leaves	Subsequent extraction with hexane and ethyl acetate	LC-MS^n	Apigenin-7-O-glucoside, homoplantaginin, rosmarinic acid, apigenin acetylglucoside, isorhamnetin-luteolin, apigenin, hispidulin, rosmanol, epirosmanol, methoxycarnosol, carnosol, rosmadial, carnosic acid, methyl carnosate	(Kontogianni et al., 2013)

A summarization of phenolic compounds constituents found in *Salvia* is presented in Table 2.

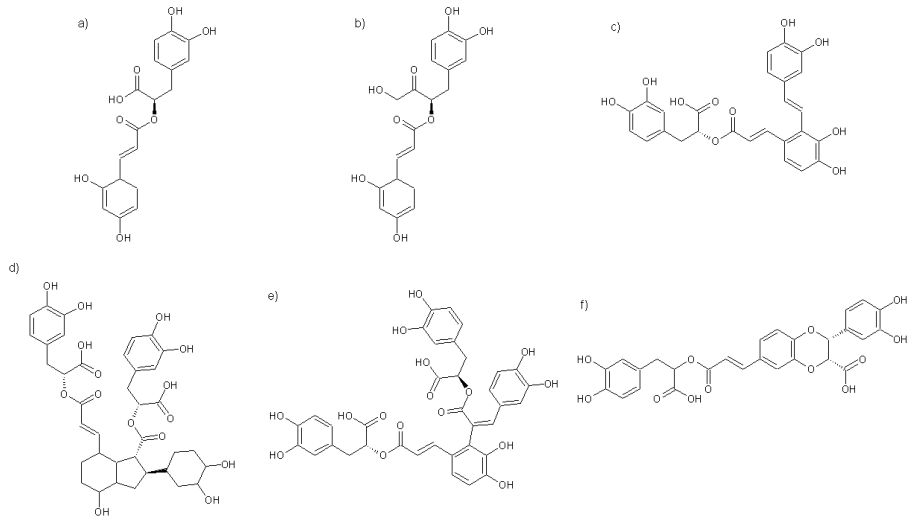

Figure 2. Common phenolic compounds found in Salvia species: a) rosmarinic acid, b) methyl rosmarinate, c) salvianolic acid A, d) salvianolic acid B, e) salvianolic acid E, f) salvianolic acid J. From the PubChem compound database (National Center for Biotechnology Information). Structures were made using ACD Labs Freeware.

Terpenes

Within the broad group of secondary metabolites, we find the terpenoids, which are the most numerous and structurally diverse also named as isoprenoids because they are formed from isoprene units (basic units of 5 carbon atoms) linked in chain (González-López, Quiñones-Aguilar, & Rincón-Enríquez, 2016; Ludwiczuk, Skalicka-Woźniak, & Georgiev, 2017). The term "terpene" is currently used to represent terpenoids (N. Yadav, Yadav, & Goyal, 2014). They are derivatives of isopentenyl diphosphate or its double bond isomer dimethylallyl pyrophosphate which are produced by two metabolic routes: the mevalonate (MVA) pathway, or from 2-C-methyl-D-erythriol 4-phosphate (MEP) pathway, and classified based on the number and structural organization of carbons presents in the structure: hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids (carotenoids) and polyterpenoids (Boronat & Rodríguez-Concepción, 2014; Kitaoka, Lu, Yang, & Peters, 2015; Ludwiczuk et al., 2017). Hemiterpenoids have a simple structure. Monoterpenes have a structure of two isoprene units (divided into three groups acyclic, monocyclic and bicyclic). Diterpenoids are based on four isoprene units, classified as linear, bi, tri, tetra, penta, or macrocyclic diterpene depending on their skeletal core; likewise, sesquiterpenoids have five isoprene units and consist of 25 carbon structure (are a relatively small group of terpenoids). Triterpenoids have a carbon skeleton based on six isoprene units (known to be a widespread distribution), and the tetraterpenoids have a structure of eight isoprene units, the most common are the carotenoids (Ludwiczuk et al., 2017).

These compounds are responsible for the specific aromas and flavors, in addition to carrying out various biological functions in plants, on the other hand, they are part of the life of the human being because they are used as flavoring, dyes, aromatics, antitumor, antioxidants, antibiotics, insecticides among other things (González-López et al., 2016; Schrader & Bohlmann, 2015). This group of compounds is found in a great variety of plants, among them the genus *Salvia* from which diterpenoids (more than 600), triterpenoids and monoterpenes have been isolated in almost all the species studied, particularly in roots, flowers, and leaves, (Jianping et al., 2018; Topçu, 2006). Although diterpenes and monoterpenes are the most studied constituents, pentacyclic triterpenes (ursolic and oleanolic acid mainly) are the highest in most species of *Salvia* (Topçu, 2006).

In the Chinese species of *Salvia*, abietane diterpenes (tanshinones, roylenones, and derivatives) are the most abundant, which are attributed to antioxidant and antimicrobial activity, on the other hand, icetexane diterpenes are reported in American *Salvia* species, in the species *Salvia officinalis*, Norabietane diterpenoids were isolated and identified, which demonstrated their anti-inflammatory effect (Jianping et al., 2018; L. Li et al., 2019). In a study conducted in the species, *Salvia nemorosa* L. found spathulenol in leaves and caryophyllene oxide in oxygenated flowers as sesquiterpenes majority (M. B. Bahadori, Dinparast, et al., 2017). Different species produced in Iran were analyzed by GC-MS, identifying a total of 58 compounds where the main compounds characterized were α-pinene, camphene, β-pinene, camphor, β-caryophyllene, germacrene D, bicyclogermacrene, caryophyllene oxide, and benzyl benzoate. In *Salvia atropatana* Bunge, *Salvia grossheimii* Sosn. And *Salvia verticillata* L., the major constituent was germacrene D, a sesquiterpene hydrocarbon, in *Salvia bracteata* Banks & Sol. it was β-caryophyllene a sesquiterpene hydrocarbon, in *Salvia dracocephaloides* Boiss. camphor an oxygenated monoterpene, *Salvia eremophila* Boiss, and *Salvia sahendica* Boiss & Buhse contained mostly α-pinene to monoterpene hydrocarbon, while in *Salvia hydrangea* DC. Ex Benth and *Salvia lahnocalyx* Hedge was caryophyllene oxide, an oxygenated sesquiterpene (Asadollahi, Firuzi, Jamebozorgi, Alizadeh, & Jassbi, 2018). In the species *Salvia herbacea*, its chemical composition was evaluated by means of NMR (nuclear magnetic resonance) in the aerial parts of the plant, finding diterpenes such as tehuanins, dehydrosalviarin, and 1α, 10α-epoxysalviarin (Bautista et al., 2013).

As we can see within a large number of species within the *Salvia* genus, there is a diversity of terpenoid compounds (Table 3), which can be present in the different parts of the plant and in different proportions depending on several factors such as production, species, part of the plant and very likely environmental conditions of growth.

STUDY CASES OF *SALVIA* BIOLOGICAL PROPERTIES

Salvia genus has been used since ancient times by different cultures around the world, due mainly to their biological and medicinal properties. In function to the species and the region of origin is the use that has been given in traditional medicine to this genus to treat various ailments (fever, carminative, spasmolytic, antiseptic/bactericidal, astringent, mouthwash, inflammations, hypoglycemic, antihyperlipidemic, care skin and hair, rheumatism, sexual debility, treating mental and nervous conditions) (Asadollahi et al., 2018; Kintzios, 2003). Currently, scientific research is developed from the constituents of *Salvia* species to evaluate their different medicinal properties (Asadollahi et al., 2018).

Table 3. Terpenoids found in some *Salvia* species

***Salvia* species**	**Part**	**Main group of terpenoid**	**Isolated compounds**	**Reference**
S. officinalis		Monoterpene Triterpene	Carvacrol, thujone, geraniol, camphor, 1-8,cineole, borneol, α- and β-pinene, ursolic and oleanolic acid	(Ludwiczuk et al., 2017)
S. miltiorrhiza	Rhizome	Diterpene	Miltirone, methylenetanshinquinone, tanshinone I, tanshinone IIa, methyl-tanshinonate, 11-hydroxymiltiodiol, cryptotanshinone, sibiriquinone A-B, dihydroisotanshinone	(Aoyagi et al., 2008; Dat et al., 2007)
S. sahendica			12-deoxy-salvipisone, sahandinone, 12-deoxy-6,7-dehydroroyleanone, 7 β-acetoxyroyleanone, Δ 9-ferruginol, sahandol, sahandone	(Ebrahimabadi, Mazoochi, Kashi, Djafari-Bidgoli, & Batooli, 2010)
S. shannoni	Leaves	Diterpene	Sepulturins A-F, infuscatin, 8-hydroxysalviarin, splenolide A, tehuanin G.	(Bautista et al., 2013)
S. tebesana	Roots	Diterpene	Tebesinone A, tebesinone B, aegyptinone A, aegyptinone B	(Eghbaliferiz et al., 2018)
S. amplexicaulis		Sesquiterpene hydrocarbon, oxygenated monoterpene	Germacrene D, viridiflorol, caryophylene oxide, β-caryoplyllene	(Mihailo et al., 2003)
S. austriaca	Roots	Diterpene	Taxodione, 15-deoxy-fuerstione, 7-(2'-oxohexyl)-taxodione, taxodone	(Kuźma, Kaiser, & Wysokińska, 2017)
S. verbenaca L	Aerial parts	Monoterpene Sesquiterpenes hydrocarbons	Viridiflorol, β-caryophyllene, α-pinene, p-cymene	(Farhat et al., 2019)
S. judaica	Leaves	Sesquiterpene	β-cubebene, ledol	(Boszormenyi et al., 2009)
S. hispanica	Aerial parts	Diterpene	Salvihispin A	(Fan et al., 2018)
S. *tomentosa*	Aerial parts	Monoterpene Diterpene	α-pinene, 1,8-cineole, cis-thujone and borneol	(Hanlidou, Karousou, & Lazari, 2014)
S. spinosa	Aerial parts	Oxygenated Sesquiterpenes	Caryophyllen oxide, spathulenol	(M. Bahadori & Mirzaei, 2015)
S. palaestina	Whole plant	Triterpene	Uresane	(Al-Jaber et al., 2012)
S. nemarosa	Aerial parts	Oxygenated Sesquiterpenes	Caryophyllen oxide, spathulenol, leden oxide	(M. B. Bahadori, Dinparast, et al., 2017)
S. namaensis	Whole plant	Diterpene	Carnosol, carnosic acid	(G. P. Kamatou et al., 2010)
S. hypoleuca	Roots	Diterpene Triterpene	Manool, 7α-acetoxyroyleanone, ursolic acid, oleanic acid, 3-cpicorosolic acid, 3-epimaslinic acid, coleonolic acid.	(Saeidnia et al., 2012)
S. forskahlei	Roots	Diterpene	Forskalinone	(Baricevic & Bartol, 2003)
S. plebeia	Whole plant	Diterpene	Plebedipene A, 2-O-deacetylplebedipene A, plebedipene B	(Jing Xu et al., 2016)
S. leucantha	Leaves	Sesquiterpene	spathulenol, β-caryophyllene, α-himachalene, and γ-cadinene	(Upadhyaya, Dixit, Padalia, & Mathela, 2009)
S. chinensis	Aerial parts	Triterpene Sesquiterpene	Blumenol A, clovane-2β, 9α-diol, pinfaenoic acid	(Y. Wang et al., 2008; Y. Wang et al., 2009)
S. prionitis	Roots	Diterpene	Dihydrotanshinone I	(M.-H. LI, PENG, & XIAO, 2010)
S. herbacea	Aerial parts	Diterpene	Tehuanins A-H, 1α,10α-epoxysalviarin, 1α,10α-epoxysalviarin-dehydrosalviarin	(Bautista, Maldonado, & Ortega, 2012)
S. hydrangea *S. urmiensis*	Aerial parts	Triterpene	Salvadione C, perovskone Urmiensolide, urmiensic acid	(M Moridi Farimani et al., 2011; Mahdi Moridi Farimani et al., 2013)

Salvia and Diabetes

Type-2 diabetes mellitus, commonly known as diabetes, is a metabolic disorder characterized by hyperglycemia and insulin resistance. Diabetes arises from carbohydrate metabolic disorders caused by factors such as deficient insulin release/action, causing hyperglycemia. Untreated hyperglycemia can cause numerous comorbidities such as cardiovascular diseases, neuropathy, nephropathy, retinopathy, and blindness (DeFronzo, Ferrannini, Zimmet, & Alberti, 2015). The International Diabetes Federation reports that by 2017, there were around 425 million people worldwide living with diagnosed diabetes, and this number is expected to increase by 48% by 2045 (International Diabetes Federation, 2017). Diabetes management includes lifestyle changes and conventional prescribed drugs. However, patients from low- and middle- income countries are often under a great economic burden to acquire conventional diabetes drugs for their treatment, and turn to traditional alternatives such as the use of medicinal plants and herbs from their region or country (International Diabetes Federation, 2017; Nazarian-Samani, Sewell, Lorigooini, & Rafieian-Kopaei, 2018).

One of the most studied herbs for its antidiabetic bioactivity is *Salvia* species (spp.), a widespread group of plants belonging to the Lamiaceae family. There are several studies regarding the antidiabetic potential of this plant, which is mainly attributed to its polyphenolic and terpenes content, which mediates its hypoglycemic potential through numerous mechanisms (Jia et al., 2019). In this section, we briefly assess the most recent findings on the studies showing the potential of *Salvia* spp as an antidiabetic agent.

Salvia species are rich in tanshinones, a group of abietane diterpenes isolated almost exclusively from *Salvia miltiorrhiza* and some other *Salvia* species (Dong, Morris-Natschke, & Lee, 2011). These compounds have been the aim of many works to evaluate their bioactive properties. In this sense, Alimpić et al. (2017) reported the anti-diabetic potential of 12 tanshinones isolated from *Salvia miltiorrhiza* Bunge through their inhibitory activity against protein tyrosine phosphatase 1B of the tanshinone cryptotanshinone, tanshinone IiB and dehydrodanshenol A with IC50 values of 5.5, 4.7 and 8.5 μM, respectively. The authors also showed by enzyme kinetic assays of PTP1B inhibition that cryptotanshinone and tanshinol B were mixed -noncompetitive type inhibitors, whereas dehydrodanshenol A was a classical-noncompetitive type inhibitor. From these results, it may be suggested that *Salvia* tanshinones may improve insulin sensitivity and insulin resistance (D. H. Kim et al., 2017).

Flores-Bocanegra, González-Andrade, Bye, Linares, and Mata (2017) reported the antihyperglycemic effect of *Salvia carcinata* infusions in mice during an oral sucrose tolerance test (31.6 – 316 mg/kg), this effect was attributed to the flavonoid and clerodane diterpene glycoside constituents with α-glucosidase inhibitory effects such as a newly identified biflavone, amarisolide, pedilatin, apigenin-7-O-β-D-glucoside and the flavone 2-(3,4-dimethoxyphenyl)-5,6-dihydroxy-7-methoxy-4H-chromen-4-one. To understand the inhibition mode of action of these compounds, a molecular docking and dynamic study revealed that amarisolide and pedalitin bind to the active site of α-glucosidase.

Salvia triloba, from Turkey, was effective in preventing the increase in glucose levels during an oral glucose tolerance test in streptozotocin/nicotinamide-induced diabetic rats at a concentration of 200 mg/kg (Çam et al., 2017). Similarly, Arabiyat et al. (2016) supplemented methanol extracts from the aerial parts of *Salvia triloba* from Jordan to high fat diet-induced hypertriglyceridemia rats, and their results showed that *S. triloba* dose at 750 mg/kg

significantly reduced the hypertriglyceridemia in experimental fasting rats. The mode of action of *S. triloba* extracts is suggested through the inhibition of lipid metabolism enzymes such as pancreatic lipase.

In an effort to understand the metabolites responsible for the antidiabetic action of *Salvia* spp, Huang et al. (2015) tested a common phytochemical found in *Salvia* species salvianolic acid B from *Salvia miltiorrhiza* in type-2 diabetic rats. Salvianolic acid B at 100 and 200 mg/kg inhibited diabetes symptoms, it was hypothesized that the action was the alleviation of insulin sensitivity, glycogen synthesis, and increased antioxidant activity.

Moreover, the most bioactive set of compounds in the carbohydrate metabolic enzymes are compounds obtained from hydroalcoholic/water-soluble extracts. This was assessed by M. B. Bahadori, Dinparast, et al. (2017) who evaluated the α-glucosidase inhibitory activity of *Salvia syriaca* n-hexane, dichloromethane, and methanol extracts; and the highest inhibitory rate of *S. syriaca* was reported for the essential oil, and the methanol extracts with IC50 values of 1.18 mg/mL and 2.13 mg/mL, respectively. The most abundant compound in each extract was spathulenol (87.4%) in the essential oil, and rosmarinic acid and rutin in the methanolic extracts, thus the inhibitory properties of *S. syriaca* may be partially attributed to these chemicals.

As previously mentioned, *Salvia* spp is rich in polyphenols and terpene compounds. Both groups of compounds have shown antioxidant and antidiabetic properties in numerous studies from diverse sources. However, it is not clear yet, which are the responsible compounds for this action or if a synergistic action is exerted. On this subject, M. B. Bahadori, Salehi, and Sonboli (2017) showed that methanolic extracts of *Salvia urmiensis* are better α-glucosidase and α-amylase inhibitors with IC50 values of 8.3 and 24.0 μg/mL, respectively, than essential oils from leaves. This indicates that essential oil constituents in *S. urmiensis* such as ethyl linoleate (19%), methyl hexadecanoate (17%), and methyl linoleate (7.5%) are not good inhibitors of these carbohydrate metabolic enzymes. Furthermore, the interaction between *Salvia miltiorrhiza* chemicals and α-glucosidase was assessed by Tang, Zhao, and Xue (2018) using an integrated approach consisting of computational analysis and experimental studies. The authors found that the depsides salvianolic acid C and salvianolic acid A in *Salvia miltiorrhiza* are potent α-glucosidase inhibitors with IC50 values of 4.31 and 19.29 μM, respectively. The computational analysis revealed that the interaction between these depsides and α-glucosidase is mediated by hydrophobic and hydrogen bonds.

To test the effect of *Salvia officinalis* L. essential oil as antidiabetic agents, Belhadj et al. (2018) used Alloxan-induced diabetic male Wistar rats. *Salvia officinalis* L. Essential oil was rich α-thujone (29%), 1,8-cineole (12%), camphor (7.2%), β-caryophyllene (6.4%), followed by β-thujone (4.6%), α-humulene (4.6%), camphene (4.2%) β-pinene (3.5%), α-pinene (3%). First, the authors reported that the *in vitro* inhibitory rate of *S. officinalis* EO on α-amylase had IC50 values of 38 μg/mL; however, this value was lower than that of acarbose with IC50 = 14.9 μg/mL. Moreover, in vivo inhibition of α-amylase was observed at a dose of 2.5 μL per diabetic rat. Also, EO treatment reduced the alanine transaminase, aspartate transaminase, and lactate dehydrogenase activities, which are key digestive enzymes that increased in diabetic rats. This might be related to the reduced glycemia and increased glycogen storage in treated rats. The mode of inhibition on α-amylase by *Salvia mirzayanii* ethanol extracts has shown to be mixed at a concentration of 5 and 20 mg/mL, while acarbose is a noncompetitive inhibitor (Moein, Jahanshai, Rahimzadeh, & Moein, 2018).

On this subject, a study by Ben Khedher et al. (2018) showed the anti-diabetic effect of *Salvia officinalis* leaf extract at 100 and 400 mg/kg per day using male mice fed on a high-fat diet for 9 weeks by improving insulin sensitivity, inhibition of lipogenesis in adipocytes and decreased inflammation levels. Interestingly, *S. officinalis* extracts showed better results than the drug control rosiglitazone, a commonly administered drug inhibitor of PPAR-y full antagonists. The reported anti-diabetic effect may be attributed to the phenolic constituents found in *Salvia officinalis*, where the most abundant compounds were rosmarinic acid, apigenin, verbascoside, pinoresinol, apigenin-7-O-glucoside, and luteolin-7-O-glucoside. Furthermore, sage extracts reduced the plasma levels of the pro-inflammatory cytokines TNF-a, KC/GCRO, and IL-12 and increased the anti-inflammatory cytokines IL-2, IL-4, and IL-10.

Decoctions of *Salvia elegans* Vahl., *Salvia greggii* A. Gray, and *Salvia officinalis* L. are anti-diabetic and anti-obesity agents as reported by Pereira, Catarino, Afonso, Silva, and Cardoso (2018), who showed that the decoctions of these *Salvia* species are inhibitors of key metabolic enzymes such as α-glucosidase, α-amylase and pancreatic lipase. *Salvia elegans* showed the highest α-glucosidase inhibition with an EC50 of 36 ug/mL, followed by *Salvia greggii* and *Salvia officinalis* with EC50=345.3 and 71.2 ug/mL, respectively. The highest bioactivity of *Salvia elegans* was attributed to the high content of caffeic acid and derivatives. Furthermore, *S. officinalis* and *S. elegans* showed no α-amylase inhibitory potential, which is desired to avoid gastrointestinal discomfort (Ercan & El, 2016; Gutiérrez-Grijalva, Antunes-Ricardo, Acosta-Estrada, Gutiérrez-Uribe, & Basilio Heredia, 2019). Moreover, *S. officinalis* and *S. elegans* were also good inhibitors of pancreatic lipase with EC50 values of 4.6 and 8.2 μg/mL, respectively.

Salvia miltiorrhiza has also shown preventive properties on the onset of diabetic comorbidities such as retinopathy. A study published by L. Zhang et al. (2013) in alloxan-induced diabetic mice showed that administration of *Salvia miltiorrhiza* at 0.06 mL/kg and 1.4 mL/kg for ten weeks, lowered the blood glucose level of treated rats. Furthermore, *Salvia miltiorrhiza* treatment can improve the recovery of blood oxygen transport and promote the absorption of retinal hemangioma, by possibly preventing retinal lipid peroxidation and retinal ischemia.

Salvia and Hypercholesterolemia

Cholesterol is a simple lipid, we can find it in the bloodstream, in body organs and nerve fibers (Lawes, Vander Hoorn, Law, & Rodgers, 2004). Cholesterol participates in various functions in the body such as the synthesis of estrogenic hormones (testosterone, estrogen, dihydroepiandrosterone, progesterone, and cortisol), some vitamins (vitamin D) and bile acids; these last help in the digestion and absorption of fats obtained in the diet (Carretero Colomer, 2008). Cholesterol is insoluble in plasma so lipoproteins must transport it from the liver to where it is required, playing an important role in health, this compound can be good or bad. The good cholesterol is high-density lipoproteins (HDL), and the bad ones are low-density lipoproteins (LDL) (Ma, 2006). When the levels of cholesterol in the blood are modified, diverse diseases can be expressed (dyslipidemias), especially when elevating the LDL cholesterol levels (LDL-C); there is hypercholesterolemia (Marduel et al., 2010). Hypercholesterolemia occurs when the total plasma cholesterol values of low-density

lipoproteins are above the 95th percentile for age and sex (López, More, & Serra, 2009; Varghese, 2014). This situation can be caused by diet or derived from a genetic disorder like family hypercholesterolemia, which can be clinically manifested from birth. However, this disease is not diagnosed and appropriately treated throughout the world, so it is estimated that there may be between 14 and 34 million cases of which only 5% is treated adequately, which increases the risk factor of suffering atherosclerosis and premature coronary disease, and are linearly related to cholesterol levels (Canalizo-Miranda et al., 2013; Marduel et al., 2010; Mehta et al., 2016; Merchán et al., 2016). Table 3 shows the levels of hypercholesterolemia, depending on the level of cholesterol (Carretero Colomer, 2008).

Table 4. Total and LDL cholesterol ranges in hypercholesterolemia

	Total Cholesterol (mg/dL)	**LDL Cholesterol (mg/dL)**
Mild hypercholesterolemia	200-250	135-175
Moderate hypercholesterolemia	250-300	175-215
Severe hypercholesterolemia	>300	>215

The treatment to reduce this condition is based on the modification of the diet (low in saturated fat and high in monounsaturated, consumption of fruits and vegetables), the lifestyle (including physical activity), and in the use of statins, resins, fibrates, nicotinic acid, inhibitors of cholesterol absorption and phytosterols (Greig & Deeks, 2016; López et al., 2009; Merchán et al., 2016; Varghese, 2014). Statin (reduces between 33% and 58% the level of LCL-C) and fibrates are the most used drugs to reduce the synthesis of cholesterol at the liver level. However, they have been related to increased risk of myopia, which also can present inadequate responses, and about 15% of patients are intolerant (Canalizo-Miranda et al., 2013; Strandberg, Kolehmainen, & Vuorio, 2014). Due to this, different researches have looked for natural sources derived from plants for the control of these diseases, considering that from our ancestors, an extensive list of species has been used as traditional medicine. The species *Salvia officinalis* L. used against different diseases were evaluated using its leaves to make capsules (500 μg of powder), which were administered to 50 patients between 40-60 years of age, who had levels of 100 and 130 mg/dL of LDL cholesterol (LDL-C). They were administered in conjunction with 10 mg of atorvastatin for two months three times. The results obtained showed that the use of *Salvia officinalis* L. in addition to the therapy with statin decreased the levels of LDL-C and total cholesterol, without apparent adverse effects, and with a possible impact on the prevention of cardiovascular complications, perhaps due to the antioxidant properties of the plant (Saeed Kianbakht, Nabati, & Abasi, 2016).

Salvia miltiorrhiza traditionally known as Chinese herb was evaluated in hyperlipidemic rats, an aqueous extract of *Salvia miltiorrhiza* was administered in three doses (50, 100 and 150 mg kg / day) and using as a positive control [3- (3- (2- chloro-3-trifluoromethylbenzyl-2,2-diphenylethylamino) proproxy) phenylacetic acid at a dose of 100 mg per kg/day, the treatments were administered for 4 weeks. The data obtained showed a significant decrease in LDL-C and total cholesterol in the plasma of the rats treated with sage extract at a dose of 100 and 150 mg kg/day. A similar result was obtained in control, which indicates that the extract has the potential for the treatment of atherosclerosis (suffering as a result of hypercholesterolemia) by decreasing LDL-C (W. Ji & Gong, 2008). Kwok et al. (2014), carried out another study in the same species of *Salvia miltiorrhiza* mixed with *Puerariae lobata*, another Chinese

medicinal herb, this study was carried out in postmenopausal women (at least 160) between 47 and 65 years of age with early hypercholesterolemia who were administered during a year capsules of the herbal mixture, women were monitored at 12-week intervals. After 12 months of treatment, there was a marked decrease in LDL-C and total cholesterol in women under the treatment with the herbal mixture, the results suggest that a dose of 1g daily may have a better effect after 6 months and that its effect is higher in women of ages over 55 years.

Likewise, Jing et al. (2009) evaluated the effect of a capsule designed with different herbs (*Rheum palmatum*, *Cassiaobtusifolia* L., *Salvia miltiorrhiza* and *Panax ginseng*) which was compared with pravastatin (10 mg), both treatments were administered to patients both men and women over 18 years of age who presented hypercholesterolemia (≥220 mg/dL of total blood cholesterol, body mass index <35 kg/m2 and triglycerides> 400 mg/dL). The results obtained after six weeks showed that the patients who consumed the capsules with the herbs presented a decrease in the content of total cholesterol (7.9%) and LDL-C (10.4%). Although the effect was less than that shown by the drug pravastatin (total cholesterol 16.3% and LDL-C 19.2%), the main advantage is that damage to the liver and muscle function are minimal compared to the drug.

Similarly, in *Salvia hispanica* (chia) the effect was evaluated after being administered to rabbits, which presented conditions of early hypercholesterolemia. The animals were fed for 6 weeks with 4 different diets (100g per day), a control with commercial feed for rabbit, another in which the food was added chia oil (10%), a third in which it was added 1% cholesterol to the food, and finally one in which 1% of cholesterol and 10% of chia are added. The addition of cholesterol was performed to induce vascular dysfunction, a subsequent consequence of hypercholesterolemia. Vascular dysfunction is characterized by 1) reduced acetylcholine-relaxation and nitric oxide (NO) -release; 2) endothelium-dependent increase of angiotensin II response; 3) lipid metabolism-alteration and moderate changes of vascular morphology. This study showed that chia oil (*Salvia hispanica*) could improve vascular dysfunction by increasing acetylcholine-relaxation, normalizer NO-release, likewise reduced contractile response to angiotensin II. This effect is attributed mainly to the content of fatty acids present mainly α-linolenic acid, in addition to other bioactive compounds such as tocopherol and flavonoids (Sierra et al., 2015).

On the other hand, Kothari and Jeyaraj (2017) evaluated the effect of chia (*Salvia hispanica*) in women with hypercholesterolemia as evaluated by anthropometric measurements and serum lipid profile. Two groups of women of 30-45 years old, one was instructed to consume 12.5 g of chia, twice daily, by 60 days, the other group was the control. The results showed an increment in all lipid parameters in the control group, while the treatment group had a reduction in the lipid parameters, this effect can be due to omega-three fats and the fiber content of chia, concluding that chia could be an effective hypolipidemic agent.

Salvia and Hypertriglyceridemia

Dyslipidemias or increased lipids in the blood are disorders derived from an increase in cholesterol (hypercholesterolemia) and triglycerides (hypertriglyceridemia), which maximize the risk of morbidity and death (Miguel Soca, 2009). Triglycerides are a type of fat that can arise due to various factors such as for overweight, physical inactivity, excessive alcohol consumption, metabolic syndrome or type 2 diabetes mellitus, as well as genetic disorders

(Berglund et al., 2012). Increase of triglycerides above the 95th percentile by age and sex is the clinic diagnostic for hypertriglyceridemia, its incidence also varies by race, more consistent in men than women, and ages of 40-59 years (Brahm & Hegele, 2013; Kushner & Cobble, 2016). This disease is generally classified as primary when it is hereditary and secondary when it occurs due to various factors (environmental or other previous illness). Familial hypertriglyceridemia is the most frequent of the primary type of hypercholesterolemias and is characterized by the fact that triglyceride levels are normal, and LDL cholesterol is high. Its prevalence is 0.5-1.0% and because it is hereditary it adopts an autosomal dominant pattern; it does not present apparent symptomatology, and its diagnosis is by means of findings of analytical alterations, that is to say the existence of a variable phenotype in the family and an increased concentration of apolipoprotein B (main structural protein of chylomicrons, VLDL, and LDL, and ligand of receptors of LDL) (Brahm & Hegele, 2013; Díaz, 2008).

According to the level of triglycerides, hypertriglyceridemia is categorized as normal with a concentration of triglycerides <2.0 mmol/L, from low to moderate with 2.0-10.0 mmol/L of triglycerides and severe when presenting > 10.0 mmol/L of triglycerides (Brahm & Hegele, 2013). The treatments are based on the degree of hypertriglyceridemia, the statins are the main recommended drugs, besides to fibrates (used in primary and secondary hypertriglyceridemia), ezetimibe and n-3 fatty acids are used as adjunctive treatments for residual and persistent hypertriglyceridemia, some other therapeutic strategies are dietary (a weight reduction of 5-10% decreases the triglycerides 20%) and modification in the lifestyle (Berglund et al., 2012; Han, Nicholls, Sakuma, Zhao, & Koh, 2016; Kushner & Cobble, 2016; Pang, Chan, & Watts, 2014). Currently, these two last points (diet and lifestyle) have been of interest in various investigations due to the concern for the side effects derived drugs used in these diseases, coupled with the interest in the use of traditional or natural medicine that has used for many years. In this sense, Arabiyat et al. (2016) carried out a study in which they obtained a methanol extract of the leaves of *Salvia triloba* (obtained from Amman, Jordan), which were used in different concentrations in rats albino which were induced hypertriglyceridemia through a diet rich in fat, rats were divided into 4 groups: 1) Control diet (normal with food for rat, 2) HDF: diet high in fat (30%), 3) Orlistat, enzyme lipase inhibitor pancreatic (5 mg/kg b.wt) + HFD, 4) Extract methanol of *Salvia triloba* (750 mg/kg b.wt) + HFD. They found that *Salvia triloba* has a promising effect hypotriglyceridemic, due to its extract showed a reduction of 66.4% in the triglycerides in the blood of group 4, compared with a 68.1% of orlistat (Group 3). Results can be attributed, according to the authors, to the phytochemical composition of the species *Salvia triloba*, which contains hydrocarbons, sterols, triterpenes, fatty acids, phenolic acids, and flavonoids. Concluding that *Salvia triloba* may inhibit gastrointestinal enzymes involved in digestion and absorption of lipids, this indicates that it has hypotriglyceridemic properties. *Salvia hispanica* (chia seed), another species used to prevent dyslipidemias, was evaluated in a study that was divided into two experiments. Male Wistar rats were fed without restriction, in the first experiment there were 72 rats divided into 3 groups which were given different treatments 1) Semi-synthetic diet (control diet), 2) Semi-synthetic diet with sucrose (SRD); 3) Semi-synthetic diet with SRD + Chia; in the second there were 96 rats divided into 2 groups fed for 3 months 1) Semi-synthetic diet (control diet), 2) Semi-synthetic diet with sucrose (SRD) this was subdivided into three groups 1) Group of rats were sacrificed after 3 months, 2) Group of rats that continued 2 more months with food plus SRD, 3) This group received SRD + chia for two more months. To carry out the analyzes (triglycerides, non-esterified fatty acids (NEFA), and total cholesterol) blood was obtained directly from the jugular of the rats in

both experiments at the end of the feeding period, the establishment of hypertriglyceridemia was observed. Insulin resistance in the diet with SRD, however adding chia as a replacement for the fat source in the diet manages to lower triglycerides more than 60% in both experiments, as well as decreases NEFA and total cholesterol, on the other hand, the addition of chia allowed to reduce visceral fat. This confirms that hypertriglyceridemia can be normalized by replacing the source of fat with chia seed (*Salvia hispanica*), as it is rich in α-linolenic acid and fiber (Chicco, D'Alessandro, Hein, Oliva, & Lombardo, 2009). Similarly, in a study conducted in people with hypertriglyceridemia, the hypolipemic effect of chia seeds (*Salvia hispanica*) was evaluated. In this study, a daily dose of 30 g of chia was administered for 4 weeks, 60% of the participants were women and 40 men aged 25 to 54 years, blood was collected every week to quantify triglycerides in patients, finding that treatment with chia achieved a significant reduction in triglycerides in patients with hypertriglyceridemia (Roca & Carrión, 2014).

Another study conducted by S Kianbakht, Abasi, Perham, and Hashem Dabaghian (2011) *Salvia officinalis* extracts were spray-dried, and subsequently, 500 mg of *Salvia officinalis* powder was placed in capsules (gelatin) and administered to 57 patients, men, and women, (approximately 50 years old). Thirty-four patients took the capsules with *Salvia* every 8 hours for two months, and the rest took capsules with placebo under the same conditions, patients were recommended to restrict the consumption of fatty foods, and perform physical activity during the experiment. Blood was obtained at the beginning and at the end to determine the levels of cholesterol, triglycerides, VLDL (low-density lipoproteins), LDL cholesterol, HDL cholesterol, creatinine, and liver enzymes. The results showed a decrease in the content of triglycerides with a percentage of change of 22.8% in patients treated with *Salvia*, compared with placebo patients in whom triglycerides were increased, this behavior was present in the variables of cholesterol, VLDL, LDL, except in HDL where patients with the treatment Sage base levels were increased in patients. In this study, it is concluded that the leaves of *Salvia officinalis* can be used for the treatment of hypertriglyceridemias, according to their composition in water-soluble compounds in conjunction with liposoluble.

Moreover, (Hernández-Saavedra et al., 2016), evaluated the effect of infusions of *Hypericum perforatum*, *Salvia officinalis*, and *Calendula officinalis* on cardiovascular risk developed in obese rats. In this experiment, the plants were pulverized to make the infusions that were administered daily to obese-induced rats, for 12 weeks. Finally, the rats were anesthetized to obtain blood and abdominal fat to perform the total cholesterol analysis, HDL cholesterol, triglycerides, LDL cholesterol. The results obtained showed that infusions of *C. officinalis*, *H. perforatum*, and *S. officinalis* significantly reduced lipoproteins and total lipids, being able to modulate them, thus showing their hypotriglyceridemic effect. Besides mentioning that the effect of *S. officinalis* may be associated with the inhibition of hepatic de novo synthesis or the activation of β-oxidation, but in the other plants, the inhibitory effect of triglycerides may be related to the pancreatic lipase activity. Another study conducted by Khashan and Al-khefaji (2015) in albino rats given doses of 100 mg/kg of aqueous and ethanol extracts of *Salvia officinalis* leaves, alloxan was used to induce diabetes, the results obtained showed a decrease in the content of triglycerides in chia-treated rats. However, the aqueous extract had a more significant effect on the reduction of lipids compared to the ethanol extract and the drug.

Salvia and Hyperuricemia

Uric acid, the final product in the metabolic degradation of purine nucleosides (adenine and guanine), can scavenge oxygen radicals and protect the erythrocyte membrane of lipid oxidation (El Ridi & Tallima, 2017; C. Li, Hsieh, & Chang, 2013). The uric acid is soluble in biological fluids, hence it is entirely soluble in human blood. This acid is eliminated by the gastrointestinal tract and kidney. The 70% of daily uric acid is disposed via kidney, and in 5-25% impaired renal (kidney) excretion leads to "*hyperuricemia*," increasing the concentration ($\leq$ 6 mg/dL in women and $\leq$ 7 mg/dL in men) of uric acid in the blood and appears to have more impact in women than men (El Ridi & Tallima, 2017; Esteva, 2009; Grayson, Kim, LaValley, & Choi, 2011). Those levels of uric acid come off the balance between the rate of production and elimination. Some factors that increase the risk of presenting hyperuricemia is the type of diet, such as the consumption of meat, fish, alcohol (depending on the type of beverage), sugary drinks, some sweet fruit, and fruit juice. On the other hand, some factors that reduce the risk are the consumption of vegetables and legumes, skimmed milk, coffee, vitamin C, and cherries (Álvarez-Lario & Alonso-Valdivielso, 2014). Hyperuricemia has been implicated in cardiovascular diseases, diabetes type 2, and non-alcoholic fatty liver disease, all the manifestations of the metabolic syndrome; besides, hyperuricemia could be a risk factor of developing gout (Bardin & Richette, 2014; El Ridi & Tallima, 2017).

Treatment for hyperuricemia involves the use of drugs or lifestyle changes (weight loss, alcohol reduction, and foods rich in purines); the administration of hypouricemic drugs is recommended in patients with frequent acute symptoms. In asymptomatic patients, the treatment will depend on the origin of the hyperuricemia if it is derived from the increased capacity to produce uric acid or is a consequence of the decrease in the body's ability to eliminate it (Esteva, 2009). The main drugs used are inhibitors of xanthine oxidase (allopurinol and febusostat), urisuric (benzbromarone), uricolitic (rasburicase), all of them present adverse reactions, several studies have focused their interest in new treatments based on natural products, in a study carried out by Huijuan et al. (2017), tested the effect of Qi-Zhu-Xie-Zhou-Fang (QZXZF) a mixture of different plants: *Astragalus mongholicus* Bunge, (huang qi, Astragalus), *Atractylodes macrocephala* Koidz., (bai zhu, rhizome atractylodis macrocephalae), *Coix lacryma- Jobi* L., (my ren, Jobstears Seed), *Pyrrosia lingua* (Thunb.) Farw., (shi wei, Pyrrosia Leaf), *Smilaxglabra* Roxb., (tu fu ling, Smilacis Glabrae Rhizoma), *Salvia miltiorrhiza* Bge., (dan shen, Dan-shen Root), *Cuscuta chinensis* Lam., (tu si zi, Chinese dodder Seed) and *Isaria cicadae* Miq (jinchan hua, Fungus Sclerotia on Cicada) in male Wistar rats to which the level of uric acid was raised to induce hyperuricemic nephropathy with 100 mg/kg adenine and 300 mg/kg potassium oxonate, subsequently the drug allopurinol (35 mg/kg per day) was administered to a group of rats, another group was administered the mixture of plants QZXZF to a dose of 1.4 g/kg per day, left another group without treatment as control (normal rats), after 3 weeks the rats were euthanized 1h after the last drug administration, and the blood samples were obtained for the determination of uric acid and CysC (Cystantin C, protein used as a biomarker of kidney function). The results showed that it was possible to induce hyperuricemia nephropathy since the levels of uric acid and Cysc C were elevated, and in turn the groups of rats treated with the drug and with QZXZF managed to decrease the levels of uric acid by 69% and 22% On the other hand, the level of Cys C was reduced with QZXZF in hyperuricemic nephropathy rats, while the drug did not present a significant effect. Their results suggest that QZXZF reduces the levels of uric acid, Cys C mild and improves renal

fibrosis, the main pathologic process of hyperuricemic nephropathy, and relate their effect on renal pathologies with the content of Dashensu a monomer derived from *Salvia miltiorrhiza* (Danshen roots).

Moreover, El Euch, Hassine, Cazaux, Bouzouita, and Bouajila (2019) conducted a study with essential oil from leaves of *Salvia officinalis* to know its anti-enzymatic effect on some diseases, one of the different enzymes studied was xanthine oxidase, which is involved in the development of hyperuricemia, by catalyzing the oxidation of hypoxanthine and xanthine to uric acid. This experiment was carried out by obtaining hydrodistillation essential oil of sage, which was analyzed by gas chromatography and gas mass spectrometry to determine its chemical composition, and then the anti-xanthine oxidase activity was carried out comparing it with the drug allopurinol. A concentration of 50 mg/L of the essential oil of *Salvia officinalis* was used, finding a percentage of inhibition of 36.89 ± 1.83% lower than that found with allopurinol, this activity is attributed to its content in α-thujone, β-thujone, α-pinene, β-pinene, β-caryophyllene, and myrcene. M. B. Bahadori et al. (2015) studied the inhibitory effect of an extract and essential oil of *Salvia spinosa* L. on the enzyme xanthine oxidase (a key enzyme in the formation of uric acid). The essential oil was obtained by hydrodistillation and the extract by maceration with methanol, dichloromethane, and n-hexane. The essential oil showed significant inhibition of the enzyme while extract effect was moderate. The results obtained were related to the content of phenolic compounds of the extract and to the terpenoid compounds (caryophyllene and spathulenol) found in the essential oil of *Salvia spinosa*. In this same context is the study carried out by J. K. Kim et al. (2017), who evaluated the effect of extracts of *Salvia plebeia* on the inhibition of the enzyme xanthine oxidase in in vitro tests and in an animal model with hyperuricemia, for which an extract with ethanol was obtained from of leaves and roots of sage, which was tested in the *in vitro* test with the enzyme (xanthine oxidase), using allopurinol as control, on the other hand, during *in vivo* evaluations, hyperuricemia (inducer potassium oxonate 250 mg/kg) was induced in male mice of 6 weeks of age, *Salvia plebeia* extracts were administered orally (50-200 mg/kg) in one group before inducing hyperuricaemia and in another group after induction, later each group blood was collected to evaluate the level of uric acid. According to the *in vitro* results obtained, the leaf extract achieved to inhibit the activity of the enzyme in a significant way while the root extract showed no significant effect, apparently attributed to the content in scutellarein, luteolin and nepetin, on the other hand the groups of mice that were first induced hyperuricemia for 1 hour and then administered the extract achieved to reduce uric acid levels, likewise the pretreatment with the extract for 7 days allowed the animals to have lower susceptibility to hyperuricemia inducer, demonstrating that *Salvia plebeia* has an anti-hyperuricemic effect.

Similarly, a compound isolated from *Salvia miltiorrhiza* root, lithosphermic acid (LSA), a major component of this species, was used to check its hyperuricemic effect in rats. In this experiment, the compound was isolated and tested *in vitro* on the enzyme xanthine oxidase. Sixty Wistar albino male rats were used for the *in vivo* test. Rats were divided into six groups: 1) control received only saline solution 2) group to which hyperuricemia was induced by administering 300 mg/kg BW of potassium oxonate, 3-5) received doses of 10, 20 and 30 mg/kg BW of LSA respectively and 6) received allopurinol (10 mg/kg BW), groups of 3-6 were injected with the hyperuricemia inducer 1 h later to the treatment. Blood was obtained from the rats, and the levels of uric acid were evaluated. The results showed that the compound LSA inhibits the formation of uric acid as well as the drug allopurinol, blocking the enzyme xanthine oxidase, in the *in vivo* test it was observed that the compound derived from *Salvia miltiorrhiza*

presented a dose-dependent effect and that a dose of 30 mg/kg BW showed a decrease equal to the drug allopurinol (X. Liu, Chen, Shang, Jiao, & Huang, 2008).

Salvia and Cancer

One of the main causes of death in the world is cancer, in 2015 there were 8.8 million deaths due to various types of cancer, the most common are lung, colon, liver, breast, uterine cervix, esophagus, both men and women (Akaberi, Mehri, & Iranshahi, 2015; World Health Organization, 2017). This disease is caused by changes in the behavior of the cells, when changes occur in genetic information, derived from various factors such as environmental, chemical, physical, metabolic and genetic (Dai & Mumper, 2010; Karp, 2011). The different types of cancer are caused by various mutations that lead to the disease, that is, when they affect either genes that stop or slow down the cell cycle or genes that induce cell growth and division (Freeman, 2013).

Currently, treatments are based on cytotoxic drugs, which inhibit the growth and proliferation of cancer cells (Chen, Guo, Bao, Lu, & Wang, 2014), However, for some years, the identification and development of drugs derived from natural products has increased, many of the anti-cancer drugs (almost 80%) are obtained from natural products, to mention some we have paditaxel, taxotere, vincristine, camptothecin, irinotecan, topotecan, and teniposide (Cragg, Grothaus, & Newman, 2009). For this reason, several scientific studies have been conducted on different types of cancer with natural products to find compounds that act as anti-carcinogens but without damaging normal cells, and thus improve the quality of life of people. (Akaberi et al., 2015). *Salvia* is one of the natural products widely studied due to its chemical composition, in China, hundreds of products derived from this genus have been approving since more than 100 pure compounds have been chemically isolated (Chen et al., 2014).

Within the various studies on the sage genus, Farimani et al. (2015), evaluated the antiproliferative effect *in vitro* of two terpenoids, urmiensolide, and urmiensic acid, both isolated from the aerial parts of *Salvia urmiensis* on two cancer lines A549 (human alveolar lung epithelial carcinoma) and MCF-7 (human breast adenocarcinoma). The extraction of the compounds was carried out by maceration with hexane at room temperature for 7 days. to subsequently carry out a fractionation of the extract and identify the compounds present, later the identified compounds were tested with the two cell lines at different times (24, 48 and 72 h) and concentrations of 1mg/mL, as positive control doxorubicin (a drug used in cancer chemotherapy) was used. The results obtained showed that the two isolated compounds had an antiproliferative effect with IC50 values of 2.8 μM for the triterpenoid urmiensolide and 1.6 μM for urmiensic acid against MCF-7 in 72 h and whereas in A549 urmiensolide showed an IC50 of 12.1 μM and urmiensic acid of 10.9 μM in the same incubation time. Another study carried out on 11 species of sage (*Salvia atropatana* Bunge, *Salvia bracteata* Banks & Sol., *Salvia dracocephaloides* Boiss., *Salvia eremophila* Boiss., *Salvia grossheimii* Sosn., *Salvia hydrangea* DC. Ex Benth., *Salvia lachnocalyx* Hedge., *Salvia mirzayanii* Rech., *Salvia sahendica* Boiss. & Buhse., *Salvia sclareopsis* Bornm. Ex Hedge and *Salvia verticillata* L.) obtained from different regions of Iran were evaluated to know their chemical composition and cytotoxic activity against cells of human colorectal adenocarcinoma (HT-29), cells of human lymphoblastic leukemia (MOLT-4) and against breast cancer cells (MCF-7).To carry out the experiment, the essential oils was extracted by hydrodistillation from the aerial parts (stems,

leaves, and flowers) of the species and evaluated its chemical composition by GC-MS (gas chromatography-mass spectrometry) and GC-FID (gas chromatography-flame ionization flame), to later evaluate its cytotoxicity using different concentrations (100-1000 μg/mL) of each sage species on the three cell lines (HT-29, MOLT-4, and MCF-7). According to the results obtained, the oils showed little cytotoxicity against cancer cell lines, only *Salvia lachnocalyx* and *Salvia sahendica* species had an anticancer effect against all cell lines with an IC50 in a range of 127.7 - 419.0 μg/mL, the lowest values were for *Salvia grossheimii* against cancer lines HT-29 and MOLT-4 (145.6 and 115. μg/mL, respectively). Similar behavior was shown by *Salvia mirzayanii* and *Salvia sclareopsis* against MCF-7 and MOLT-4. They also report anticancer activity of almost all species on MOLT-4; these results may mainly be due to non-volatile compounds (Asadollahi et al., 2018).

M. B. Bahadori, Eskandani, De Mieri, Hamburger, and Nazemiyeh (2018) evaluated the antiproliferative effect of compounds isolated from the aerial parts of four species of *Salvia* (*Salvia spinosa, Salvina santolinifolia, Salvia syriaca,* and *Salvia nemarosa*) on cancer cells A549 (human alveolar lung epithelial carcinoma). The compounds were isolated by maceration with different organic solvents (n-hexane, dichloromethane, methanol), and its cytotoxicity was tested. Isolated compounds antiproliferative activity in cancer cells A549, and possible mechanisms were tested. According to the results, only the diterpene clerodane of the species *Salvia nemarosa* showed an effect on the viability of lung cancer cells A549 with an IC50 inhibitory concentration of 35 μg/mL in 48 h. Also, morphological changes indicative of cell death were observed. The compound CDA caused cell cycle arrest, which disables the cell to repair the damage caused in the DNA, and apoptosis occurs or an abnormal division of DNA. The results showed that the clerodermic acid (CDA) isolated from *Salvia nemarosa* possesses antiproliferative activity since it has strong geno- and cytotoxicity and induces apoptosis in A549 cells. Benniou, Langat, Mulholland, Benayache, and Benayache (2018), isolated 3 triterpenoid compounds (2α, 3β, 11α-trihydroxyolean-18-ene, 3β-acetoxy, 2α, 11α-dihydroxyolean-18-ene, and 2α-acetoxy, 3β, 11α-dihydroxyolean-18-ene) from the aerial parts (leaves and flowers) of *Salvia phlomoides,* to which their anticancer activity was evaluated in a single dose of 10 μM against the NCI60 panel of human tumor cell line derived from nine cancer cells (leukemia, lung, melanoma, colon, nervous system, ovary, renal, prostate, breast cancer). According to the results, the inhibitory effect was low with the compounds 2α, 3β, 11α-trihydroxyolean-18-ene and 2α-acetoxy, 3β, 11α-dihydroxyolean-18-ene, while the compound 3β-acetoxy, 2α, 11α - dihydroxyolean-18-ene showed a growth inhibition of the 38% against RPMI-8826 leukemia cell line, 36% against the non-small cell lung cancer NCI-H460 and 34% against the non-small cell lung cancer NIC-H522 and 30% in colon cancer HT29 cell line. Another group of researchers studied the species *Salvia leriifolia* from which two extracts were obtained, one soluble in water and the other insoluble, of which different compounds were isolated, which were tested against two different cell lines of cancer, HeLa (cervical cancer cell) and PC3 (prostate cancer cell), compounds were evaluated in various concentrations in a range of 20.0 and 1.25 μM for 48 h, using cycloheximide as standard for normal fibroblast cell line. The compounds found were Salvialeriol, a diterpene type abietane, as well as 6-hydroxysalvinolone and deacetylnemorone, and two known triterpenes, 2-acetoxylupeol, and lupine-2,3-diol. All the compounds showed antiproliferative activity against the two cancer lines (HeLa and PC3). However, the compounds 6-hydroxysalvinolone, deacetylnemorone, and lupine-2,3-diol their effect was more significant with IC50 ranging from 2.6 to 8.0 μM in cervical cancer cell line and from 2.8 to 6.15 μM in a prostate cancer cell

line. On the other hand, the compounds *Salvia*leirole and 2-acetoxypanol presented moderate cytotoxicity (Choudhary et al., 2013).

A study in *Salvia tebesana* root reported the isolation of two new diterpenoids (tebesinone A and tebesinone B), and two more compounds aegyptinone A and aegyptinone B, they were used against cancer cell of human breast adenocarcinoma (MCF-7), melanoma (B16F10), prostate (PC-3) and colon (C26) to evaluate the cytotoxic effect. The results obtained showed that all the compounds isolated from *Salvia tebesana* had cytotoxic activity against the cancer line. However, the compounds aegyptinone A and tebesinone B presented the highest cytotoxicity against MCF-7, B16F10, PC-3, and C26 cell lines (IC50 2.1-10.3 μM), whereas tebesinone A showed no effect. Likewise, aegiptinone B showed high activity against PC-3 cell line (IC50 5.79 μM). This suggests that the compounds with the most antiproliferative effect with some types of cancer are aegyptinone A and tebesinone (Eghbaliferiz et al., 2018). The isolated compounds from *Salvia multicaulis* (salvimulticanol, salvimulticaoic acid, 2-oxocandesalvone, candesalvone B, 6-β-hydroxycandesalvone B, and candesalvone B methyl ester, were evaluated against human acute lymphocytic leukemia (CCRF-CEM), breast cancer cell MDA- MB-231-pcDNA3, colon cancer cells (HCT116), glioblastoma cells (U87MG). The 2-oxocandesalvone compound showed higher activity against CCRF-CEM with an IC50 of 11.58 μM, while with the other cancer cell lines it was in a range of 1.3-23.84 μM of IC50 The rest of the compounds found had less cytotoxic activity, the authors attribute the variability of the cytotoxic effect to the variations in the structure of the different compounds (Hegazy et al., 2018). Ceratol, ceratodiol, 1-ketoaethiopinine, ferruginol, and 12-deoxysalvipisone, compounds isolated from *Salvia ceratophylla* root, were evaluated against human lymphoblastic leukemia (MOL-4) and human breast adenocarcinoma (MCF-7), finding that ceratodiol, 1- ketoaethiopinine and 12-deoxysalvipisone showed cytotoxic activity against the two cancer lines compared with a reference cytotoxic (cisplatin), the compound ceratol has low toxicity against MOLT-4 and no against MCF-7. The cytotoxic effect of ketoaethiopinine and 12-deoxysalvipisone is attributed to its structure, which has α, β-unsaturated carbonyl (Mirzaei, Firuzi, Chandran, Schneider, & Jassbi, 2019). Similarly Zengin et al. (2018) evaluated the cytotoxic effect of several species *Salvia blepharochlaena, Salvia euphratica* var. leiocalycin, and *Salvia verticillata* subsp. amasica on the cancer lines A549 (human alveolar lung epithelial carcinoma) and MCF-7 (human breast adenocarcinoma). The results obtained showed strong cytotoxicity of *S. euphratica* extract in both cancer lines with an IC50 of 44 and 176 μg/mL in MCF-7 and A548 cells, respectively, followed by the extract obtained from *S. blepharochlaena*. The cytotoxic effect of the plants of the *Salvia* genus is related to their chemical, composition mainly to the terpenoids. Likewise in a study conducted by S. Li, Zhaohuan, Guangshun, Guanhua, and Guangji (2016), extracted diterpenoids tanshinone from *Salvia miltiorrhiza* and evaluated their effect on human lung cancer cell line (PC 9) and breast cancer cell line (MCF-7), their results showed a decrease in the viability of the cell lines of PC 9 and MCF-7 with an IC50 of 7.4 and 4.4 μg/mL respectively, according to the morphological studies the treated PC 9 cells showed damage in the cellular membrane, deformed and dark cells, which indicates apoptosis. Another result was that the levels of the proteins PARP, procaspase-3 and procaspase-9 decreased when increasing the concentration of diterpenoids, while cleaved-caspase-3/9 protein gradually increased. In addition, a decrease in the expression of the ATF4 protein was observed and an increase in the expression of the proteins p-eIF2α, p-JNK, and caspase-12 as a function of the dose of diterpenoid on the cancer lines (PC 9 and MCF-7), which indicated an induction to apoptosis.

Russo et al. (2013) evaluated the anticancer activity of essential oils of *Salvia officinalis* L. on three human melanoma cell lines (A375, M14, A2058. Sage from 18 regions of southern and central Italy (S1-S18) were collected. The essential oils obtained by hydrodistillation were tested at different concentrations (12.5-200 μg/mL) on the cellular lines. The results obtained showed that the essential oils affected the development of human melanoma cancer cells, and the effect was presented according to the origin of sage, S6, S18 and in particular the essential oil S13 (IC50 8.2, 12.1 and 11.7 μg/mL in M14, A375 and A2058 cells, respectively) exhibited the highest inhibitory and apoptotic effect. Their anticancer results were related to the compounds found in the essential oils α - and α -thujone isomers in synergy with other compounds such as camphor. Alimpić et al. (2017) evaluated the biological activity of aqueous and ethanol extracts of *Salvia amplexicaulis* L. on human colon tumor HCT-116 cells; the applied dose was 1.0 to 500 μg/mL. The results showed significant effects in the toxicity in human carcinoma HCT-116 cell line, being more significant effect the one presented by the aqueous extract, however both present cytotoxic activity after 24 h, with an IC50 of 114.1 μg/mL and 164.5 μg/mL in the aqueous and ethanol respectively.

Salvia and Alzheimer

Alzheimer's disease (AD) is characterized by neuronal loss, abnormal aggregation of amyloid peptides (Aβ) plaques, hyperphosphorylation of tau proteins and intraneuronal accumulation of neurofibrillary tangles, as well as the presence of inflammatory mediators, such as cytokines or transcription factors (Bolós, Perea Juan, & Avila, 2017; Paulson et al., 2008). Commonly used drugs exert side-effects, which leads to the search for natural compounds to treat AD. Medicinal plants and their main bioactive compounds might be a promising alternative for the treatment of neurodegenerative ailments, such as AD (Kumar & Nisha, 2014; Omar, Scott, Hamlin, & Obied, 2017). The phytochemical components of *Salvia* spp., namely rosmarinic acid, salvianolic acid A (Sal A) and B (Sal B), along with tanshinones (Tan) and Tan derivatives, provide them with anti-AD and cognitive-enhancing potential activities, and make this plant genus an excellent candidate as source for the development of anti-AD drugs (Hügel & Jackson, 2014; Sharifi-Rad et al., 2018).

Several studies have demonstrated that extracts or phytochemicals from *Salvia* spp. exert action against biomarkers of AD. For instance, methanolic extracts from dried leaves of *S. triloba* have been evaluated to establish a scientific basis for the use of this plant to treat neuroinflammatory diseases, such as AD in a Sprague-Dawley rat model (Ahmed et al., 2013). The oral treatment of rats with extracts from *S. triloba* at 750 mg/kg and 375 mg/kg significantly reduced the increase of acetylcholinesterase (AChE) activity in the brain and serum caused by $AlCl_3$. Furthermore, *S. triloba* extracts attained to reduce the levels of C-reactive protein (CPR), MCP-1, and NF-κB induced by $AlCl_3$ in the AD-rat model. In general methanolic extracts of *S. triloba* showed effective anti-inflammatory activity against neuroinflammation associated with AD (Ahmed et al., 2013).

Inhibition of cholinesterases is considered as an effective approach for the management of AD (McGleenon, Dynan, & Passmore, 1999). Several studies have been carried out to understand and estimate the anticholinesterase activity of *Salvia* spp (Demirezer et al., 2015; Duru, Tel, Öztürk, & Harmandar, 2012; Loizzo et al., 2010). Besides, this activity has been related to the presence of salvianolic and rosmarinic acids in the plants belonging to the *Salvia*

genus (Habtemariam, 2018). For example, butanol extracts from aerial parts of *S. sclareoides*, with a high content of rosmarinic acid, showed inhibition on the AChE activity by binding with the enzyme (Marcelo et al., 2013). It has been mentioned that essential oils extracted from flowers of *S. urmiensis* showed high anti-AChE and anti-butyrylcholinesterase (BChE) activities (M. B. Bahadori, Salehi, et al., 2017). On the other hand, extracts from the aerial parts of *S. syriaca* exhibited moderate anti-AChE and anti-BChe activities (M. B. Bahadori, Dinparast, et al., 2017). This contradictory information might be due to the composition of the extracts and part of the plant under study. In a more extended study, the inhibition of cholinesterase activity of 14 *Salvia* spp was evaluated (*S. argentea*, *S. bracteata*, *S. caespitosa*, *S. cryptantha*, *S. glutinosa*, *S. indica*, *S. microstegia*, *S. multicaulis*, *S. pinnata*, *S. quezelii*, *S. syriaca*, *S. tobeyi*, *S. verticillata* subsp. *amasiaca*, and *S. viscosa*). It was demonstrated that the ethanol and dichlorometane extracts from the leaves of *S. cryptantha* were the most efficient against AChE and BChE (Orhan et al., 2013).

Amyloid peptides, $A\beta_{1-40}$ and $A\beta_{1-42}$, are excellent molecular targets to develop therapies and drugs potentially effective against AD. It has been demonstrated that rosmarinic acid, the major component of aerial parts of *S. sclareoides*, binds to $A\beta_{1-42}$ oligomers, therefore promoting the disaggregation of Aβ aggregates. These results support the claim of a protective effect of *Salvia* against the neurotoxicity caused by Aβ peptide (Airoldi et al., 2013). Tan IIA, a diterpene present in *S. miltiorrhiza*, protects rat cortical neurons from the neurotoxic effects of $A\beta_{25-35}$. Pretreatment of cells with Tan IIA (0.01-50 μM) prior to exposure to $A\beta_{25-35}$ (30 mM) reduced the cell apoptosis, increased the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, diminished the malondialdehyde (MDA) and intracellular ROS production, also reduced the mitochondrial membrane potential loss, the activity of caspase-3 and increased the Bcl-2/Bax ratio. These results suggest that Tan IIA alleviates the toxicity induced by $A\beta_{25-35}$ in neurons, through its antioxidative potential, and therefore it is a promising agent for the treatment of AD-associated to oxidative stress (T. Liu, Jin, Sun, Xu, & Hu, 2010). Extracts from *S. miltiorrhiza* (0.2 mg/mL), which contained Tan IIA and Sal B, also showed neuroprotective effects against $A\beta_{25-35}$-induced apoptosis in SH-SY5Y cells *via* inhibiting oxidative stress and diminishing the mitochondrial-dependent apoptotic pathway (H. Yu et al., 2014). It has been demonstrated that Sal A (40-100 μM) significantly reduces $A\beta_{42}$ aggregation by chelating metal ions and inhibiting the formation of ROS in human neuroblastoma SH-SY5Y cells. The mechanism proposed is that Sal A binds to the C-terminus of Amyloid peptides and stabilizes the α-helical conformations (Y. Y. Cao et al., 2013).

Neuronal cell death caused by apoptosis is one of the main reasons for the cognitive deterioration in AD. *Salvia choloroleuca*, *S. mirzayani*, and *S. santoliniflia* methanolic extracts interfered with apoptosis in rat PC12 cells. Pretreatment of cells with different concentrations of the extracts (10-100 μg/mL) attenuated Bax/Bcl-2 ratio, reduced the accumulation of intracellular ROS and the loss of mitochondrial membrane potential; also, the extracts prevented the cleavage of caspase-3 and increased glutathione level in H_2O_2-induced apoptosis in PC12 cells. This information seems to suggest that the antioxidant components present in the three *Salvia* spp. extracts might affect the apoptotic pathways concerning the mitochondria (Alamdary et al., 2012). Methanol extracts from roots of *Salvia aristata* (100 μg/mL) also showed the ability to suppress the apoptosis of SH-SY5Y cells induced by H_2O_2 by reducing the disruption of mitochondrial membrane potential, inhibiting the gene expression of Bax, caspase-3, and caspase-9, as well as increasing the gene expression of Bcl-2. This anti-apoptotic

and neuroprotective effect might be related to the phenolic and flavonoid content of the *S. aristata* extracts (Esmaeili, Alilou, & Sonboli, 2015).

Several additional studies have been conducted to establish the mode of action of extracts or phytochemicals of *Salvia* spp. against amyloid peptide-induced AD models (Foolad & Khodagholi, 2013; P. Jiang, Li, Xiang, & Jiao, 2014; Y. W. Lee et al., 2013; T. Yu et al., 2018). *Salvia* plants affect numerous biological processes that might influence on neurological and cognitive functions. The discovery of new treatments or drugs to reduce AD-related illnesses using this genus of plants seems promising. Nevertheless, more research is needed to find the best candidate for this application.

Salvia and Parkinson's Disease

Recently, important attention has been paid to investigate herbs, plant extracts or plant bioactive compounds to develop therapies or drugs for the prevention/treatment of Parkinson's Disease (PD) (Ittiyavirah & Hameed, 2014; Srivastav, Fatima, & Mondal, 2017; Vijayakumar, Prabhu, Rajalakhsmi, & Manogar, 2016). *Salvia* spp., especially *Salvia miltiorrhiza*, have been studied due to their anti-parkinsonian and anti-neurotoxicity activities (Hu et al., 2016; X.-z. Li, Zhang, Liu, & Lu, 2013; Song et al., 2012). Sal A and Sal B, rosmarinic acid, Tan I and Tan IIA, as well as dihydrotanshinone (DT), have been identified in the roots of *Salvia* spp., such as *S. miltiorrhiza* (Danshen) and *S. yunannensis* (Zidanshen) (J.-L. Cao et al., 2016; Lin et al., 2018; Ni et al., 2019). These compounds have also been found in the flowers, leaves, and stems of *S. miltiorrhiza* (Lin et al., 2019).

1-Methyl-4-phenylpyridinium (MPP^+) is a neurotoxin used to induce a syndrome similar to PD. Treatment of human neuroblastoma SH-SY5Y cells with MPP^+ 500 µM triggered the reduction of cell viability, the condensation, and fragmentation of nuclei, the increase of intracellular ROS concentration, in Bax/Bcl-2 ratio and caspase-3 activity. MPP^+ also caused a reduction in mitochondrial membrane potential and the release of cytochrome *c*. All these factors, which are related to PD, were inverted when cells were pretreated with Sal A (50 µg/mL) (X.-J. Wang & Xu, 2005). Similarly, Sal A prevented the apoptosis of SH-SY5Y cells exposed to H_2O_2 (200 µM). Pretreatment of cells with Sal A (10 nM) suppressed the cell death, the loss of the mitochondrial membrane potential, the upregulation of AMPK and p-AKT expression induced by the H_2O_2 exposure (H.-a. Zhang et al., 2012).

Sal B exerted a protective effect, in a dose-dependent manner (0.1-10 µM), in human neuroblastoma SH-SY5Y cells against 6-hydroxydopamine(6-OHDA)-induced apoptosis (Tian et al., 2008). Pretreatment of cells with Sal B prevented nuclear apoptosis, inhibited the increase of intracellular ROS level, and the decrease in mitochondrial membrane potential. Also, it suppressed intracellular calcium concentration elevation. Furthermore, Sal B reduced the 6-hydroxydopamine-induced increase of caspase-3 activity and eliminated the reduction in PKC phosphorylation caused by 6-OHDA; also, Sal B prevented the 6-OHDA-induced reduction in the Bcl-x/Bax ratio and activation of ERK. These results suggest that the neuroprotective effect of Sal B might be due to a rise in antioxidant potential (Tian et al., 2008). In a similar study, it was demonstrated that Sal B protected SH-S5Y cells from the neurotoxic effect of MPP^+. Sal B (10-100 µM) reduced apoptosis, diminished the loss in mitochondrial membrane potential, and also reduced the increase of intracellular ROS level and in the

Bax/Bcl-2 ratio, as well as the activation of caspase-3 induced by the neurotoxin MPP^+ (Zeng et al., 2010).

It has also been demonstrated that Tan I (1-20 μM) reduced the expression levels of neuroinflammation factors, such as nitric oxide, tumor necrosis factor-α, IL-1β, and IL-6, as well as inhibited the activation of NF-κB in lipopolysaccharide (LPS)-activated BV-2 microglia cells. Furthermore, Tan I (10 mg/kg) improved motor functions, normalized striatal levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), reduced the loss of neurons in the substantia nigra pars compacta (SNpc) in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced male C57BL/6 mice model of PA (S. Wang et al., 2015). Additionally, Tan IIA (25 mg/kg) prevented degeneration of nigrostriatal dopamine-containing neurons, avoided loss of SNpc, improved motor performance and striatal dopamine content in MPTP-treated mice. Furthermore, Tan IIA inhibited the MPTP-activation of the expression of NADPH oxidase and iNOS in microglial cells in the SNpc (Ren et al., 2015). Another way in which Tan I and Tan II A exert their anti-parkinsonian activity might be by inhibiting the aggregation of α-synuclein, a protein that when aggregated, is toxic and is present in patients undergoing PD (K. Ji et al., 2016).

Dihydrotanshinone (DT) (10 mg/kg) and cryptotanshinone (10 mg/kg), two main phytochemicals isolated from *S. castanea*, exert neuroprotective effects by inhibiting dopaminergic cell loss and enhancing the poor locomotor performance induced by MPTP (40 mg/kg) in a PD mouse model. Furthermore, DT and cryptotanshinone increased the mRNA expression levels of SOD1, SOD2, Gpx-1 and Nrf2 in C57BL/6 mice (G.-y. Cao et al., 2018)

The results mentioned provide scientific evidence to assume that phenolic acids and terpenes from *Salvia* spp. might be promising agents in treating neurodegenerative diseases associated with oxidative stress, such as Parkinson's disease; and that the neuroprotective effect of these compounds is strongly associated to their antioxidant properties.

CONCLUSION

According to the literature review, genus *Salvia* has a medicinal effect against different diseases related to metabolic syndrome, cancer, and neurodegenerative diseases. Those positive effects could be derived from its content in bioactive compounds like polyphenolics and terpenoids, and the synergy between them. Further systematic studies are needed involving both bioactivity and bioavailability evaluation of phytochemical constituents of *Salvia* spp to elucidate mode and mechanisms of action.

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Chapter 9

PHYTOCHEMICAL COMPOSITION OF BEETROOT AND ITS POSSIBLE EFFECT ON HUMAN HEALTH

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ABSTRACT

Beta vulgaris L. belongs to the Chenopodiaceae family, and it's generally known as beetroot or garden beet and has several varieties with bulb colors ranging from yellow to red. It is considered as one of the ten most essential vegetables around the World, due to the presence of essential components such as fiber, minerals, vitamins, nitrate, ascorbic acid, carotenoids, phenolic acids, polyphenols and betalains making its consumption highly beneficial to a human body. Traditionally, beetroot is usually consumed in juice, or boiled for salads, and processed for different uses as food, however nowadays it is recognized as a functional food, due to the properties it has shown as an antioxidant, anti-inflammatory, and for its anticoagulant activity among others. The objective of this chapter is to provide a brief overview of the phytochemicals and bioactivities present in beetroot and their possible benefits on human health.

Keywords: beetroot, human health, phytochemicals

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INTRODUCTION

Beetroot is a cultivated form of *Beta vulgaris* L. and describes several cultivars of edible taproots that are grown throughout America, Europe, and Asia. Beetroot is known by various names around the World: Ziluobo in Chinese, Kaensai in Japanese, Sveklastolovaia in Russian, Beetroot in English, Betterave in French, Chukandar in Hindi, Beta in Swedish and Remolacha de mesa in Spanish (Chawla et al. 2016). The edible portion of beetroot is the root, having an average height of 1–2 m; generally, globous or cylindrical shaped with red-purple/golden or yellow/red-white in coloration depending on their variety. Leaves of beetroot arise from the crown of hypocotyl and are varied in size, shape, and color. Seeds are known as multi-germ seeds. Stems are decumbent, erect, and multi-branched. The flower is tiny, with five petals. Beets are available throughout the year, low temperature (10-15^{o}C) promotes the development of deep red pigmentation in the beetroot. Red beetroot (*B. vulgaris*) seeds, leaves, and roots are rich in phenolic compounds, whose concentration is dependent on the stage of plant development (Ninfali and Angelino 2013). Red beetroot (*B. vulgaris* var. *rubra*) contains a large number of betalains, a group of numerous water-soluble nitrogen-containing pigments, among to the betalain group, exist two classes of compounds: the yellow-orange (betaxanthin) and the red-violet (betacyanins) (Stintzing and Carle 2004) compounds that display potent antioxidant, anti-inflammatory and chemo-preventive activity *in vitro* and *in vivo*. Beetroot provides nitric oxide (NO) availability and has emerged as a potential strategy to prevent and manage pathologies associated with diminished NO bioavailability, notably hypertension and endothelial function. Beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies related to oxidative stress. This chapter provides a brief review of the phytochemicals and bioactivities present in beetroot and their association with the benefits in human health.

TAXONOMY

Beta vulgaris belongs to the Chenopodiaceae family within the order Caryophyllales (Table 1).

Table 1. Taxonomical classification of beetroot adapted from (Chawla et al. 2016)

Taxonomic subdivisions	Description
Kingdom	Plantae
Subkingdom	Tracheophyta
Super division	Spermatophyta
Class	Magnoliopsida
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Chenopodiaceae
Genus	*Beta* L.
Species	*B. vulgaris* L.

ORIGIN

Beta have been domesticated since the earliest beginnings of agriculture, cultivated before the tenth century. It originated in the 8th century from Mesopotamia and was indigenous to Asia Minor and Europe. Several varieties of beetroot, such as yellow beets were originated in 1700, and Prussians developed sugar beets in 1800. Now, red beets are more popular, and these are native to the Mediterranean region. These are widely cultivated in Europe, America, and throughout Asia (Chawla et al. 2016).

Cultivated beets are derived from section *Beta*, one of the four sections in this genus. Section *Beta* includes six subspecies within *B. vulgaris*: subsp. *vulgaris* (sugar beet, table beet, mangel or fodder beet), subsp. *cicla* (Swiss chard, leaf beet, spinach beet), ssp. *maritima* (wild sea beet), ssp. *adanensis*, ssp. *trojana*, and ssp. *macrocarpa* (Ahmad et al. 2010).

The table beet was originally cultivated as a leaf vegetable in Asia and by the Romans (Ford-Lloyd and Williams 1975). By the 17th century, table beet was cultivated in Europe, and it spread to many other regions of the world. The primary root shapes used since this period are round and globe-shaped roots, which are the most common type; flattened globe or Egyptian types; and cylindrical types, which have a specific value in the processing market.

The primary root colors available since the 17th century have been red and yellow-rooted types, though significant variation exists in root color. The sugar beet, a close relative of table beet, is designated as the same subspecies as table beet and is a crop of modern origin. Sugar beet was developed from a fodder beet population known as "White Silesian" during a search for alternative sources of sucrose when France was unable to obtain sugarcane sugar due to a British blockade during the Napoleonic wars (Fischer 1989). The selection for high sucrose beetroot was practiced by several breeders during the 18th and 19th centuries, including one of Marggraf's students named Archard, and their methodologies were among the first to describe mass selection in a scientific manner (Goldman and Navazio 2008).

PRODUCTION

As it is known, there exist many cultivars of beetroot, but there are four major groups: garden beet group (beetroot), leaf beet group (rhubarb chard, spinach beet, Swiss chard, silverbeet), sugar beet group (sugar beet) and fodder beet group (mangel-wurzel, mangold). Among these, sugar beet is the major agricultural crop, because it provides about 30% of the world's sugar; meanwhile, fodder beet cultivars are also important as a source of cattle-feed (Elbandy and Abdelfadeil 2008). The beetroot is a crop with an excellent potential yield, mostly in temperate climates, where has the highest yields; for instance, in 2013, the global average yield of the crop was 58.2 tons per hectare (Mabberley 2017, Birk 2010). About beetroot production, sugar beet heads the statistics, only in 2017, about 301 million metric tons of sugar beet were produced (FAO 2017). Since many years ago, Russia leads production; for example in 2017 produced around 52 million tons that account for 17.25% of the world's sugar beet production. France follows it since 2013, but in 2017 produced 34.3 million tons.

That year, the top 5 countries (others are Germany, the United States of America, and Turkey) account for 57.55% of sugar beet world's production (Table 2).

Table 2. Sugar beet production trend from 2013 to 2017

2013			2017		
Rank	**Country**	**Production**	**Rank**	**Country**	**Production**
1	Russia	39.2	**1**	Russia	51.9
2	France	33.6	**2**	France	34.3
3	United States	29.8	**3**	Germany	34.0
4	Germany	22.8	**4**	United States	32.0
5	Turkey	16.5	**5**	Turkey	20.8
6	China	12.1	**6**	Poland	15.7
7	Ukraine	10.8	**7**	Ukraine	14.8
8	Poland	10.6	**8**	Egypt	12.1
9	Egypt	10.0	**9**	China	9.3
10	United Kingdom	8.0	**10**	United Kingdom	8.9
	World	250.2		World	301.0

Production: Millions of tons

Although the season for beet is June to October, it is available throughout the year, owing mainly to improved horticultural practices. Beetroot is a root vegetable containing carotenoids, nitrates, vitamins, carotenoids, phenolic acids, and flavonoids and water-soluble pigments called betalains that have been divided into betacyanins that have red-violet and betaxanthine with orange-yellow color. All these components have multiple benefits to the health of the human being. Several researchers have reported that beetroot is an important source of health-promoting phytochemicals (Elbandy and Abdelfadeil 2008). The phenolic acids and flavonoids, carotenoids, and betalains of beetroot have antioxidant, anti-inflammatory, anticarcinogenic, and hepatoprotective activities have anti-diabetic, cardiovascular disease lowering and antihypertensive among other bioactive activities (Clifford et al. 2015, Jain, Gautam, and Naseem 2011). Therefore, utilization of beetroot as an ingredient in different food products imparts beneficial effects on human health and provides an opportunity for the development of various functional foods.

PHYTOCHEMICALS

Phenolic Acids

Beetroot is characterized by its high content of antioxidant compounds (Figure 1), which have been related to the prevention of certain chronic diseases (Hobbs, George, and Lovegrove 2013). Within these, phenolic acids belonging to phenolic compounds group, are derived from the secondary metabolism of the plants. The chemical structure of phenolic acids includes one aromatic ring, and at least one hydrogen is substituted by one hydroxyl group. Phenolic acids are divided into two groups; hydroxycinnamic acids (structure C_6-C_3) and hydroxybenzoic acids (structure C_6-C_1), the antioxidant capacity of phenolic acids depends on its structure, for example, position and number of hydroxyl groups (Nade, Kawale, and Patel 2015, Vasconcellos et al. 2016, Heleno et al. 2015).

The presence of phenolic acids in beetroot has been reported by various researchers (Balasundram, Sundram, and Samman 2006, Kazimierczak et al. 2014) who evaluated beetroots (*B. vulgaris*) produced organically and conventionally, identifying chlorogenic acid, gallic acid, p-coumaric acid with a total content of phenolic acids between 20.01-242.3 mg/Kg fresh weight, being the conventional type significantly higher than the organic one. Likewise, Singh et al. (2016) analyzed some fruits and vegetables marketed in India, finding in beetroot two phenolic acids, gallic acid, and caffeic acid in concentrations of 25.7 and 22.6 mg/100 g dry weight, respectively, but there were not detected ferulic acid, sinapic acid, and protocatechuic acid.

Additionally, Ertekin, Nazli, and Guzel (2017) identified chlorogenic acid (25 mg/L) and ferulic acid (57 mg/L), in red beet, but did not detect gallic acid, caffeic acid, and p-coumaric acid. In like manner, Mattila and Hellström (2007) found ferulic acid (highest concentration), protocatechuic acid, vanillic acid, ρ-coumaric acid, ρ-hydroxybenzoic acid, and syringic acid in red beet. In this respect, the distribution and content may vary depending on the variety and the analyzed part, among other factors. Concerning the above, some differences were observed in concentrations of phenolic acids (gallic acid, ferulic acid, chlorogenic acid, caffeic acid, vanillic acid, and syringic acid) in roots and stems of red beet (*B. vulgaris* var. *conditiva*). Vanillic acid (1.7 mg/g dry extract) was found as the highest compound in roots and syringic acid (3.2 mg /g dry extract) in stems (Ben Haj Koubaier et al. 2014).

In another study conducted by Zein, Hashish, and Ismaiel (2015), the content of antioxidant compounds in two types of extraction (water and methanol) of leaves of Swiss chard (*B. vulgaris* var. *cicla*) and beetroot (*B. vulgaris* var. *rubra*), was determined. Authors observed a change in the content of phenolic acids depending on the extract, that is, the extracts of Swiss chard leaves had a higher content of caffeic, vanillic and salicylic acids, while beetroot leaves the major compounds corresponded to vanillic acid, pyrogallol and protocatechuic.

Additionally, the presence of 4-hydroxybenzoic acid (0.012 mg/g dry extract), chlorogenic acid (0.018 mg/g dry extract) and caffeic acid (0.037 mg/g dry extract) have been reported in the root of *Beta vulgaris* cv. Detroit Dark (Georgiev et al. 2010). On the other hand, Kujala et al. (2002) analyzed different parts (peel, flesh and crown) of beetroot (*Beta vulgaris*) of 4 cultivars (cv. Platronde Egyptische, cv. Forono, cv. Little Ball and cv. Rubia), finding the highest concentrations in peel and identified two ferulic acid conjugates, feruloyl-glucose (8.9-24.4 µg/g dry weight) and β-D-fructofuranosyl-α-D-(6-*O*-(*E*)-feruloylglucopyranoside (22.4-68.4 µg/g dry weight).

It has also been reported that beetroot pomace (containing parts of flesh, crown, and peel) of different cultivars (cv. Cardinal-F1, cv. Egyptian, cv. Bicor and cv. Kestrel) contains some phenolic acids like ferulic, vanillic, ρ-hydroxybenzoic and caffeic acid (Singh et al. 2016). Similarly, it has been reported that ferulic acid (132.52 mg/100 g) is the main phenolic acid in beetroot pomace, in addition to other phenolic acids such as vanillic acid (5.12 mg/100 g), ρ-hydroxybenzoic acid (1.13 mg/100 g), caffeic acid (7.11 mg/100 g) and protocatechuic acid (5.42 mg/100 g) (Vulić et al. 2012).

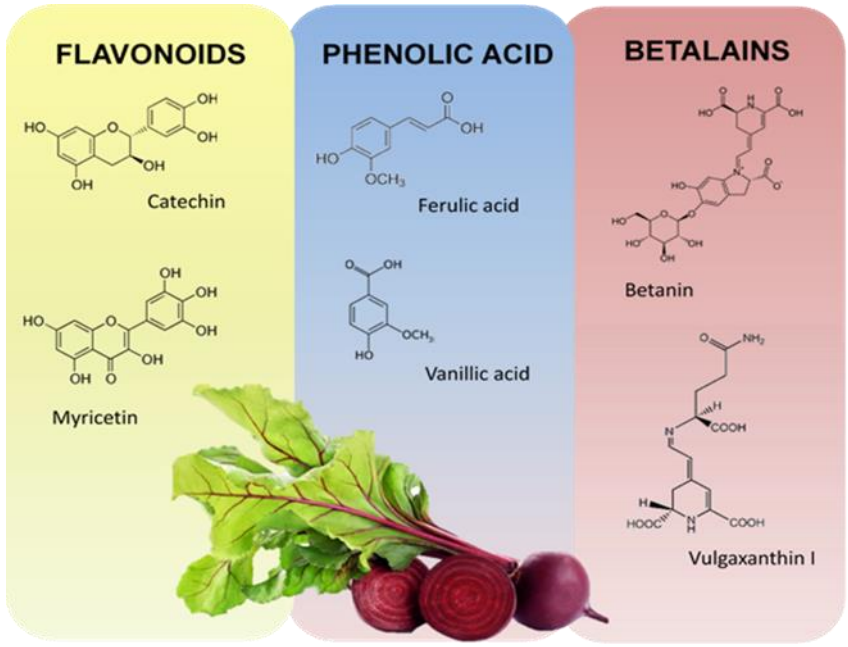

Figure 1. Chemical structures of common antioxidant compounds found in beetroot.

Table 3. Content of phenolic compounds in different parts of beetroot

Plant part	Phenolic compound content	Reference
B. vulgaris var. *cicla* (seeds)	Glucopyranosyl-xylosyl-rhamnetin Glucopyranosyl-glucopyrasyl-rhamnetin Vanillic acid, Xylosylvitexin, 2,4,5-Trihydroxybenzaldehyde Dihydroxybenzaldehyde	(Vulić et al. 2012)
B. vulgaris var. *cicla* (roots)	Vitexin, vitexin-2-O-xyloside and vitexin-2-O-rhamnoside	(Gennari et al. 2011)
B. vulgaris var. *cicla* (leaves)	Anthocyanins (0.47 µg mol/g extract), total phenolic content (31.09 µg pyrocatechol/mg extract), Flavonoids (11.88 µg catechin/mg extract)	(Sacan and Yanardag 2010)
Beet root pomace	Ferulic acid (0.950 mg/g) Vanillic acid (0.040 mg/g) ρ-Hydroxybenzoic acid (0.009 mg/g) Caffeic acid (0.070 mg/g) Protocatechuic acid (0.027 mg/g)	(Singh et al. 2016)
Eden Organic Beetroot Juice	Total phenolic content 2126.28 (µg Ferulic Acid Equivalente Equivalent/mL)	(Wootton-Beard et al. 2014)
James White Organic Beetroot Juice	Total phenolic content 3024.79 (µg Ferulic Acid Equivalent/mL)	(Wootton-Beard et al. 2014)

Flavonoids

Flavonoids are a group of phenolic compounds widely distributed in nature, around 5000 compounds have been identified and are synthesized from phenylalanine, tyrosine, and malonate (Pietta 2000). Flavonoids are C15 compounds, and all of them have a phenylbenzopyrone structure (C6-C3-C6). The arrangement of the central pyran ring (C3) determines how the compounds are classified mainly in flavones, flavanols, isoflavones, flavanones, and flavanonols (Pietta 2000). In general, flavonoids fulfill various functions such as attracting pollinators, signaling plant hormones, protecting against stress and functioning as phytoalexins (Taylor and Grotewold 2005).

In recent years the consumption of flavonoids has been linked to the prevention of some chronic degenerative diseases due to their antioxidant, anti-inflammatory, antitumor, and antimicrobial properties, among others (Martínez-Flórez et al. 2002, Panche, Diwan, and Chandra 2016). Therefore, the identification and quantification of flavonoids present in plants are of great interest. As mentioned above, beetroot belongs to the Amaranthaceae family (order Caryophyllales), which is characterized by the production of betalains and exclusion of anthocyanins (Polturak and Aharoni 2018). However, several studies have shown that beetroot has an interesting content and flavonoid profile. For example, Guldiken et al. (2016) reported a total flavonoid content in red beetroot (*Beta vulgaris* L.) of 260 mg/100 g fresh weight. Singh et al. (2016) found 221.6 mg/100 g and (Mohdaly et al. 2010) a content between 0.46-1.24 mg/g dry weight in sugar beet pulp (*B. vulgaris* cv. Gloriato).

A similar profile of flavonoids showed red beet (*B. vulgaris* var. conditiva) in root when identifying 3 flavonoids (quercetin, myricetin, and kaempferol), while in stems 4 flavonoids were identified (rutin, quercetin, myricetin, and kaempferol) (Babagil et al. 2018, El-Beltagi et al. 2018). In the same way, Pyo et al. (2004) analyzed Swiss chard red and white, identifying some flavonoids such as catechin, myricetin, quercetin, and kaempferol in leaf and stem, being kaempferol the highest compound. On the other hand, the major flavonoid in beetroot pomace was catechin (37.96 mg/100 g^{-} dry weight), followed by epicatechin (0.39 mg/100 g dry weight) and rutin (0.25 mg/100 g dry weight) (Heleno et al. 2015).

Other flavonoids, such as betagarin, betavulgarin, cochliophilin A, and dihydroisorhamnetin have been identified in beetroot (*Beta vulgaris*) (Georgiev et al. 2010).

Moreover, xylosylvitexin, glucopyranosyl-glucopyranosyl-rhamnetin, and glucopyranosyl-xylosyl-rhamnetin have been reported in seeds of *B. vulgaris* var. *cicla* (Vulić et al. 2012).

Similarly, Burri et al. (2017) analyzed diverse horticultural sources not used frequently, identifying some flavonoids like xylosylvitexin, rutin, glucopyranosyl-glucopyranosyl-rhamnetin, and glucopyranosyl-xylosyl-rhamnetin in Leaves of beetroot. Likewise, Ninfali and Angelino (2013) analyzed the main flavonoids present in Swiss chard leaves (*B. vulgaris* ssp. cicla) finding vitexin-2”*O*-rhamnoside, 2”-xylosylvitexin, isorhamnetin-3-gentiobioside, and rutin. In general, it could be inferred that beetroot is a good source of flavonoids, with an important amount and variety in the profile of these polyphenolic compounds.

Betalains

Betalains are a group of nitrogenous natural pigments; these compounds are characterized by being soluble in water and can be classified into two classes of compounds; betaxanthins (yellow-orange) and betacyanins (red-violet) (Ninfali and Angelino 2013, Tanaka, Sasaki, and Ohmiya 2008). Betalains, as well as other pigments, can function as visual attractants for pollinators and animals that facilitate its dispersion, besides it has been reported that they can be a response to some types of stress and have other physiological functions (Moreno et al. 2008, Strack, Vogt, and Schliemann 2003). Betalains are part of the secondary metabolism of plants and its biosynthesis, in general, has two ways; betaxanthins which are the result of the condensation of betalamic acid and several amino compounds, while betacyanins are obtained by condensation of betalamic acid and c-DOPA (dihydroxyphenylalanine) derivatives (Gengatharan, Dykes, and Choo 2015, Stafford 1994, Brockington et al. 2011).

In most of the families of Caryophyllales betalains replace the anthocyanins (Azeredo 2009). In this context, the primary sources of betalains are Swiss chard (*Beta vulgaris* ssp. *cicla*), amaranth (*Amaranthus* spp.), prickly pears (*Opuntia* spp.), pitahaya (*Hylocereus* spp.), and beetroot (*B. vulgaris* ssp. *vulgaris*), highlighting beetroot (red and yellow), for being the most important and the most marketed in the world (Chauhan et al. 2013).

Several researchers have described an amount and variety of important betalains in beetroot; however, this will depend on certain factors such as variety, maturity, geographical conditions, and environmental factors, among others. As reported by Montes-Lora et al. (2018) who analyzed the betalains content in three stages of maturity of *B. vulgaris* (cv. Pablo), finding a significantly higher content of betalains in the middle state of maturity and identifying several compounds of betacyanins (betanin, isobetanin and gomphrenin I) and betaxanthins (muscaarin, vulgaxanthin I, aminobutyric acid-betaxanthin, indicaxanthin, isoleucine-betaxanthin, leucine-betaxanthin and phenylalanine-betaxanthin).

Additionally, Koss-Mikołajczyk et al. (2019) analyzed the betalains content in beetroot white and red pulp and managed to identify vulgaxanthin I, dopamine-betaxanthin, phenylalanine-betaxanthin, tyrosine-betaxanthin, tryptophan-betaxanthin, 2-decarboxy-neobetanin, 17-decarboxy-neobetanin, betanin and isobetanin in red beetroot corresponding to betanin (3.234 mg/g dry weight) as the highest compound, whereas in white beetroot betalains were not detected. Similarly, Lee et al. (2014) analyzed the effect of the type of production (greenhouse or field) on the composition of nine beetroot cultivars. Betalains were in higher concentration in beetroot produced in the field. Betanin was the main compound in the red varieties, while in the yellow variety was vulgaxanthin I. In another study it was determined the betalains content in red beetroot (*Beta vulgaris* ssp. *vulgaris*) depending on the harvest time (autumn and spring), observing a greater amount in spring (8.38 mg/g fresh weight) than in autumn (4.62 mg/g fresh weight) (Bucur, Ţarălungă, and Schroder 2016).

The variation of betalains has also been investigated depending on the plant part, finding interesting results as reported by Slatnar et al. (2015) who studied the profile of betalains in the peel, pulp, and petiole of three cultivars of beetroot two red (Pablo F1 and Taunus) and one yellow (Bolfor). The highest content was observed in peel (5.33-31.04 mg/g dry weight) followed by pulp (0.35-8.65 mg/g dry weight) and petiole (0.85-11.10 mg/g dry weight). Also, the highest concentrations corresponded to beetroot red cultivars. Fifteen betacyanins and derivatives were identified in the peel, 9 in pulp and 13 in the petiole, being betanin and isobetanin with the highest concentration, while in yellow beetroot no presence of betacyanins

was observed. In the case of betaxanthins, red beetroot presented 3 in peel and 3 in the pulp, while yellow beetroot showed 7 betaxanthins in the peel, 4 in pulp and 5 in the petiole, being vulgaxanthin I the highest for all cultivars.

Similar results were reported in red beet (*B. vulgaris* ssp. conditiva), by identifying vulgaxanthin I, betanin and isobetanin in root and stems, showing a higher concentration of betacyanins and betaxanthins in root (53 y 46 mg/g) than in stems (11 and 10.4 mg/g) (Balasundram, Sundram, and Samman 2006). In another study of red beetroot (*Beta vulgaris* ssp. *vulgaris*) a similar behavior was observed when presenting a higher content of betalains in peel (10.76 mg/g dry weight) than in pulp (7.56 mg/g dry weight) and identifying 9 betacyanins (betanin, isobetanin, betanidin, 17-decarboxy-neobetanin, neobetanin, isobetanidin, 2-decarboxy-neobetanin, 2,17-bidecarboxy-betanidin and 6´-*O*-feruloyl-betanin) and 2 betaxanthins (vulgaxanthin I and dopamine-betaxanthin) (Sawicki et al. 2019).

Other researchers focused on the use of waste from the food industry, analyzed the content of betalains in beetroot pomace obtained from a juice processing factory, finding the content of betacyanins of 4.09 mg betanin equivalents/g and betaxanthins of 7.32 mg vulgaxanthin-I equivalents/g (Georgiev et al. 2010). In the same way, Kushwaha et al. (2018), analyzed several extraction variables to obtain betalains and phenolic compounds of beetroot pomace and found that the optimal extraction conditions correspond to solid to liquid ratio 1:15, temperature 50.4°C, extraction time 10 min and pH 2.50, with responsibility for total phenolics of 156.54 mg/100 mL and betalains of 17.17 and 15.04 mg15.04 mg/L for betacyanins and betaxanthins, respectively.

Carotenoids

Carotenoids are pigmented compounds responsible for red, yellow, and orange colorations of fungi, some animals and insects, and plants mainly. In plants, carotenoids participate in the process of photosynthesis and protect against photo-oxidation (Delgado-Vargas, Jiménez, and Paredes-López 2000). The carotenoids are isoprenoids, and their general structure consists of eight isoprene units where their order is inverted in the center of the molecule, the different carotenoids are derived from the modifications in the structure by cyclization, oxygen insertion, double bonds and change in chain (elongation or shortening) mainly (Delgado-Vargas, Jiménez, and Paredes-López 2000, Yamamoto and Bassi 1996). Several studies have shown that carotenoids have a protective effect against diseases such as some types of cancer and cardiovascular diseases (Rao and Rao 2007). Therefore, his study is of great interest.

The carotenoids and flavonoids are the natural pigments mostly distributed in the plants; however, in the order of the Caryophyllales, some families are characterized by containing betalains as the natural pigments responsible for the colorations (Fiedor and Burda 2014). Therefore, many of the researches carried out in beetroot focus mainly on the study of betalains. In this sense, there is little information in the content and profile of carotenoids in beetroot.

Some researchers reported the content of total carotenoid of 1.31 mg/100 g (Ramos et al. 2018) and 225.3 µg/100 mL of extract (Singh et al. 2015). Similarly, Rebecca et al. (2014) analyzed the content of carotenoids of different vegetables, finding in beetroot a concentration of 1.9 mg/100g fresh weight, being lower than that in carrots, spinach, red capsicum, and yellow capsicum.

Other researchers, as Dias et al. (2009) found β-carotene (2.5 mg/100 g fresh weight) and lutein (4.4 mg 100/g fresh weight) in leaves of *B. vulgaris* var. *vulgaris*. Likewise, Mamatha, Sangeetha, and Baskaran (2011) reported the content of 0.31, 0.02, 0.02 mg/100 g dry weight, for lutein, zeaxanthin, and β-carotene, respectively. Additionally, they have been found in leaves and stems of beet (*Beta vulgaris*) β-carotene (1.36 mg/100 g fresh weight) and lutein (1.96 mg/100 g fresh weight). Also, lutein (2.08-5.13 mg/100 g fresh weight) and β-carotene (1.56-4.48 mg/100 g fresh weight) have been reported in *B. vulgaris* var. flavescens and *B. vulgaris* var. *vulgaris* (Reif et al. 2013).

Phenolic Amides

Although the importance of the study of phenolic amides has grown in recent years, since they have shown some antioxidant, anti-inflammatory, antiproliferative and antigenotoxic effects (Boz 2015), in relation to other compounds with similar properties these are found in a smaller proportion, so their presence in food is of great interest (Lee et al. 2015), this compounds are scarcely studied.

In beetroot, some phenolic amides have been identified like *N-trans*-feruloyltyramine and *N-trans*-feruloylhomovanillylamine (Kim et al. 2003). Likewise, they have been found in seeds of *B. vulgaris* var. *cicla* four phenolic amides (*N-cis*-feruloyl 3-O-methyldopamine, *N-cis*-feruloyl tyramine, *N-trans*-feruloyl 3-O-methyldopamine, and *N-trans*-feruloyl tyramine) (Dai and Mumper 2010).

BENEFICIAL EFFECTS OF BEETROOT ON VARIOUS DISEASES

Beetroot contains many betalains; among the betalain group, exist two classes of compounds: betaxanthin and betacyanins (Stintzing and Carle 2004), phenolic acids, and flavonoids compounds that possess potent antioxidant, anti-inflammatory and chemo-preventive activity *in vitro* and *in vivo*. Beetroot beside provides nitric oxide (NO), which has a notable effect on hypertension and endothelial function. Beetroot is also being considered as a promising treatment in pathologies associated with oxidative stress. Thus, beetroot has phytochemicals and bioactivities with the benefits in human health (Figure 2).

Antioxidant Activity

Various methods have been used to evaluate the antioxidant activity of extracts from the different parts of beetroot. The authors, in general, attribute this activity to the presence of compounds of phenolic nature (Edziri et al. 2019, Kavalcová et al. 2015). Table 4 shows the different methods that have been used to determine the antioxidant activity of various parts of beets.

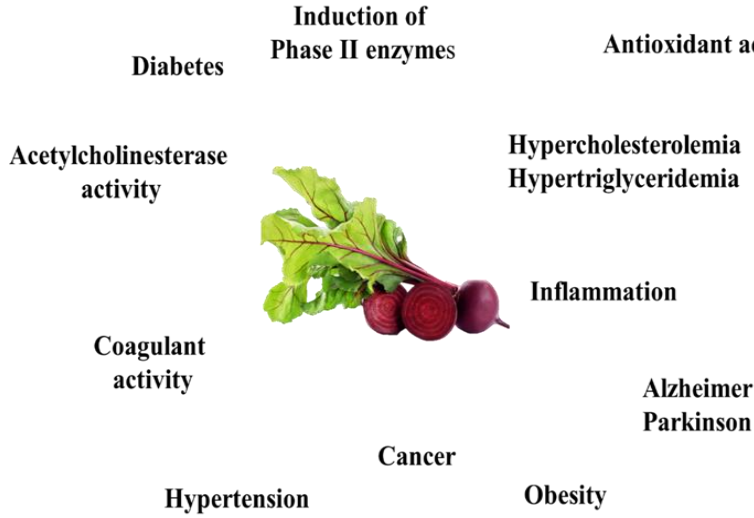

Figure 2. Beneficial effects of beetroot on various diseases.

Table 4. Methods that have been used to determine the antioxidant activity of various parts of beets

Plant part	Type of extraction	Methods	Reference
Root	95% Ethanol	ORAC	(Nahla, Wisam, and Tariq 2018)
Root (*Beta vulgaris* var. *rubra*)	80% ethanol	DPPH$^{\cdot}$	(Dai and Mumper 2010)
Root (*Beta vulgaris*)	Water 95% Ethanol	Reducing power Chelating capacity	(Wettasinghe et al. 2002)
Red beetroot	50% ethanol	DPPH	(Ravichandran et al. 2012, Bavec et al. 2010)
Root (*Beta vulgaris*)	95% Ethanol	Inhibition of β-carotene bleaching	(Zade, Charde, and Charde 2011)
Beet root pomace	50% ethanol	DPPH Superoxide anion radicals	(Vulić et al. 2012)
Beetroot, Chips, Powder	Juice Water	Folin-Ciocalteu assay	(Vasconcellos et al. 2016)
Root	95% ethanol	DPPH	(Lee et al. 2015)
Hairy root Beet root	70% ethanol	ORAC DPPH	(El-Beltagi et al. 2018)
Red Beet	Water	DPPH	(Kavalcová et al. 2015)
Leaves of Chard (*Beta vulgaris* var. *cicla*)	Boiled water for 30 min	Hydroxyl radical scavenging activity	(Zein, Hashish, and Ismaiel 2015)
White Organic Beetroot	Juice	FRAP ABTS CUPRAC	(Wootton-Beard et al. 2014)

ABTS$^{\bullet+}$: 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
DPPH: 2.2-diphenyl-1-picrylhydrazyl
ORAC: Oxygen radical absorbance capacity
CUPRAC: Copper II reducing antioxidant capacity

Anti-Diabetes Effects

Currently, diabetes type 2 is considered a worldwide epidemic, it is predicted to reach 360 million cases by the year 2030 (Wild et al. 2004), because of that, several investigations have a focus on the anti-hyperglycemic effects of plant materials (Rahimi et al. 2005).

As mentioned before, beetroot is a rich source of polyphenols and betalains. These compounds are suggested to modify postprandial glycemia by inhibiting carbohydrate digestion and absorption, stimulation of insulin release from pancreatic cells, modulation of hepatic glucose output, activation of insulin receptors, or modulation of glucose uptake in insulin-sensitive cells, which have a high potential to reduce the impact of diabetes type 2 (Wild et al. 2004).

To evaluate the influence from beetroot bioactive components on the glycemic response, either by direct inhibition of glucose uptake or by indirect action affecting insulin sensitivity, Wootton-Beard et al. (2014) measured the postprandial glucose and insulin responses attributed to the intake of either beetroot juice, a control beverage matched for macronutrient content or a glucose beverage in healthy adults. According to their results, the beetroot juice treated group showed a significant decrease in glucose response in the early phase (0-30 min) and postprandial insulinemia until 60 min phase. The effect was attributed to betanins (particularly neobetanin), and nitrate, even more than phenolics since the flavonoids and phenolic acids contents in beetroot juice were considered too low to account for the observed effects.

In a study conducted by Olumese and Oboh (2016), blood samples were collected from subjects on Day 0 (Phase I: Control). The subjects were given a 10% beetroot juice (BRJ) solution daily for six weeks, and blood samples were collected (Phase II: Intervention). After that, a two-week washout period (Phase III: washout period, no BRJ administered). 10% Beetroot administered to the subjects for this study contained 9808 mg Gallic Acid Equivalent/100 mL of polyphenols and 8334.0 mg Quercetin Equivalents/100 mL of flavonoids. Administration of beetroot juice for six weeks led to significantly reduced blood glucose levels from 76.1 mg/dL to 49.8 mg/dL, the changes observed were in the intervention or Phase II groups, there were no significant differences between the blood glucose levels in Phase I (Control group) and phase III (washout period).

The development of diabetes is among others associated with the activity of gut enzymes, catalyzing the degradation of starch α-amylase and α-glucosidase. Therefore, their inhibitors are believed to be useful in managing the early stages of diabetes by modulating breakdown of carbohydrates.

The mechanism for the hypoglycemic action of the extract has been tentatively attributed to saponins that inhibit gluconeogenesis and glycogenolysis (Bradley et al. 2013). Other evidence suggested that the hypoglycemic activity of Beetroot extract may be due to flavonoids, through the inhibition of glucose transporters.

Anti-Obesity Properties

Obesity is often accompanied by insulin resistance, and both are characterized by low nitric oxide (NO) bioavailability. Low NO bioavailability is related to insulin resistance, presumably due to an interruption of lipid metabolism, increased fat mass, and/or decreased in glucose delivery. Because of that, improve the stimulation of NO generating pathways may be an

excellent strategy to increase insulin sensitivity, so, according to several authors, the ingestion of dietary nitrate may be an attractive option (Bradley et al. 2013). The intake of dietary nitrate (NO_3) is not enough, but the generation of NO increases when NO_3 is reduced to NO_2 by commensal bacteria in the oral cavity. Later, NO_2 is converted in NO in the stomach, which complements endogenous NO production from L-arginine; this is known as the nitrate-nitrite-NO pathway (Beals et al. 2017).

Beals et al. (2017) treated obese and nonobese adults with beetroot juice (approx. 17 mmol NO_3) and a dose of glucose (25 g), with or without prior antibacterial mouth wash to inhibit NO_3 reduction to NO_2. Those authors found that blood glucose concentration after beet juice plus glucose consumption was greater while insulin sensitivity was lower in obese adults compared with nonobese. Also, inhibition of oral bacteria nitrate reductase activity with mouthwash did not influence blood glucose or insulin in either group, but mouthwash decreased insulin sensitivity only in obese adults, they demonstrated that the metabolic response to beet juice is more benign in overweight adults when nitrate reductase activity is not inhibited.

Moreover, obesity is related to low-grade chronic inflammation, and the induction of metabolic disorders, such as weight gain, high blood glucose levels, and high total cholesterol levels even may cause liver damage strongly associated with oxidative stress (Lorizola et al. 2018). In this regard, a positive effect of beetroot and its derivatives on oxidative stress and inflammation has been demonstrated. Those effects are presumably due to the antioxidant activity of phenolic compounds such as gallic acid, vanillic acid, chlorogenic acid, ferulic acid, caffeic acid and syringic acid and flavonoids like myricetin, quercetin, rutin, and kaempferol, also by enhancing endogenous antioxidant defenses, helping to protect cells from oxidative damage (Koss-Mikołajczyk et al. 2019).

Phenolic compounds are present in the entire plant, thus, vegetal material non edible could be used as a good source of interest compounds, as it was demonstrated by Lorizola et al. (2018), they evaluated the effect of beetroot stalks and leaves ethanol extracts on oxidative stress, using an experimental model of high-fat diet-induced obesity in Swiss male mice.

Lorizola et al. (2018) concluded that even beetroot stalks and leaves did not affect final weight gain, the supplementation with it prevented many of the alterations that resulted from obesity in mice, decreasing fasting glucose and cholesterol levels. Those authors associated their results with the action of vitexin-2-O-rhamnoside-related compounds. They also observed that beetroot stalks and leaves enhance the activity of hepatic antioxidant enzymes, reducing the liver damage caused by a high-fat diet.

Anti-Hypertension Characteristics

High blood pressure is a frequent public health problem and is a major cause of heart disease; there are frequent studies that show beetroot significantly decreases systolic and diastolic blood pressure. Beetroot's effect on the blood vessels is highly related to its high inorganic content, it also contains a high number of phytochemical compounds that includes ascorbic acid, carotenoids, flavonoids and phenolic acids (Lundberg, Weitzberg, and Gladwin 2008).

Beetroot has been related to a hypotensive effect, mostly because its high content of inorganic NO_3 (250 mg/kg of fresh weight) (Kerley, Dolan, and Cormican 2017), this may vary according to the form of consumption, it has been observed that the content of NO_3 is more

significant in the juice than chips or dried powder (Vasconcellos et al. 2016). Several studies have shown that NO metabolites are significantly lower in hypertension, but it has been proposed that inorganic NO_3 can be recycled *in vivo* to form NO, which is an important physiologic mediator which regulate blood pressure, representing an important alternative source of NO to the L-arginine-NO-synthase pathway (Bahadoran et al. 2017, Ambriz-Perez et al. 2016).

In this sense, many studies have been performed, under different conditions such as exposition time, beetroot juice doses, age of patients, etc. Bahadoran et al. (2017) executed a systematic review and meta-analysis to clarify the effect of beetroot juice supplementation on systolic blood pressure and diastolic blood pressure. A positive correlation of beetroot juice doses and exposition time with the most significant differences in blood pressures and, also, a smaller effect after supplementation with higher NO_3 doses was found; suggesting that the hypotensive effect of the beetroot goes beyond NO_3 content. Moreover, beetroot exerts its beneficial effects through other bioactive compounds such as betalains (betacyanins and betaxanthins), flavonoids, and polyphenols (Bahadoran et al. 2017).

Phenolic compounds are related to the inhibition of the NF-κB metabolic pathway (Leyva-Lopez et al. 2016). This pathway has typically been suggested as one of the mechanisms responsible for inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) upregulation. Nevertheless, Matuskova et al. (2013) carried out a study in male Wistar-Kyoto rats brains where NF-κB pathway seems to have a different behavior, they observed that increased NF-κB expression in the brain was associated with downregulation of eNOS and iNOS leading to decreased NOS, with a concomitant decrease in NO, what is one of the main causes of hypertension.

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 (Ben Haj Koubaier et al. 2014). Several studies (Jajja et al. 2014, Webb et al. 2008) observed that ingestion of a single dose of beetroot juice (500 mL; 23 mmol nitrate) by fourteen healthy subjects reduced systolic BP (SBP) and DBP by ten and eight mmHg. Kapil et al. (2010), showed that consumption of beetroot juice (250 mL: 5 mmol nitrate) reduces SBP by 5 mmHg in nine healthy subjects. In a similar study, Coles and Clifton (2012) demonstrated that the ingestion of beetroot juice (500 mL, 9mmol nitrate) resulted in a DBP reduction of 8 mmHg.

Asgary et al. (2016) examined the effect of raw beet juice (RBJ) and cooked beet (CB) on BP of hypertensive subjects. Twenty-four hypertensive subjects aged 25 to 68 years old were divided into two groups. One group took RBJ for two weeks, and the other group took CB. After two weeks of treatment, both groups had a washout for two weeks then switched to the alternate treatment. Each participant consumed 250 mL per day of RBJ or 250 g per day of CB each for two weeks; after this time they concluded that d that short-term supplementation with either RBJ or CB can effectively improve BP, but RBJ has a greater effect compared with CB. This result suggested that RBJ could be an effective supplement in patients with hypertension except for diabetic patients who should be examined before the administration of beet-derived preparations. Another study demonstrated that in free-living people consuming an unrestricted diet and a single dose of 500 g of beetroot and apple juice, a trend to lower blood pressure by 4–5 mmHg at 6-h was observed but only in men (Coles and Clifton 2012).

A reduction in SBP in the magnitude of 5 mmHg has been correlated to a cardiovascular mortality reduction of approximately 10% at the population level.

Anti-Hypercholesterolemia Activity

Al-Dosari et al. (2011) examined the possible anti-hypercholesterolemic, the potential of lyophilized aqueous *Beta vulgaris* extract (BVE) in cholesterol-rich diet-induced hypercholesterolemia in rats. BVE at the doses of 250 and 500 mg/kg body weight for 70 consecutive days showed a significant decrease in total cholesterol and triglycerides. The acute toxicity test of BVE showed no mortality or morbidity in rats; they concluded that the lipid-lowering potential of beetroot may be due to flavonoids and/or saponins, which were found in beetroot.

In another study, (Wroblewska, Juskiewicz, and Wiczkowski 2011) evaluated the effect of beetroot crisps in the rat diet at 0.3, 1 or 3%, which corresponds to a daily intake of 18, 60 and 180 g beetroot crisps for adult consumers with an average body weight of 70 kg. The experimental diets were administered for four weeks to 8 at male Wistar rats aged approximately four weeks. The standard basal diet containing 8% soybean oil and 0.3% cholesterol was supplemented with different amounts of beetroot crisps: 0, 0.3, 1, or 3%. The dyslipidemia basal diet was prepared with the aid of lard instead of oil, an extended amount of cholesterol - 1%, and 0.5% cholic acid, and was supplemented with the same amounts of crisps. Betalains analysis in red beet crispy showed that betacyanins content was 4.10 mg/g, whereas betaxanthin content was 2.80 mg/g. Betacyanins were present as betanin (57%), isobetanin (35%), and neobetanin (1.4%). In the case of betaxanthins, the predominant compound was vulgaxanthin (70%). Beetroot crisps did not significantly affect final body weight, but final body weights were significantly influenced by fat type supplementation. Diet supplementation with 0.3 and 1% crisps decreased the triacylglycerol level by about 20%. The findings indicate that beetroot has a significant effect on rats possibly exerted by the phytoconstituents.

Anti-Hypertriglyceridemia Activity

Hypertriglyceridemia is considered another critical CVDs risk factor and is known as the significant risk factor for atherosclerotic cardiovascular disease (ASCVD), reasons why its prevention and reduction should be of the utmost relevance. The nitrate-nitrite-NO pathway has shown a reduction in abnormal triglycerides (TGs) levels, among other beneficial effects, this means there exists a pathway for increasing NO bioavailability within the body through supplementing NO or NO_3 abundant food component (Stokes et al. 2009, Peng et al. 2017). Zand et al. (2011) prepared a supplement formulation called Neo40 Daily®, with beetroot and Hawthorn berry since they have the highest nitrate content and nitrite reductase activity, respectively. Patients older than 40 years presenting three or more cardiovascular risk factors, were treated with the supplement twice a day by 30 days. After this period authors observed a significant increase in both plasma nitrite and nitrate, which indicates an increase in NO availability, also they found a significant reduction (27%) in elevated triglycerides level in the 72% of patients analyzed. They conclude that bioactive compounds present in the supplement could be a key in the NO homeostasis and make the nitrate-nitrite-NO pathway more efficient. Therefore, they could modify risk factors for CVDs and ASCVD.

Anti-Cancer Properties

Numerous *in vitro* studies have demonstrated the activity of various bet root extracts against the tumorigenic process and proved that the diets high in phytochemicals which include fruits and vegetables, contribute toward reducing the risk for certain types of human cancers (Kapadia et al. 1996). Bioactive compounds in beetroot impart anticancer effects through various complementary and overlapping mechanisms of action, including the induction of metabolizing enzymes; modulation of gene expression; and their effects on cell proliferation, apoptosis, and subcellular signaling pathways. The ability to inhibit the growth of cancer cells is often proposed as a feature confirming that plant bioactive phytochemicals can serve as chemopreventive agents (Doughari 2012).

There are several reports about the benefits of betalains in humans. In an experiment by Kapadia et al. (1996), beetroot was used by two groups of patients who have 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.

Besides, 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.

Koss-Mikołajczyk et al. (2019) found that extracts of red beetroot showed rather low or no cytotoxic effect towards human colon cancer HT29 cells, but white beetroot extract diminished cell survival by 50% after 24 h incubation.

Because red variety did not show as significant cytotoxic effect as the white variety extract, it can be presumed, that the red type was devoid of cytotoxic compound(s) present in the other variety, and that betalains were not responsible for growth inhibitory effect towards HT29 cells.

Another investigation reported that antitumoral activity oral consumption of water containing 78 mg/mL of the extract of red beetroot showed inhibitory activity against *N*-nitrosomethylbenzylamine (NMBA)-induced tumors in the esophagus of rats. Moreover, the plant extracts improved apoptosis and decreased angiogenesis and inflammation in cancerous cells in rats (Lechner et al. 2010).

In three different experimental, Kapadia et al. (2003) reported that a shallow dose of betanin (0.0025%) from beetroot acts as a potent chemopreventive agent in tumor models in mice. On the other hand, *Beta vulgaris* ethanolic extract exhibit significant anticancer activity against lung (A549) but a slight effect against colorectal adenocarcinoma Caco-2 cell lines at the high concentrations of ethanolic extract (800 μg/mL) (El-Beltagi et al. 2018).

The studies mentioned above show that *Beta vulgaris* contains phenolic groups, flavonoids, and betalains. Polyphenolic compounds might inhibit cancer cells by xenobiotic-metabolizing enzymes that alter metabolic activation of potential carcinogens (Lopez-Martinez, Parkin, and Garcia 2011).

The mechanism of action of anticancer activity of phenolic compounds could be by disturbing the cellular division during mitosis at the telophase stage. Phenolics can reduce the amount of cellular protein, mitotic index and colony formation during cell proliferation of cancer cells.

Anti-Inflammatory Effects

The anti-inflammatory potential of aqueous extract of leaves of *B. vulgaris* var. *bengalensis* were assessed in an *in vivo* model by carrageenan-induced rat paw edema method for acute inflammation. Edema was induced by injecting 0.1 ml of a 1% (w/v) solution of carrageenan into the sub plantar aponeurosis of the right hind paw of the Albino Wistar rats. The vehicle, extracts (500 mg/kg and 1000 mg/kg of aqueous extract), and the standard drug (indomethacin 10 mg/kg) were administered orally 60 min before the injection of the carrageenan. The volumes of edema of the injected and the contralateral paws were measured 5 hrs. after the induction of inflammation using a plethysmograph to calculate the percentage of anti-inflammatory activity. The development of carrageenan-induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin, and the delayed phase is sustained by the leukotrienes and prostaglandins (Joosten et al. 2006, Sitonio et al. 2013). These studies revealed that compounds present in aqueous extracts (flavonoids, saponins, and tannins) from leaves were active against inflammation-induced, although the effect was less pronounced than that of indomethacin. These findings suggested that beetroot-containing diets might have the potential to improve health.

In a different study, Zade, Charde, and Charde (2011) performed the anti-inflammatory activity by carrageenan-induced rat paw edema method. The acute toxicity study of ethanolic extract of roots of *B. vulgaris* does not show any signs of toxicity up to 3g/kg body weight. Since there was no mortality at a higher dose 1/10th of the maximum dose of extract tested for acute toxicity was screened for evaluation of wound healing activity i.e., 300mg/kg.

The determination of anti-inflammatory activity is based on plethysmographic measurement of edema produced by sub-plant injection of carrageenan in hind paw of the rat. The increase edema in animal treated standard (Ibuprofen gel) and *Beta vulgaris* extract were composed with an increase in edema of untreated control animals at a constant interval of 1, 2,3, and 4 hrs. The percentage inhibition of edema at a known range in treated animals was used to calculate the percent inhibition of edema of control. The study revealed that the BV extract showed significant inhibition of edema. The maximum activity showed during 2nd and 3rd hrs., the results are highly significant as compared to standard. The anti-inflammatory activity of beta vulgaris may be due to inhibition of release of histamine, serotonin and kinins in the first hour after an injection of carrageenan and also retard the release of prostaglandin and like substances in 2-4hr 13, shows anti-inflammatory activity of *B. vulgaris* extract (Charde et al. 2010).

Neuroprotective Properties

In the developed world, life expectancy is increasing, with an increment of many age-related diseases, such as neurodegeneration (Franceschi et al. 2018). Substantial evidence supports the hypothesis that oxidative stress plays a major role in the pathogenesis of neurodegenerative diseases. Oxidative stress is generally caused by the excessive accumulation of ROS in cells and has been implicated in the development of many degenerative diseases, including Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Alzheimer's disease (Liu et al. 2017, Rooke et al. 2011). Phytochemicals have been reported

to exert a beneficial effect in this type of condition. Beetroot is a nitric oxide (NO) generator having potential to improve cerebrovascular flow.

Anti-Parkinson Effects

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder of the central nervous system (CNS) and is the second most common neurodegenerative disorder after Alzheimer's disease (Nade et al. 2013).

Nade et al. (2015) demonstrated the anti-Parkinson activity of *B. vulgaris* in experimental animals. Parkinson was induced by administration of reserpine (5 mg/kg/day for 5 consecutive days), haloperidol (1 mg/kg), and tacrine (2.5 mg/kg).

The symptoms of the disease, such as tremors, akinesia, rigidity, catalepsy, and vacuous chewing movements (VCMs) were evaluated. The foot shock-induced aggression (FSIA) model was used to confirm anti-parkinsonian activity. The methanolic extract of *Beta vulgaris* (MEBV) was administered at doses of 100, 200, and 300 mg/kg (Nade et al. 2013). The combination of L-dopa and carbidopa was used as a standard drug. They found that pretreatment with MEBV (200 and 300 mg/kg) significantly reduced the intensity of muscular rigidity, duration of catalepsy, akinesia, the number of tremors, VCMs, and increase fighting behavior. MEBV significantly increased locomotor activity and grip strength. Oxidative stress is one of the major reasons for nerve damage in many neurodegenerative disorders (Nade et al. 2013). In Parkinson's disease, oxidation of dopamine by monoamine oxidase-B, and aldehyde dehydrogenase generates hydroxyl free radicals in the presence of ferrous ions (basal ganglia are rich in iron).

Anti-Acetylcholinesterase Activity

Oxidative stress plays a crucial role in neurodegenerative diseases such as Alzheimer's disease (AD) via lipid peroxidation of the cell membrane of the neurons. Pathogenesis of AD has not been clarified; however, the most accepted theory is known as the 'cholinergic hypothesis', which is based on insufficiency in the amount of neuro-mediator acetylcholine, which is broken down by acetylcholinesterase (Colović et al. 2013, Schliebs and Arendt 2006). Therefore, AChE inhibitors have become the widely used treatment against AD; however, these inhibitors are effective only against the mild type of AD and possess adverse side-effects (Peksel, Arisan, and Yanardag 2013). Thus, efforts have focused on plant phytochemicals as natural sources of effective AChE inhibitors with little or no side effects which could be used as a dietary intervention in the management of this disease.

Acetylcholinesterase enzyme inhibitor deficiency is one of the hallmarks of Alzheimer's disease and responsible for most of its symptoms, such as declining memory and cognition (Nade et al. 2013, Maczurek et al. 2008). Colović et al. (2013) evaluated the anti-acetylcholinesterase inhibitory activity of chard was found to increase dose-dependently. a high AChE inhibition (20.98%) was seen in 6 μg/mL (EC_{50} values were found 14.43 μg/mL). Sterols, terpenoids, oils, flavonoids, alkaloids, and other phenolic compounds possess anti-acetylcholinesterase properties (Singh and Rishi 2005, Ji and Zhang 2008). These constituents, flavonoids may have a contribution to the occurrence of antioxidant and anti-

acetylcholinesterase activity of chard, they concluded that that chard extract could be used as an accessible source of natural antioxidants and antiacetylcholinesterase with consequent health benefits.

Anticoagulant Activity

Studies documenting the anticoagulant activity of *B. vulgaris* are scarce. The anticoagulant activities were evaluated by the Prothrombin time (PT) test and activated partial thromboplastin time (aPTT). Plasma (100 µl) for from healthy volunteers was mixed with 50 µl of extracts, or beetroot juice were incubated at 37°C for 5 min at 37°C. Then, 200 µl of PT assay reagent (rabbit brain extract and calcium chloride) pre-warmed at 37°C for 10 min was added and a digital coagulometer recorded the clotting time. Normal saline was used instead of the extracts as a negative control. Heparin (1 IU/mL) was used as a positive control. The aPTT test was used to evaluate coagulation factors VIII, IX, XI, XII, and prekallikrein in the intrinsic coagulation pathway, and PT is used to evaluate the coagulation factors V, VII, and X in extrinsic coagulation pathway (Lapikova et al. 2008). The results of PT and aPTT of *Beta vulgaris* extracts and juice are shown in Figure 3.

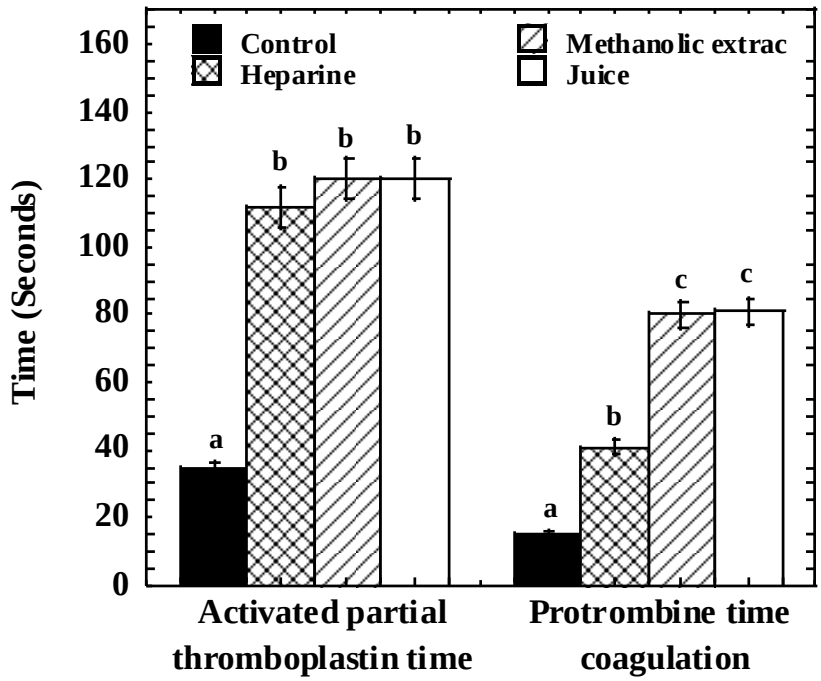

Figure 3. Effect of juice and beet methanol extracts on the blood coagulation time.

The highest prolongations of PT were among the juice and methanolic extract with 80 and 80.9 s, respectively. These extracts had better anticoagulant activity than the positive control heparin. Besides, the prolongation of aPTT indicates an inhibition of the intrinsic and/or common pathway of coagulation. The juice and methanolic extract of *Beta vulgaris* had an

aPTT of 120 s indicating they had significant anticoagulant activities. No data are documenting the anticoagulant activity of *Beta vulgaris*. The authors suggested that beetroot could be used to treat venous fragility or varicose veins. This activity can be attributed to the high amount of total polyphenol and flavonoids as demonstrated in other studies (Pawlaczyk et al. 2013, Mao et al. 2009).

Antinociceptive Properties

When tissue damage occurs, the body releases pro-inflammatory substances such as prostaglandins that play a vital role in mediating pain and inflammation. Hence by reducing the production of these prostaglandins, the analgesic effect can be produced. For the evaluation of anti-nociceptive activity, chemical and thermal methods of nociception were used in mice. In the chemical process, acetic acid writhing test and thermal methods, hot plate, and tail-flick tests were performed. *Beta vulgaris* lyophilized powder 300 mg/kg inhibited the abdominal constrictions induced by acetic acid and increased the pain threshold of mice towards the thermal source in a dose-dependent manner. The activity exhibited by the extracts was comparable to that of the standard drug aspirin (300 mg/kg), Sarfaraz and Ikram (2019) conclude that lyophilized powder of *Beta vulgaris* root possesses marked analgesic activity by acting both centrally and peripherally. However, further studies are required to establish the exact mechanism of action.

CONCLUSION

Studies have provided evidence that many chronic diseases can be prevented by including fruits and vegetables such as beetroot in the diet as a functional food. Epidemiological studies and clinical trials have been carried to detail information about the role of phenolic acids, flavonoids, and betalains present in beetroot in human health. The antioxidant activity, anti-inflammatory, and vascular-protective effects offered by beetroot and its constituents have been demonstrated by several *in vitro* and *in vivo* human and animal studies.

Beetroot has gained popularity and is consumed mainly as a juice, and as a supplement where studies have been conducted in rats and has been shown to reduce blood pressure, reduce inflammation, relieve oxidative stress, preserve the integrity of the endothelium, present decrease some of the effects of Parkinson's disease and restore the cerebrovascular hemodynamics.

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Chapter 10

BARBERRY: MEDICINAL PROPERTIES AND USAGE FOR METABOLIC DISORDERS PREVENTION

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ABSTRACT

Barberry (*Berberis vulgaris*) is a widely distributed species in Europe, Africa, Asia, and North America. This species has been used in traditional medicine for the treatment of gastritis and peptic ulcers, gallstones, kidney stones, and liver problems, rheumatism, diarrhea, and skin infections, but also to improve immune function. Within this chapter, its applications on diabetes –T2DM-, obesity, hypertension, dyslipidemias, hyperuricemia, cancer, Alzheimer's, and Parkinson's diseases, will reviewed. Berberine appears to have the most dramatic effects on blood insulin levels, cholesterol levels, inflammatory processes, and tumorigenesis. As these underlie or are components of a variety of metabolic disorders, berberine has the potential to have wide-reaching utility in clinical settings. Despite the lack of clinical studies, its relevance in traditional medicine has located berberine as a well-known supplement for the aid of these and other health conditions.

Keywords: berberine, diabetes, obesity, dyslipidemias, anti-inflammatory, cancer

INTRODUCTION

The genus *Berberis* belongs to the Berberidaceae, and it comprises about 500 species around the World. Barberry (*Berberis vulgaris* L.) is a thorny bush which grows from 1 m up to 3 m. Primary stems are long with short branches, glabrous, with three-fid spines; leaves are simple with serrated margins. Flowers are produced in racemes, small and yellow; berries are red or purple and fleshy. This species is native to Europe, Africa, and Asia, and it is also known as berberry, paundice berry, mountain grape, Oregon grape, pepperidge, or sow berry.

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Nowadays, barberry is naturalized in North America, but as it is susceptible to *Puccinia graminis*, barberry distribution has been severely affected (Whittemore 1993+). Barberry is used as ornamental, as natural fences, and its fruits are used for jams other dishes. Its roots and bark have been used in traditional medicine due to its many phytochemicals, but berberine and berbamine mainly (Bober et al. 2018).

Berberine (BBR) is an ammonium salt with origins in traditional Chinese medicine and Ayurveda in India. An organic heteropentacyclic compound, an alkaloid antibiotic, a botanical anti-fungal agent, and a berberine alkaloid, berberine has been used as an herbal therapy for more than 2500 years (Tomosaka et al. 2008). Its molecular formula is $C_{20}H_{18}NO_4^+$ (See Figure 1).

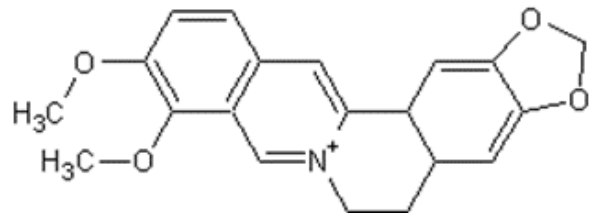

Figure 1. Berberine chemical structure.

Inflammation, hypertension and uterine bleeding (Arayne, Sultana, and Bahadur 2007), edema, gastrointestinal problems, diarrhea, hepatitis, hemorrhoids, menstrual pain, whooping cough, and liver dysfunction are examples of the diversity of problems that berberine has been used to prevent and treat in traditional medical systems, and which are still in use by upwards of 80% of the World's population (Imanshahidi and Hosseinzadeh 2008). In more modern times, research has also shown strong evidence for berberine's pharmacological effectiveness in treating hyperglycemia, dyslipidemias, allergies, and even for its efficacy in preventing certain cancers—perhaps due to its anti-inflammatory effects (Potdar, Hirwani, and Dhulap 2012).

Contemporary studies both confirm what traditional medicine systems have known for millennia about BBR's pharmaceutical potential—that it has powerful antioxidant, anti-inflammatory, antidiarrheal, and antimicrobial properties (Yin, Zhang, and Ye 2008, Mohammadi et al. 2014, Wang et al. 2010, Freile et al. 2003)—and provide insight into how this isoquinoline alkaloid can be used therapeutically as well.

Berberine has the potential for a broad range of therapeutic uses, several of which are directly relevant to metabolic syndrome: Therapeutically normalizing blood glucose levels, correcting cholesterol imbalances, and improving impaired insulin sensitivity. Together berberine's physiological effects result in improved insulin resistance, and as a result, this helps to lower blood pressure and reduce body fat as well (Imanshahidi and Hosseinzadeh 2008).

Elevated blood glucose—leading to reduced insulin sensitivity and type 2 diabetes mellitus (T2DM)—dysregulated lipids, hypertension, and obesity are all significant risk factors associated with the development of and define metabolic syndrome.

The implications for the demonstrated pharmacologic and therapeutic values of BBR might be much broader than metabolic syndrome given berberine's mechanisms of action. For example, in addition to the conditions above typically presumed to have a metabolic component—T2DM, obesity, hypertension, and dysregulated lipids—there are other conditions

whose metabolic aspects are only more recently coming under study. These include hyperuricemia, cancer, and Parkinson's disease, as well as certain cognitive disorders such as Alzheimer's disease. If these have a significant metabolic component, as it is becoming increasingly apparent may be true, then it stands to reason that these disorders may be susceptible to berberine's pharmacologic and therapeutic actions as well.

Table 1. Possible mechanisms of berberine's action in different diseases

Type 2 diabetes mellitus (T2DM)	• Increased insulin activity • Increased AMP-activated protein kinase activity • Increased antioxidant levels • Increased superoxide dismutase and catalase activation • Increased gut microbiota activity • Increase in insulin receptor mRNA • Pancreatic β-cell regeneration • Inhibition of T Helper17 and T Helper1 cell differentiation • Decreased free radical damage • Reduction in fasting blood glucose and malondialdehyde • Antioxidant effects
Obesity	• Reduced fasting blood sugar • Reduced postprandial blood sugar • Reduced fasting insulin levels • Reduced expression of retinol-binding protein4 • Reduced food intake and weight • Reduced serum glucose levels • Reduced total cholesterol and triglyceride levels • Antihypertensive
Hypertension	• Antihypertensive affecting pro-inflammatory cytokines IL-6, IL-17, and IL-23 • Vasodilatory effects due to inhibition of angiotensin-converting enzyme and stimulation of nitrous oxide and cyclic guanosine monophosphate release in vascular tissues
Dyslipidemias	• Increased cardiac fatty acid transport protein-1, fatty acid transport proteins, fatty acid beta-oxidase, and peroxisome proliferator-activated receptor-γ • Decreased proliferator-activated receptor-α mRNA and protein expression • Interference with enterocytes' absorption of intestinal cholesterol
Hyperuricemia	• Downregulation of the NLRP3 gene and IL-1β
Cancer	• Inhibition of tumor-associated microorganisms • Regulation of gene expression • Interaction with DNA and RNA • Inhibition of *N*-acetyltransferase, cyclooxygenase-2, and telomerase • Suppression of tumor cell proliferation • Facilitation of apoptosis of cancer cells
Alzheimer's disease	• Inhibition of nuclear factor-kappaB activation • Reduced malondialdehyde production and increased superoxide dismutase activity • Suppression of protein-coding gene MMP-9 and the protein EMMPRIN • Indirect action through a reduction in expression of other metabolic disorders
Parkinson's disease	• Reduced proinflammatory cytokine activity and acute-phase proteins • Reduced dopaminergic system damage • Reduction in caspase-3 activation • Increased activity of tyrosine hydroxylase-positive neurons • Increased protection against dopaminergic neuron injury • Activation of mitogen-activated protein kinases

This chapter will discuss the potential pharmacologic and therapeutic uses of berberine in this broader context of considering conditions for which a metabolic component is well established, such as disorders associated with metabolic syndrome, and other conditions where emerging evidence suggests that it may be prudent to consider metabolic interventions including berberine administration, and where doing so may be less mainstream in Western medicine.

TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases and is considered a complex metabolic disorder. As evidence of its seriousness, it is considered an independent cardiovascular risk factor (García-Fontana et al. 2016) as such T2DM is thought to be the main driver behind a large percentage of cardiovascular disease. While conventional therapies exist, about 80% of the population in the developing world rely on traditional medicine (Chung et al. 2012, Quartey et al. 2012); there, as well as in other countries, there are economic benefits to seeking less expensive alternatives to Western medicine (Robinson and Zhang 2011, Lan et al. 2015).

One such alternative, which has shown great promise in the prevention and treatment of T2DM is berberine. The antidiabetic and glucose-normalizing effects of BBR are well established for T2DM, both in in vitro and in vivo studies (Yin, Xing, and Ye 2008) and to a lesser but equally important degree, in human clinical trials as well. This section will focus on *in vivo* and human clinical trials.

In Vivo Animal Studies

Animal studies suggest varying mechanisms of action for berberine, and it is yet unclear which, or if all, mechanism(s) play a role in its effectiveness against T2DM. For example, diabetic rats injected with 50 or 100 mg/kg berberine for four weeks showed significant improvement in T2DM and other markers. Berberine seemed to result in increased glucose tolerance, and AMP-activated protein kinase (AMPK) activity and the researchers suggested this mechanism as responsible for BBR's efficacy (Zhang et al. 2012). In a study of non-obese, diabetic rats given 200mg/kg oral berberine over two weeks, on the other hand, there were similar results in terms of T2DM markers; however, in this study, there was evidence that berberine played a role in inhibiting T helper17 and T helper1 cell differentiation. The researchers thus concluded that such T helper inhibition was important for T2DM control (Cui et al. 2009).

In another *in vivo* study, two groups of T2DM rats with hypercholesterolemia (HCh) were injected with 150 and 300mg/kg berberine, respectively, for four months. T2DM markers also improved. The researchers found evidence that there was a related increase in insulin activity, pancreatic β-cell regeneration, helpful antioxidant levels, and a decrease in oxidative degradation of lipids that reduces free radical damage to lipid cells (Zhou et al. 2009).

Yet another mechanism of action is suggested by a study of T2DM mice given 100 mg/kg daily of BBR over two weeks. A reduction in fasting blood glucose (FBG) and

malondialdehyde (MDA) was seen along with an increase in superoxide dismutase (SOD) and catalase (CAT) activation (Chatuphonprasert, Lao-ong, and Jarukamjorn 2014). There is also evidence that berberine might modulate gut microbiota topically in the gastrointestinal tract where it is poorly absorbed, and thus it could have an anti-diabetic effect through a non-systemic anti-infective mechanism (Han, Lin, and Huang 2011).

Human Clinical Trials

In a representative clinical study of two diabetic populations—patients newly diagnosed with T2DM and those with poorly controlled T2DM—were significantly improved after oral administration of 0.5 g/three times per day berberine for three months. Clinically significant improvements were found in hemoglobin A1c (HbA1c), fasting blood glucose (FBG), postprandial blood glucose (PBG), triglycerides (TG), total cholesterol (TC), and low-density lipoprotein-C (LDL-C) levels (Yin, Xing, and Ye 2008).

Results of still other clinical trials suggest the same significant reductions in TG, TC, and LDL-C (Zhang et al. 2008), with some finding additional desirable effects including clinically substantial reductions in blood glucose, insulin, and apolipoprotein B levels. For example, T2DM patients administered 3 g/d berberine fruit extract orally for three months showed clinically significant reductions not only in TG, TC, and LDL-C, but in glucose, insulin, and apolipoprotein B as well (Shidfar et al. 2012).

These effects may be due to berberine's apparent propensity for increasing insulin receptor mRNA, which decreases blood glucose levels (Zhang et al. 2010). A daily dose of 1 g/d berberine in both T2DM patients and dyslipidemic (Mahady et al. 2003) patients reduced free fatty acids after three months, which the researchers speculated could be causal for the observed antidiabetic effect of berberine.

Data suggest that the mechanisms of action in the maybe be several. Berberine may favorably alter insulin sensitivity and insulin secretion, glucolipid metabolism in the liver, reduce the intestinal absorption of glucose, modulate gut microbiota. Berberine any also play an important role as an antioxidant and through its anti-inflammatory effects, and effects on lipid metabolism (Pang et al. 2015).

OBESITY

Obesity is increasingly prevalent in Western countries as well as in developing countries. Indeed, worldwide, the incidence of obesity is increasing at a rate unseen in most disorders in modern times. It knows no gender, age, or racial bounds, and socioeconomic class or country are increasingly becoming less predictive of who will experience obesity (Firouzi et al. 2018).

The exponential rise in obesity worldwide has been attributed to the Westernization of diets across the globe, as well as to marketing strategies that increasingly target children. Consequential to the rise in obesity is a concurrent rise in the incidences of cardiovascular disease and T2DM, and related stroke and hypertension, and obesity are often seen comorbid with metabolic syndrome. Data are suggesting that obesity may be an independent factor in

several cancers (Kumanyika et al. 2008). All told, it was estimated that 2.6 people worldwide current die as a result of obesity (Kral and Heymsfield 1987).

The equation that defines the reason for obesity is considered to be relatively simple: Obesity results when there is an imbalance between energy intake and energy expenditure (Firouzi et al. 2018). As simple as this equation may be, however, it is estimated that obesity at a minimum result in years-of life-lost ranging from 0.8-8.4 years among obese individuals relative to people who are not overweight, depending on age and gender (Grover et al. 2015). Obesity is, therefore, unquestionably a severe health threat with far-reaching implications for health and economics.

Current treatment recommendations for obesity most commonly include increased physical activity and calorie reduction—put, lifestyle, and diet. These are straightforward, cost little, and resources for achieving them are easily within reach for most people. Still, interventions are often unsuccessful. When unsuccessful, more drastic measures may need to be considered such as gastric bypass and other of a variety of related invasive techniques (Després and Lemieux 2006), these interventions are less straightforward, and they sometimes carry significant risks, costs can be prohibitive, and other resources to obtain them, such as availability, are important factors.

Considering the evidence for berberine's therapeutic efficacy relative to T2DM, it stands to reason that berberine might also be efficacious in reducing markers of obesity because there are many overlapping factors, such as glucose tolerance. However, while current literature includes *in vivo* and at least one clinical trial, direct evidence of the effects of berberine on obesity in humans is limited as research stands today.

In Vivo Animal Studies

The preponderance of studies that include body weight and other aspects of obesity as measures when assessing the efficacy of berberine are found in *in vivo* animal studies. Insulin-resistant obese rats, for example, were fed a high-fat diet and treated daily with berberine 0.2 g/kg body weight. After treatment for eight weeks, fasting blood sugar, postprandial blood sugar, and fasting insulin levels were significantly reduced, as was the expression of retinol-binding protein4 (Hwang et al. 2009).

In another study, obese rats on a high-fat diet were treated daily with 0.75, 1.5, or 3 mg/kg/day bodyweight for 36 days. After the treatment period significant reductions in weight gain, food intake, serum glucose levels, as well as total cholesterol and triglyceride levels were found (Hu and Davies 2010). A decrease in weight gain among diabetic rats was also observed after they were treated with 50 or 100 mg/kg body weight of berberine for four weeks (Zhang et al. 2012).

It appears that in *in vivo*, as well as in some related *in vitro* studies, the mechanisms of action relative to obesity are similar to its effects with T2DM: Normalizing insulin resistance and glycemic control, modulating lipid activity, and as an antihypertensive (Firouzi et al. 2018).

Human Clinical Trials

Human clinical trials investigating berberine's efficacy in treating obesity are fewer. Those that do exist have tended to look at markers that may be associated with obesity, or at obesity indirectly through measures evaluating weight loss or gain after treatment with berberine. For example, out of ten studies published between 2009 and 2018 as identified in a review article on berberine in the treatment of obesity and metabolic syndrome, only seven were human clinical trials and of those seven only two considered weight or body mass index (BMI)—typical measures of obesity—as direct markers (Firouzi et al. 2018). Thus, it is primarily necessary to extrapolate from other data as to how berberine may be useful in the treatment of obesity.

In one clinical study, berberine's effect on non-alcoholic fatty liver disease was investigated. However, obesity was included as a direct marker given the close relationship between obesity and non-alcoholic fatty liver disease. A total of 80 patients was assigned to either the case group or the control/placebo group. Case patients received 750mg berberine daily for three months. Among other positive markers relative to the potential of berberine as a therapeutic marker, weight was significantly reduced in the case group but not the control group.

Another study reviewed the efficacy of berberine on metabolic and cardiovascular risk factors, including diabetes and hypercholesterolemia, both of which could be related to obesity. It was concluded that significant evidence for improved glycemic control and lipid profiles exists after patients are treated with berberine (Derosa, Maffioli, and Cicero 2012).

In a study on the effect of berberine in patients with metabolic syndrome, BMI was reduced in patients receiving berberine versus placebo; however, the difference between the treatment and control groups was insignificant (Zilaee et al. 2015). In another study, however, weight loss between treatment and placebo groups was clinically significant when looking at weight alone (Kashkooli et al. 2015).

Finally, traditional Chinese medicine products, including berberine, were reviewed for their possible efficacy in the treatment of obesity. The authors concluded that berberine and other natural botanicals likely had significant therapeutic effects on obesity based on a review of the literature. The significant effects of berberine included appetite reduction and improved satiation markers, blocking lipid absorption and digestion, a reduction in lipid synthesis, increased lipid oxidation, and an overall improvement in dyslipidemias (Zhang, Zhu, and Jiang 2014). As related to obesity specifically, appetite reduction, increased satiety, and the resultant decreased overall caloric intake is likely to have significant effects. If directly related to treatment with berberine, these would suggest good potential for berberine as a noninvasive treatment for obesity that is also easy to access, inexpensive, and easy to monitor.

HYPERTENSION

Hypertension is considered one of the leading cardiovascular risk factors and is a hallmark characteristic of metabolic syndrome. Hypertension affects at least 20% of worldwide adults, and its incidence is increasing annually (Shin, Shim, and Park 2014, Hall 2003). Like obesity,

hypertension appears to be on an out-of-control trajectory and with global health and economic impacts, as well as significant effects on quality of life.

The incidence and costs associated with hypertension are predicted to rise exponentially in the years to come. An estimated 40.5% of US residents alone are expected to have some form of cardiovascular disease, including that caused by hypertension, by the year 2030 (Heidenreich et al. 2011). Prevention strategies are thus critically important for public health and economic reasons as the hypertension-related incidences of heart attack, heart failure, and kidney disease, and their chronic care, continue to rise at a seemingly uncontrolled rate (Psaty et al. 2003, Carlsson et al. 2013).

Evidence suggests that hypertension is characterized by inflammation (Dzielak 1992, Trott and Harrison 2014), with pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-17, and IL-23 as likely factors (Kang 2013). There is also evidence that blocking inflammatory cytokines can protect blood pressure (Trott and Harrison 2014). Therefore, therapeutic agents targeting inflammation may be useful in preventing the devastating effects of hypertension.

Berberine has shown some promise as an antihypertensive and hypotensive agent, although as with obesity, data showing its efficacy for treating hypertension in humans are limited. As with its effect on other disorders, though, berberine appears to have various mechanisms by which it might affect blood pressure. The preponderance of evidence once again comes from *in vivo* animal models.

In Vivo Animal Studies

In a recent study, hypertensive rats were administered 100 mg/kg body weight per day of berberine. The researchers found that rats treated with berberine had a delayed onset of hypertension and also a reduction in its severity. The researchers attributed these effects in part to berberine's effects on the pro-inflammatory cytokines IL-6, IL-17, and IL-23 (Guo et al. 2015).

In another study, hypertensive rats were injected with an aqueous solution of berberine extract. The researchers found that arterial blood pressure was significantly reduced after treatment. They also found evidence of vasodilation in an *in vitro* arm of the same study where the researchers examined the effects of aqueous berberine on cut aorta rings. The researchers concluded that berberine has antihypertensive and vasodilatory effects (Hassanabad et al. 2005).

Examining yet another mechanism of action, berberine administered intravenously to rats resulted in lowered arterial blood pressure in a dose-dependent way. This appeared to be at least partly due to inhibition of the angiotensin-converting enzyme, the presence of which is implicated in vasoconstriction, and to stimulation of nitrous oxide and cyclic guanosine monophosphate release in vascular tissues, both of which are known vasodilators (Kang et al. 2002).

Human Clinical Trials

A few human clinical trials have investigated the effects of berberine in patients with essential hypertension alone as well as those with hypertension and comorbid disorders such as congestive heart failure and metabolic syndrome.

Treating essential hypertension, or high-normal blood pressure in the absence of comorbid disorders can be challenging. Drug treatment is often not recommended because the side effect profiles of currently available drugs are significant relative to the potential benefits of treatment. Thus, nutraceuticals such as berberine are increasingly being explored as potentially attractive alternatives because they tend to be free of side effects and in *in vivo* models have been shown to have likely antihypertensive and hypotensive properties.

One study with of berberine as a nutraceutical with potential in this regard investigated 30 patients with essential hypertension and who had a low overall cardiac risk. They were given a nutraceutical combination that included berberine either with or without *Orthosiphon stamineus* (java tea or cat's whiskers) for four weeks. *Orthosiphon stamineus* is known to have anti-inflammatory effects. The results showed that the addition of *Orthosiphon stamineus* was important in the preparation, as a significant reduction in blood pressure was only seen in patients taking the combined preparation (Trimarco et al. 2012).

Another study showed mixed results for the efficacy and safety of berberine in patients with congestive heart failure, in which blood pressure was also monitored (Marin-Neto et al. 1988). Twelve patients were given intravenous berberine infusions at rates of 0.02 or 0.2 mg/kg body weight per minute for 30 minutes. The higher infusion rate was associated with improved cardiac performance, including blood pressure control, and the researchers speculated that blood pressure modulation might be due to vasodilation and an increase in the strength of heart muscle contractions. However, four patients developed ventricular tachycardia in the hours after berberine infusion. Thus, caution was recommended with berberine therapy, and the need for further study into the possible relationship between berberine and ventricular tachycardia was noted (Marin-Neto et al. 1988).

A meta-analysis of the efficacy of berberine in the treatment of T2DM, hyperlipemia, and hypertension found that berberine along with lifestyle intervention was more effective than lifestyle intervention alone or placebo in reducing elevated blood pressure, and that berberine in combination with a hypotensive pharmaceutical agent was more effective than the pharmaceutical agent alone (Lan et al. 2015).

Finally, in a previously mentioned study (Zilaee et al. 2015), patients with metabolic syndrome were treated with berberine 600mg/d or given placebo for six weeks. After six weeks, the treatment group showed a significant reduction in both systolic and diastolic blood pressure compared to controls.

According to *in vivo* and human clinical trials, the potential efficacy of berberine as an antihypertensive or hypotensive agent appears promising. However, more research is needed, especially in human trials. While positive effects on blood pressure have been seen, it may be that berberine is most effective in combination with other agents and interventions. And in some patients, particularly in those with risk factors for tachycardia, some contraindications may be found.

DYSLIPIDEMIAS

Dyslipidemias include a range of related disorders characterized by an overabundance of "bad" cholesterols, such as total cholesterol, low-density lipoprotein cholesterol, and triglycerides, and/or an under-abundance of high-density lipoprotein, or "good" cholesterol. Cholesterol dysregulation is a major risk factor for cardiovascular disease and thus contributes to the risk for and causes of the leading agents of death worldwide (Chait and Eckel 2016).

While statins are the first line of treatment for dyslipidemias, significant side effects of these drugs exist (Law and Rudnicka 2006). These may include muscle pain and muscle damage, liver toxicity, risk of or exacerbation of T2DM, and even neurological side effects such as memory loss or confusion. While these do not affect everyone taking statins, the incidences are significant enough to cause concern and, in some cases, to make statin therapy undesirable.

Nutraceuticals and nutritional therapies are being increasingly studied as possible alternatives to statin therapy. Initial studies have shown that these interventions have the potential to offer therapeutic benefits without unwanted side effects. Berberine, for instance, and in particular, has shown great promise in this regard (Lan et al. 2015).

As with other metabolic disorders, the use of berberine to prevent and treat dyslipidemias has been studied in both *in vivo* and *in vitro* trials, and in human clinical trials as well. Unlike with some other metabolic disorders where data are lacking, however, berberine's effectiveness against dyslipidemias has explicitly been reasonably widely studied. This is important because a preponderance of studies have widely shown that there is a significant benefit to managing dyslipidemias when addressing cardiovascular disease (Goff et al. 2006). Thus, berberine may be a good candidate as a nutraceutical against dyslipidemia and therefore presenting another therapeutic angle from which to intervene against cardiovascular disease.

In Vivo Animal Studies

The mechanisms by which berberine appears to affect cholesterol vary, which is similar to what has been seen in studies into the effects of berberine on other metabolic disorders. For example, in one study, rats who were nutritionally induced with high cholesterol, as well as hyperglycemia, were fed berberine 30 mg/kg body weight per day. At the end of the six-week trial, a cardiac protective effect of berberine was observed: Total cholesterol and triglycerides were significantly reduced. The researchers found that these improvements were associated with increases in cardiac fatty acid transport protein-1, fatty acid transport proteins, fatty acid beta-oxidase, as well as peroxisome proliferator-activated receptor-γ, along with a decrease in proliferator-activated receptor-α mRNA and protein expression. All of these are mechanisms that act to reduce cardiac lipid accumulation (Dong et al. 2011).

Similarly, in another study, hamsters were fed a high fat and high cholesterol diet for two weeks, before and after which serum cholesterol was measured. After a ten-day treatment period when serum cholesterol was again measured, significant dose-dependent decreases in total cholesterol and low-density lipoproteins were found in treatment hamsters as compared to control hamsters. For example, hamsters given 50 mg/kg/d berberine showed a 26% reduction in low-density lipoproteins whereas hamsters given 100 mg/kg/d showed a 42% reduction in

low-density lipoproteins. The mechanisms of action were found to be related to increases in both low-density lipoprotein receptor mRNA and protein levels in hamster livers as shown in subsequent *in vitro* liver sampling from three animals that had been killed for this purpose. The researchers further concluded that the mechanism of action of berberine is different than for statins and, after further study, speculated that because of this difference, combination therapy may be more effective than berberine or statin therapy is given alone (Kong et al. 2004, Kong et al. 2008).

Another possible mechanism of berberine's action to normalize cholesterol was shown in a study of rats that were fed a high fat, moderate cholesterol diet and given 50, 100, or 150 mg/kg/d of berberine for eight weeks. Cholesterol absorption rates and plasma lipids were measured. While no significant dose-dependent relationship between berberine and cholesterol effects was observed, total cholesterol and non-high-density lipoprotein cholesterol (i.e., triglycerides and low-density lipoproteins) levels decreased by 29%-33% and 31%-41% respectively, which was clinically significant compared to controls. The cholesterol absorption rate, on the other hand, decreased by 40%-51%. The researchers concluded that, in part, berberine effectively interfered with enterocytes' ability to absorb cholesterol in the intestines (Wang et al. 2014).

Human Clinical Trials

Human trials have also consistently demonstrated the benefits of berberine in normalizing plasma lipid levels. In one study, 32 hyperlipidemia patients given 0.5 g berberine twice a day experienced a 29% reduction in total cholesterol, a 35% reduction in triglycerides, and a 25% reduction in low-density lipoproteins after a three treatment course (Kong et al. 2004).

An interesting and important effect was found in another study where 35 patients with liver disease (chronic hepatitis or cirrhosis) were given berberine 0.5 g twice per day and compared with matched controls who received silymarin 70 mg three times per day for three months. Both doses were established as therapeutic, respectively, in other studies. Blood samples were taken at baseline and the end of treatment showed that patients in the berberine group showed a significant reduction in cholesterol of 15.1%, triglyceride of 20.7%, and low-density lipoprotein of 20.8%, whereas changes on similar measures in the silymarin group were negligible.

Also interesting was that the berberine group also showed an increase in high-density lipoprotein, or good cholesterol, as well as relative to the silymarin group (Zhao et al. 2008). This is an important finding as it is generally difficult to increase high-density lipoprotein levels and doing so is desirable in healthy individuals as well as in patients. Other studies have tended to show mixed results with regard to berberine's ability to raise increase high-density lipoprotein levels (Zhu and Li 2007, Shanobin and Lirong 2007).

HYPERURICEMIA

Hyperuricemia is considered an acquired metabolic disorder that is characterized by an abnormally high level of uric acid in the blood. A common complication of hyperuricemia is

gout, which is an inflammatory arthritic process related to the high serum uric acid levels that cause crystals to be deposited in the joints (Liu et al. 2016). There is some evidence that gout is related to obesity and hypertension (Kuo et al. 2015). While the initial presentation of gout may be acute with episodes of painful joints and may remit on its own, hyperuricemia advances over time, and gout in its chronic form can be quite debilitating.

There is evidence that the global incidence of gout has increased over the past 50 years (Kuo et al. 2015) but unevenly, with the highest incidence in Pacific countries and in particular in developed versus undeveloped countries. There may be a genetic predisposition as well.

Dietary factors have always been seen as important. Diets that are associated with higher body mass index, triglyceride levels, urea, and c-reactive protein levels tend to be associated with higher uric acid levels. In men, specific indicators are body mass index and muscle mass. In women, the most important factors are waist circumference, creatinine levels, muscle mass, and high-density lipoprotein levels (de Oliveira et al. 2013).

Conventional therapies for hyperuricemia include urate-lowering drugs; however, these are minimally effective in general. Others, such as those that attenuate IL-1, and thus inflammation, can be quite expensive. Hospitals in China prescribe plant-based medicines with some success, primarily modified Simiaowan, which has impressive results that are well documented (Liu et al. 2016, Shi et al. 2013), but that have not been used more widely outside China.

While berberine is known for its anti-inflammatory activity as mentioned already, its application in treating gout is virtually unstudied. *In vivo* and human clinical trials have not been reported in the literature, and limited *in vitro* studies have been undertaken.

In Vitro Studies

A recent study sought to shed light on the possible effect that berberine may have on inflammation related to hyperuricemia and gout by examining its effect on monosodium urate crystal-induced inflammation in human cells studied *in vitro* (Liu et al. 2016).

Messenger RNA levels of the NLRP3 gene and IL-1β were measured. These were chosen because the NLRP3 gene provides instructions for making the protein cryopyrin along the NLRP3 inflammasome pathway. Cryopyrin is found in cartilage-forming cells such as might be affected by hyperuricemia. As expected, in the pretreatment sample, both the NLRP3 gene and IL-1β expressions were increased significantly in the model group compared to the normal group. However, when treated with 6.25 μM or 25 μM berberine, the researchers found that the expressions of the NLRP3 gene and IL-1β were decreased significantly. The researchers thus concluded that berberine could alleviate inflammation associated with the monosodium urate crystals characteristic of hyperuricemia. They postulated that this is due to the downregulation of NLRP3 gene and IL-1β expressions (Liu et al. 2016). Berberine's inactivation of the NLRP3 inflammasome pathway appears to be a factor.

Further research is needed, of course, especially looking at berberine's potential action against hyperuricemia in *in vitro* studies and in human clinical trials. Given the limited evidence available on possible mechanisms, as well as evidence that hyperuricemia-related disability is on the rise, it is reasonable that investigators focus on this potentially rich area of study.

CANCER

Our understanding of cancer is evolving. Once thought to be a disease perhaps caused by genetic mutations, it is more and more becoming better understood as among the range of metabolic disorders, sharing with other metabolic disorders roots in disturbed energy production involving respiration and fermentation. As with T2DM, for instance, glucose and glutamine as fermentable metabolites seem to be implicated. And therapies that focus on increasing respiratory metabolites and decreasing fermentable metabolites are increasingly recognized as key to treatment and possibly laying a foundation for prevention (Seyfried et al. 2014).

If cancer is a metabolic disorder related to an excess of fermentable metabolites, which presumably are also associated with inflammation, then it is reasonable to wonder if berberine might be useful in preventing, ameliorating, or treating cancer given the anti-inflammatory, antimicrobial, and antioxidative effects, among others, that berberine has shown in the face of other metabolic disorders. A comprehensive review of the anticancer properties of berberine concluded that berberine shows evidence of suppressing tumor growth and metastasis, is useful when combined with conventional medication therapies, and even may improve multidrug resistance (Sun et al. 2009). As with other studies investigating berberine about metabolic disorders, however, the preponderance of evidence relative to cancer is seen in *in vivo* and *in vitro* studies with human clinical trials still few excepting anecdotal evidence from Chinese medicine and Ayurveda practices.

Several possible mechanisms for berberine's efficacy are suggested including inhibition of microorganisms that tend to be associated with tumor development, regulation of gene expression, interaction with DNA and RNA, inhibition of cancer-related enzymes such as *N*-acetyltransferase, cyclooxygenase-2, telomerase, suppression of tumor cell proliferation, and facilitation of apoptosis of cancer cells, or cell death (Wu et al. 2016).

ANTIMICROBIAL ACTIVITY OF BERBERINE

According to one review article (Imanshahidi and Hosseinzadeh 2008), studies have consistently shown the antimicrobial effects of berberine, particularly against antibacterial and antifungal agents such as. *Staphylococcus aureus* and different *Candida* spp., *Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis,* and *Leishmania donovani.* Further, an *in vitro* study (Mahady et al. 2003) that assessed the efficacy of berberine against 15 strains of *Helicobacter pylori* found significant inhibitory growth effects.

The significance is that *Helicobacter pylori* are associated with the development of some types of cancer, such as gastric cancer. Extrapolating from the findings, the researchers suggest that there is a potential link between berberine's antimicrobial activity and anticancer action (Mahady et al. 2003).

BERBERINE AND REGULATION OF GENE EXPRESSION

Research in colorectal cancer has found that some cancerous cell lines and tissues have enhanced expression of the *c-Ki-ras2* protooncogene (Wang et al. 1987), which, along with tumor suppressor genes, are thought to be the primary genes on which mutations lead to cancer (Chial 2008).

In one study, researchers found that berberine was able to create a morphological change that turned teratocarcinoma cells into neuronal cells through the downregulation of the *c-Ki-ras2* proto-oncogene (Chang, Gao, and Wang 1990). Other researchers has shown that berberine administration is associated with tumor suppressor gene p53 activation in vascular muscle cells (Li et al. 2017), while a study with *both in vitro* and *in vivo* arms demonstrated that berberine is associated with growth inhibition and apoptosis of human lung cancer cells, with could be further facilitated cooperatively with p53 (Katiyar et al. 2009). In aggregate, studies suggest that the action of berberine's pharmacological effects relative to its apoptosis-promoting and antiproliferative effects is characterized by a slow, smooth course at concentrations as low as ≤ 25 mmol/l) (Sun et al. 2009).

BERBERINE'S INTERACTION WITH DNA AND RNA

A recent study showed that the treatment of osteosarcoma MG-63 cells with berberine resulted in significant apoptosis of treated MG-63 cells. The action was time- and concentration-dependent and the binding resulted in DNA damage in MG-62 osteosarcoma cells that altered the cell cycle in ways that were not seen in control cells (Zhu et al. 2014). This follows other research demonstrating that berberine has a propensity to bind with DNA and RNA to form respective complexes. The binding, in turn, damages the DNA and RNA and leads to inhibition of the respective cell cycles, and apoptosis (Krey and Hahn 1969, Creasey 1979, Islam and Kumar 2008).

BERBERINE'S INHIBITION OF N-ACETYLTRANSFERASE

Chemically-induced carcinomas are often related to exposure to environmental chemicals, including occupational chemicals. Arylamines, perhaps the best-studied toxins, are found in a wide variety of products such as oxidants, epoxies, explosives, fungicides and pesticides, colorants, and polyurethane (Chung 2015) and are metabolized by N-acetyltransferase. The metabolite can have one of two functions: It can aid in detoxification or cause toxicity that is associated with cancerous tumors (Butcher, Tiang, and Minchin 2008).

Given how common arylamines are and their importance in both detoxification and tumor induction, it is reasonable to study if there are ways to selectively inhibit their cancer-related activity. In several such studies, it was found that the administration of berberine to cancer cells of various types led to inhibition of N-acetyltransferase in the cells. Study cells included human bladder tumor cells, colon tumor cells, leukemia cells, and brain tumor cells in a dose-dependent manner (Wang et al. 2002, Chung et al. 1999, Lin et al. 2012, Chung et al. 2012).

These results imply that berberine may be useful therapeutically in reducing the proliferation of a variety of cancer cells that are dependent on N-acetyltransferase activity.

BERBERINE'S INHIBITION OF CYCLOOXYGENASE-2

COX-2, or cyclooxygenase-2, is implicated in some cancers, including colon, skin, prostate, liver, and lung carcinomas (Sun et al. 2009). Inhibition of COX-2, therefore, is a possible angle for the prevention and treatment of a variety of cancers.

Berberine's role as a COX-2 inhibitor is thought to be established, and therefore its anti-inflammatory activity on COX-2 could have a role in anticancer activity. For example, one study, which was built on previous investigations by the same researchers, showed that berberine treatment induced apoptosis in cells via COX-2 inhibition. The findings confirmed their previous investigations (Kuo, Chi, and Liu 2005). However, another study in the same year found that berberine did not inhibit COX-2 activity (Seaver and Smith 2004). Still, other research suggested that it is possible that the enzyme itself is not inhibited by berberine, but that other biochemical effects result in transcriptional suppression of COX-2 (Kuo, Chi, and Liu 2004). While berberine's role in inhibiting cyclooxygenase-2 action is unclear, then, there is evidence of its potential as a COX-related modulator of cancer, which will be further established in ongoing and future research.

BERBERINE'S INHIBITION OF TELOMERASE

Berberine's possible role in the inhibition of telomerase in cancer cells is poorly understood. Telomeres act to preserve the stability and viability of cells, including cancer cells (Sun et al. 2009), and given the cell growth that is characteristic of cancer, it is hypothesized that telomerase upregulation is involved. This is mainly due to evidence that the vast majority of malignant tumors contain telomerase and have characteristics of being immortal (Shay and Keith 2008, Kim et al. 1994).

If the immortality that telomerase may impact on cells plays a role in the widespread cell division characteristic of cancer malignancies, then an agent that interrupts telomerase could be a therapeutic agent. Data show that berberine does appear to inhibit telomerase activity (Jin 2007); however, it does not appear to be as straightforward as berberine being an actual inhibitor (Wu et al. 1999). Thus, the relationship between berberine and inhibited telomere activity in cancer cells remains unclear.

ALZHEIMER'S DISEASE

Alzheimer's disease has been recognized in the literature since 1906 but it was not until the 1960s that it became better understood for the importance it plays in age-related dementia (Lage 2006). It is a significant public health problem of enormous consequence, with more than 5.8 million Americans affected and an annual economic burden of more than $290 billion, excluding costs to family members that are estimated to be worth another $234 billion. It is the

6th leading cause of death in the United States and the 5th leading cause of death over age 65 years (Alzheimer's Association 2019).

While its history is long and stories are plenty about its devastating effects, there is still much to learn about Alzheimer's disease, and pharmacological intervention remains in its infancy despite that several drugs are currently being used clinically and in research. At best the results are limited, and side effects are common, however, and no medications as yet identified seem helpful for the prevention of Alzheimer's disease or towards a cure (Cai, Wang, and Yang 2016).

Acetylcholinesterase inhibitors such as donepezil, galantamine, and rivastigmine, and N-methyl-D-aspartate antagonist memantine have been approved in the United States, but results are modest at best (Atri 2019). Thus, Alzheimer's disease today remains irreversible in its neurodegenerative process as it progressively impacts memory and cognition over a course of years in an already vulnerable older population. With aging baby boomers and people living longer, it stands to reason that incidence and costs will increase until effective prevention strategies and treatments, or cures, are found.

Extracellular amyloid plaques and intracellular neurofibrillary tangles, along with inflammation, oxidative stress, and iron deregulation seem characteristic of Alzheimer's disease, although no clear pathology is yet apparent. Thus, it appears that any potential interventions must, in some way interfere with multifactorial processes (Carreiras et al. 2013).

In particular, neuroinflammation seems like a reasonable target for anti-inflammatory approaches; however, this hypothesis is complicated. Data seem to suggest that while long-term users of anti-inflammatory drugs do indeed have some measure of protection against Alzheimer's disease, administering anti-inflammatory drugs to patients with mild Alzheimer's disease seems ineffective. It may be that anti-inflammatories only have a preventive rather than treatment effect, or a treatment effect only before a patient becomes symptomatic (Pimplikar 2014).

Clinical studies to date into treatment strategies for Alzheimer's disease have shown generally poor results overall despite successful mouse trials (Pimplikar 2014). Focusing mostly on reducing amygdaloid plaques, it is thought that a possible explanation for these failures is that such therapies need to begin decades prior to symptomatic expression an Alzheimer's disease; thus the most effective approach might be something akin to a vaccination. Trials are underway to explore the potential value of this approach, although still using mouse models (Roberson et al. 2007, Gong and Iqbal 2008).

Mouse and human clinical trials are also to look at berberine's possible role against neurodegeneration related to Alzheimer's disease (Zhang et al. 2009, Ahmed et al. 2015). Some measure of success has been seen in both *in vivo / in vitro* and clinical settings (Cai, Wang, and Yang 2016).

Given berberine's plethora of actions in humans, and especially as an anti-inflammatory, it seems reasonable to explore its possible efficacy in preventing or treating Alzheimer's disease. In fact, it has been used for 3,000 years in China and Ayurvedic medicine to treat many ailments (Cai, Wang, and Yang 2016), and its value against a range of metabolic, cardiovascular (Chang, Chen, and Hatch 2015), inflammatory (Jin, Khadka, and Cho 2016), and neurodegenerative (Jiang et al. 2017) diseases has been well established. Besides, there is evidence for its potential neuroprotective effects in stroke (Zhou et al. 2008, Pires et al. 2014) and in general (Kysenius and Huttunen 2016).

BERBERINE AS A POTENTIAL THERAPEUTIC AGENT IN ALZHEIMER'S DISEASE

One theorized mechanism of berberine's effect is that it inhibits the formation of amyloid plaques. Such accumulation is considered a hallmark of Alzheimer's disease (Cai, Wang, and Yang 2016). A study that supported this theory found that *in vitro* berberine suppresses the amyloid-beta-induced inflammatory response in microglia, apparently by its propensity to inhibit nuclear factor-kappaB activation (Jia et al. 2012). The researchers suggested that this is evidence that berberine's anti-inflammatory action is potentially useful for the treatment of neurological diseases including Alzheimer's disease.

Another theorized mechanism by which berberine may be effective against Alzheimer's disease is that it inhibits oxidative stress. Oxidative stress is also considered to be a hallmark pathology associated with Alzheimer's disease and may have a role in a vicious cycle whereby oxidative stress causes chronic neuroinflammation, which then causes more oxidative stress that could play a role in the formation of amyloid plaques (Cai, Wang, and Yang 2016).

As one might imagine, this process sets up a self-perpetuating cycle that would never end and with likely irreversible neuronal and cellular consequences. Many studies have shown promising results in this regard, suggesting that berberine may act to inhibit oxidative stress by reversing increased malondialdehyde production and decreased superoxide dismutase activity, thus restoring a balance that appears impaired in Alzheimer's disease (Haghani, Shabani, and Tondar 2015, Lee et al. 2006).

Neuroinflammation is also implicated in Alzheimer's disease. It is hypothesized that neuroinflammation has multiple potential roles in contributing to and enhancing the effects of Alzheimer's disease. These include a role in amyloid plaque formation as well as various neuronal and even immune-impairing functions as a result of chronic inflammation (Cai, Wang, and Yang 2016). The interplay between various factors that neuroinflammation may modulate is complex with far-reaching effects.

About inflammation itself, there is ample evidence that berberine has anti-inflammatory properties, which have been discussed elsewhere in this chapter and widely in the literature. As to its potential as an anti-inflammatory against Alzheimer's disease multiple studies have shown possible effects. These are the same as for oxidative stress, where berberine may act to reverse increased malondialdehyde production and decreased superoxide dismutase activity (Haghani, Shabani, and Tondar 2015, Lee et al. 2006).

Finally, berberine may act to decrease the effects of risk factors that play a role in the development of Alzheimer's disease. Metabolic disorders that have potential vascular effects, such as high cholesterol, high blood pressure, T2DM, and obesity, may be non-genetic factors related to the pathogenesis that underlies Alzheimer's disease by way of formation of atherosclerotic plaque. It stands to reason, then, that addressing these risk factors could impact the development of Alzheimer's disease by ameliorating the propensity for the formation of atherosclerotic plaque (Cai, Wang, and Yang 2016). Two recent studies demonstrated that berberine effectively reduces inflammatory responses, which helps reduce the likelihood of amyloid plaque development.

In the first study, berberine was found to have an anti-atherosclerotic mechanism that the data suggested may be due to multiple factors including normalizing plasma lipids, reducing

overall inflammatory processes, modulating blood sugar, and having an inhibitory effect on vascular smooth muscle proliferation (Wu, Wang, and Liu 2010).

In the other study, berberine was found to suppress the expressions of protein-coding gene MMP-9 and the protein EMMPRIN, both of which are implicated in inflammation-related disease progression (Huang et al. 2012).

PARKINSON'S DISEASE

Following Alzheimer's disease, Parkinson's disease is the second most common neurogenerative disorder. It was first described in 1817, at which time its motor features were emphasized, and still, today, it is classified as a movement disorder (Gao et al. 2003). Motor features include resting tremor, rigidity, bradykinesia, and postural instability, which are relatively well known and have been studied extensively (Jankovic 2008). Parkinson's disease's cognitive effects, however, were not mentioned in early classification (Massano and Bhatia 2012). Research suggests that Parkinson's disease results from a progressive deterioration of the nigrostriatal dopamine system, and while therapies can ameliorate symptoms to some degree halting the disease progress is not yet possible (Gao et al. 2003).

Non-motor symptoms, such as constipation, depression, and an impaired ability to smell and detect odors (Hawkes, Del Tredici, and Braak 2010, Aarsland et al. 2004), in addition to being early warning signs suggesting the need for further evaluation, can also provide clues as to where research for prevention and treatment of Parkinson's disease might well focus its efforts.

We know now of the relatively broad spectrum of cognitive problems experienced by patients with Parkinson's disease as well; however, from a diagnostic perspective, these are nonspecific. These include mild cognitive impairment and dementia (Massano and Bhatia 2012). Dementias occur in around 80% of Parkinson's disease patients (Hely et al. 2008) while hallucinations and delusions occur in 45%-65% of patients with comorbid dementia, and at a statistically more significant rate than in the general population of Parkinson's disease patients (Aarsland et al. 2004).

Although nonspecific, these symptoms remain important because the cognitive symptoms carry the significant quality of life implications, and often can present themselves many years before the characteristic motor features are evident. Thus, even non-specific cognitive sequelae may aid in the early detection of Parkinson's disease and therefore allow for meaningful intervention (Massano and Bhatia 2012, Chaudhuri and Schapira 2009).

In addition to these relatively minor and nonspecific symptoms, more pronounced dementias and even hallucinations tend to show in later stages of the disease and are useful in distinguishing Parkinson's disease from other disorders with similar features but in which psychosis, for instance, is uncharacteristic. This includes Alzheimer's disease.

Mood and anxiety disorders occur in a large number of Parkinson's disease patients as well, and these again increase in frequency and intensity as the disease progresses. As with hallucinations, mood and anxiety symptoms vary with the presence of dementia. Anywhere from 40%-58% of Parkinson's disease, patients with comorbid dementia also suffer some degree of depressed mood, and 30%-49% experience significant anxiety (Aarsland et al. 2004). On the other hand, and perhaps a distinguishing characteristic for differential diagnosis, patients

with Alzheimer's disease are more likely to exhibit irritability, anger, and aggression, whereas patients with Parkinson's disease are more likely to experience more simple presentations of depressed mood and anxiety (Engelborghs et al. 2005). Common and less distinguishing among Parkinson's disease patients is apathy (Aarsland et al. 2004).

Non-cognitive, non-motor features have been identified as well. REM sleep disorders may result from lesions to the brain stem that are characteristic of Parkinson's disease (Boeve et al. 2007). Symptoms of REM sleep disorders include talking during sleep, kicking, or even jumping out of bed due to muscle atonia (Olson, Boeve, and Silber 2000). Autonomic symptoms, such as dysregulated heart rate, blood pressure, digestion, and body temperature have also been seen in later stages (Hely et al. 2008, Hawkes, Del Tredici, and Braak 2010); however, clear evidence of a relationship between these and advanced Parkinson's disease has yet to be presented (Massano and Bhatia 2012).

Reliable biomarkers for Parkinson's disease have yet to be identified; thus, in the end, proper diagnosis of this complex disease falls back to clinical skills and methods to sort through the symptoms in a way that allows for proper differential diagnosis (Massano and Bhatia 2012).

We know that about 15% of patients with Parkinson's disease inherit the disorder due to alteration of any of several specific genes, including LRRK2, SNCA, PARK2, PARK7 or PINK1 (Kahale et al. 2014). In other cases, oxidative stress may be implicated (Wei et al. 2018), and still others postulate the importance of an inflammatory process (McGeer and McGeer 2004, Gao et al. 2003). The latter two possible causes, which may contribute to what constitutes the majority of cases of idiopathic (i.e., non-genetic) Parkinson's disease (Gao et al. 2003), are in common with other metabolic disorders reviewed in this chapter.

Genetic etiology notwithstanding, it stands to reason that berberine could be useful in treating Parkinson's disease if it has an oxidative and/or inflammatory component. As with the other metabolic disorders reviewed in this chapter, berberine's potential value has been studied primarily *in vivo* and *in vitro* relative to Parkinson's disease, to seek clarity on the Parkinson's disease process and possible courses of treatment.

Neuro-Inflammation in Parkinson's Disease

Postmortem human brain studies and animal studies alike have pointed to the importance of a neuroinflammatory process as at least one of the biological mechanisms of Parkinson's disease. In one study, for example, large numbers of HLA-DR-positive glycoprotein, along with abnormal aggregates of protein inside nerve cells, and high free melanin were found in the basal ganglia of all postmortem brains studied with Parkinson's disease, whereas these were present to a significantly lesser degree in Alzheimer's disease patients and matched controls. These factors are indicative of unusually high levels of cellular inflammation (McGeer et al. 1988).

In another postmortem investigation, brains of three subjects who had self-administered synthetic heroin and subsequently developed unremitting parkinsonian-like symptoms until death several later were studied. Evidence of moderate to severe depletion of pigmented nerve cells in the substantia nigra was found, a strong indication of a significant neurodegenerative process. Interestingly, this also provided evidence that even a short-term neuronal insult in this area can set in motion a self-perpetuating, degenerative process that, like with Parkinson's disease, is resistant to meaningful intervention (Langston et al. 1999). There is also evidence to

suggest that the inflammatory process may be triggered by brain insult decades before disease onset, including environmental exposure such as to agrochemicals (Liu, Gao, and Hong 2003).

Similarly, an *in vivo* postmortem study of rat brains with induced Parkinson's disease showed that a progressive degeneration of the dopamine system resulted from a broad microglial cell response toward striatal and nigral cells located in the basal ganglia (Cicchetti et al. 2002). In another, rats were injected with lipopolysaccharide, which was found to cause a significant and permanent level of inflammation in the substantia nigra cells characterized by a clustering of macrophage cells that indicated that the cells had mobilized to fight a foreign inflammatory process. The resultant damage to the dopaminergic system was observed as remaining after one year and was thus considered permanent (Herrera et al. 2000). A third study found that intraneural lipopolysaccharide injection, which was administered to provoke degeneration of dopaminergic neurons, induced an inflammatory process. The inflammation resulted in significant caspase-3 activation, an indication of cellular damage, in the rat midbrain, and associated with glial cells (Burguillos et al. 2011).

Oxidative Stress in Parkinson's Disease

Oxidative stress has also been investigated as having a potentially important role in the pathogenesis of Parkinson's disease, albeit with less consistent results and fewer studies than for neuroinflammation. Due to the nature of related investigations, using blood samples, for instance, there have been many more studies with living human subjects than with neuroinflammation, which generally requires postmortem brain analyses.

A metanalysis of related research with human subjects found a preponderance of evidence to support the role of oxidative stress in Parkinson's disease. The analysis included 7,212 patients with Parkinson's disease and 6,037 healthy controls. Most significantly it was found that, relative to healthy controls, patients with Parkinson's disease had significantly higher blood concentrations of 8-Oxo-2'-deoxyguanosine, malondialdehyde, nitrite and ferritin—all established markers of oxidative stress to DNA and known risk factors for diabetes, atherosclerosis, and cancer—as well as lower blood concentrations of catalase, uric acid, glutathione and total cholesterol, all of which could be protective oxidative stress (Wei et al. 2018). The authors concluded that the analysis strengthened the notion that Parkinson's disease is associated with increased oxidative stress on several important dimensions.

Three major sources of oxidative stress have been identified. These are related to dopamine metabolism, mitochondrial dysfunction, and neuroinflammation itself. While the specific mechanism by which oxidative stress may be related to Parkinson's disease remains unknown, it is thought that oxidative stress may result largely from these sources. If these sources of stress are minimized, therefore, it stands to reason that the reactionary oxidative process can be reduced along with inflammation associated with microglial activation (Blesa et al. 2015).

Oxidative stress and neuroinflammation are intricately interwoven, with each possibly underlying the other in some cases, and being a reaction to it in other instances (Hwang et al. 2005). Thus, it is possible that some interventions can target both. Berberine, with its antioxidant and anti-inflammatory activities (Ahmed et al. 2015), maybe one such agent.

BERBERINE AS A POTENTIAL THERAPEUTIC AGENT IN PARKINSON'S DISEASE

As noted earlier in this chapter, berberine is an isoquinoline alkaloid that has been shown in numerous studies to be broadly effective as an antidiarrheal, antibacterial, antifungal, antitumor, antidyslipidemic, and even as an antiprotozoal. It has been used for thousands of years in China and in Ayurveda. It is present in some different herbs and in perhaps up to 500 deciduous and evergreen plants (Ahmed et al. 2015). Its potential value against Parkinson's disease is a reasonable question to investigate given the established efficacy of berberine against inflammatory and oxidative conditions, including other neurodegenerative diseases such as Alzheimer's disease.

As an antioxidant, and in its simplest, it appears that berberine mitigates the action of free radicals such as superoxide ions and nitric oxide and helps to protect cells from the damage caused by free radicals (Li et al. 2014). As an anti-inflammatory agent, berberine appears to reduce pro-inflammatory cytokine activity and thus acute phase proteins (Jeong et al. 2009).

In one study of the possible neuroprotective effect of *Coptis chinesis*, within which berberine is the main alkaloid, researchers investigated the effects of a watery extract of *Coptis chinesis* on a human cell model and a mouse model of Parkinson's disease. They found that in the human model, *Coptis chinesis* significantly increased cell viability and reduced cell death and also significantly increased the 'energy charge' or intracellular ATP concentration compared to control. Berberine was found to be one of the two main active compounds in the equation; however, the full *Coptis chinesis* extract had a stronger neuroprotective effect than berberine alone. In the mouse model, *Coptis chinesis* significantly improved motor functions in a dose-dependent way compared to control and significantly increased the activity of tyrosine hydroxylase-positive neurons, which catalyze L-DOPA, a precursor to dopamine (Friedemann et al. 2016). The researchers thus concluded that *Coptis chinesis*, including berberine as one of its main alkaloids, could be foundational to the development of Parkinson's disease treatment.

An *in vivo* mouse model with promising results found that 50 mg/kg body weight of berberine prevented memory loss and balance loss in Parkinson's disease-induced mice compared with controls. Besides, the treatment group exhibited significantly less dopaminergic system damage and less cell hippocampal cell death, or apoptosis (Kim et al. 2014).

In an *in vitro* study, human neuroblastoma SH-SY5Y cells—which are commonly used to study neurodegenerative disorders—were pretreated with berberine a significant reduction in cell death was seen. This was attributed to three main effects: A reduction in oxidative stress, a reduction in caspase-3 activation that would suggest apoptosis if activated, and ultimately a reduction in the rate of cell death. Neuroprotective effects were also seen, including upregulation of mechanisms by which to protect against dopaminergic neuron injury from oxidative stress, as well as activation of mitogen-activated protein kinases as further protection (Bae et al. 2013). The researchers, while looking specifically at Parkinson's disease in this study, concluded that berberine may be an effective therapeutic against dopaminergic neuronal diseases in general.

SAFETY OF BERBERINE

In addition to its efficacy, several studies have addressed the safety of berberine for clinical use. A search of the literature finds no reports of serious complications, although concerns at least warranting further study have occasionally been reported, such as with a possible relationship between berberine administration and ventricular tachycardia (Marin-Neto et al. 1988).

A metanalysis encompassing 27 available randomized controlled clinical studies and that included 2569 patients was conducted. Data comprised studies within seven subgroups: berberine versus placebo or berberine with intensive lifestyle intervention versus intensive lifestyle intervention alone; berberine combined with oral hypoglycemic versus hypoglycemic alone; berberine versus oral hypoglycemic; berberine combined with oral lipid-lowering drugs versus lipid-lowering drugs alone; berberine versus oral lipid-lowering drugs; berberine combined with oral hypotensive medication versus hypotensive medications alone; and, berberine versus oral hypotensive medications. While the authors noted that the studies were generally of limited quality relative to the clinical findings of berberine's efficacy, there were no reports of serious adverse reactions attributable to berberine therapy among any T2DM patients enrolled in any of the 27 studies (Lan et al. 2015).

In another study (Zhang et al. 2008), which was not included in the above metanalysis, mild to moderate constipation in five study patients was noted but otherwise, there were no adverse effects seen among the 116 patients who received berberine 1.0g daily for three months against T2DM and dyslipidemia. The researchers concluded that berberine therapy administered in this manner is safe and effective against T2DM and dyslipidemia.

In a meta-analysis of 17 more recent clinical research trials that included 1198 patients, the overall efficacy and safety of berberine for treating T2DM and related metabolic conditions were investigated. Dosages ranged from 0.5g/d to 1.5g/d for various metabolic symptoms. While efficacy was found for this spectrum of conditions, no adverse effects attributable to berberine therapy were reported within the dosage range (Wei, Zhu, and Wang 2015).

In a representative combination *in vitro* and *in vivo* study using mouse models, berberine was found to have no acute toxicity when administered for the suppression of tumor growth and metastasis (Sun and Ouyang 2007).

All in all, data support that berberine when used at therapeutic doses ranging from 0.5g/d to 1.5g/d in humans is without the risk of serious side effects and thus considered safe as a standalone therapy and in combination with other drugs to treat metabolic symptoms and conditions.

CONCLUSION

Berberine has been widely studied in *in vivo* and *in vitro* research and less so in human clinical trials. Despite the lack of clinical trials, there is convincing evidence that berberine is a safe and effective treatment consideration for many metabolic sequelae (see Table 2). While the mechanisms of action vary (see Table 1), research data suggests a few general targets of this versatile and powerful ammonium salt that can be found in numerous plants.

Table 2. Metabolic disorders toward which berberine has been found to have therapeutic efficacy

Type 2 Diabetes mellitus (t2dm)	(García-Fontana et al. 2016, Chung et al. 2012, Quartey et al. 2012, Robinson and Zhang 2011, Lan et al. 2015, Yin, Zhang, and Ye 2008, Zhang et al. 2008, Zhou et al. 2009, Chatuphonprasert, Lao-ong, and Jarukamjorn 2014) (Han, Lin, and Huang 2011, Yin, Xing, and Ye 2008, Shidfar et al. 2012, Zhang et al. 2010, Pang et al. 2015).
Obesity	(Firouzi et al. 2018, Kral and Heymsfield 1987, Kumanyika et al. 2008, Dong et al. 2011, Grover et al. 2015, Després and Lemieux 2006, Hwang et al. 2009, Zhang and Ye 2012, Hu and Davies 2010, Derosa, Maffioli, and Cicero 2012, Zilaee et al. 2015, Kashkooli et al. 2015, Zhang, Zhu, and Jiang 2014).
Hypertension	(Guo et al. 2015, Hassanabad et al. 2005, Kang et al. 2002, Trimarco et al. 2012, Marin-Neto et al. 1988, Lan et al. 2015, Zilaee et al. 2015).
Dyslipidemias	(Dong et al. 2011, Kong et al. 2004, Kong et al. 2008, Wang et al. 2014, Wei et al. 2012, Zhu and Li 2007, Shanobin and Lirong 2007).
Hyperuricemia	(Liu et al. 2016, Shi et al. 2013).
Cancer	(Imanshahidi and Hosseinzadeh 2008, Mahady et al. 2003, Wang et al. 1987, Sun et al. 2009, Zhu et al. 2014, Krey and Hahn 1969, Chung 2015, Butcher, Tiang, and Minchin 2008, Wang et al. 2002, Chung et al. 1999, Lin et al. 2012, Chung et al. 2012, Kuo, Chi, and Liu 2005, Seaver and Smith 2004, Kuo, Chi, and Liu 2004, Shay and Keith 2008, Kim et al. 1994, Wu et al. 1999).
Alzheimer's disease	(Pimplikar 2014, Roberson et al. 2007, Gong and Iqbal 2008, Zhang et al. 2009, Ahmed et al. 2015, Cai, Wang, and Yang 2016, Chang, Chen, and Hatch 2015, Jin, Khadka, and Cho 2016, Jiang et al. 2017, Kysenius and Huttunen 2016, Haghani, Shabani, and Tondar 2015, Lee et al. 2006, Wu, Wang, and Liu 2010, Huang et al. 2012).
Parkinson's disease	(McGeer et al. 1988, Langston et al. 1999, Liu, Gao, and Hong 2003, Cicchetti et al. 2002, Herrera et al. 2000, Burguillos et al. 2011, Wei et al. 2018, Blesa et al. 2015, Hwang et al. 2005, Ahmed et al. 2015, Li et al. 2014, Jeong et al. 2009, Friedemann et al. 2016, Kim et al. 2014, Bae et al. 2013).

Berberine appears to have the most dramatic effects on blood insulin levels, cholesterol levels, inflammatory processes, and tumorigenesis. As these underlie or are components of a variety of metabolic disorders, berberine has the potential to have wide-reaching utility in clinical settings.

Its effects can be seen in the blood, in cells, and even at the DNA and RNA levels. Its mechanisms of action vary widely, and perhaps this is why berberine therapy alone, or as adjuvant therapy, appears to have such broad applicability.

While formal research is relatively new, berberine has been used therapeutically for thousands of years in Chinese and Ayurvedic medicine systems. Data are plentiful within these systems to show the value of berberine in treating a range of metabolic and nonmetabolic problems. As is so often the case, Western science is catching up with and improving on the knowledge that has already been put into practice for millennia. If trends hold, then-current research data are only beginning to prove the utility of this important compound. Ongoing and future studies will add to our understanding and determine the safest and most efficacious uses for $C_{20}H_{18}NO_4^+$—berberine.

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Chapter 11

THEOBROMA CACAO: COCOA BEAN SHELLS AS A SOURCE OF PHENOLIC COMPOUNDS TO PREVENT METABOLIC SYNDROME

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ABSTRACT

Phenolic compounds are associated with a reduction of risk factors related to metabolic syndrome. Cocoa bean shell is one of the main byproducts generated from cocoa processing and is an important source of phenolic compounds. This chapter discusses (i) the optimum parameters to extract polyphenols from cocoa bean shell and (ii) the development of a method for the identification of phenolic acids in cocoa bean shell infusion using capillary electrophoresis. Extraction was performed using reflux, and ultrasound-assisted extraction and the phenolic acid profile was validated according to the guidelines established by the International Council for the Harmonization of Technical Requirements for the Registration of Pharmaceutical Products for Human Use (ICH). Results showed that the optimum conditions to obtain polyphenols were in reflux using water as a solvent for 5 minutes (262.76 ± 20.66 mg GAE/ 100 g). Further, satisfactory results were obtained for the phenolic profile. Nevertheless, no phenolic acids (gallic, syringic, benzoic, chlorogenic, and caffeic) were detected in the infusion of cocoa bean shell. This study revealed that cocoa bean shell represents a high potential of use in the

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food industry, principally in the field of functional food development and also this work provides a new alternative, which allows identifying quickly and effectively the phenolic acids that may be present in an infusion.

Keywords: cocoa beans shells, phenolic components, metabolic syndrome

INTRODUCTION

Metabolic syndrome is a constellation of risk factors that increment the risk of cardiovascular diseases and diabetes type II. Excess weight, hyperglycemia, elevated blood pressure, low HDL cholesterol concentration, and high level of triglycerides are part of the syndrome (Ford et al. 2003). The syndrome is highly related to oxidative stress and inflammation (Gregorio et al. 2016). Oxidative stress is a condition in which there is an imbalance between the production and inactivation of reactive oxygen species (Roberts and Sindhu 2009). Nowadays, antioxidant compounds derived from plant species are highly regarded as alternative therapies to prevent metabolic syndrome. Antioxidants can act as radical scavengers or chain breakers. Further, compounds such as polyphenols act as antioxidants and are associated with anti-inflammatory effects and reduction of the risk factor for metabolic syndrome. Polyphenol rich-food includes red wine, berries, green tea, and cocoa (Pastor-Villaescusa, Sanchez Rodriguez, and Rangel-Huerta 2018).

Cocoa *(Theobroma cacao)* is the primary material to prepare chocolate, which has a high demand for consumption worldwide. The processing causes a massive amount of byproducts. Cocoa bean shell is one of the main by-products of cocoa processing and generates a disposal problem (Martínez et al. 2012). Large quantities of this material are eliminated as a residue from the production of products and derivatives of cocoa. The use of cocoa bean shell as an additive in human food have been studied, principally for the fiber content (Redgwell et al. 2003). However, studies have reported that CBS contains polyphenols (Martínez et al. 2012), which are compounds that exhibit important biological activities such as antimutagenic, anticarcinogenic, antioxidant and other beneficial health properties (Plaza et al. 2016, Bhattacharya, Mukhopadhyay, and Giri 2011, Nsor-Atindana et al. 2012). The antioxidant qualities are related by the content of the total phenolic compounds present in the cocoa bean shell. However, there are no studies reported in the literature on the identification of the phenolic compounds responsible for the biological effect assigned to it. There are only chemical - biological studies published to the cocoa shell as well, such as its fermentation, enzymatic, nutritional processes, among others, such as the elaboration of dietary foods for animals (Alemawor et al. 2009).

Bioactive compounds, including polyphenols, have been extracted using traditional methods for instance: maceration, reflux, and soxhlet (Aires, Carvalho, and Saavedra 2016, Araujo 2009, Ćujić et al. 2016, Kaneria and Chanda 2012). Nevertheless, these methods consume large quantities of solvents and are time-consuming (Heleno et al. 2016). New technologies such as ultrasound-assisted extraction (UAE) has been recently employed for polyphenols (Ghitescu et al. 2015) and has proved to be more efficient than the conventional method (Yang and Zhang 2008). Hence, the aim of this investigation is (i) to compare the use of method of extraction, reflux and ultrasound-assisted extraction to determinate an optimum method of extraction, solvent choice and time process to obtain a high yield of polyphenols

from cocoa bean shell and (ii) determinate the phenolic acid profile of infusions prepared with cocoa bean shell.

THEORETICAL PERSPECTIVES

Metabolic Syndrome

The metabolic syndrome, also known as insulin resistance syndrome, is a common metabolic disorder that includes several conditions such as glucose intolerance, insulin resistance, obesity, dyslipidemia and hypertension, that increase the risk of a person to develop cardiovascular disease and Type 2 diabetes mellitus (Cornier et al. 2008). The metabolic syndrome is considered as a state of chronic inflammation. The National Cholesterol Education Programme – Adult Treatment Panel III (NCEP-ATP III) defines the following criteria for the metabolic syndrome: elevated waist circumference ≥ 102 cm in men and ≥80 cm in women, high triglycerides blood concentration ≥1.694 mmol/L or 150 mg/dL, low HDL-c blood concentration <1.036 mmol/L or 40 mg/dL (men) and <1.295 mmol/L or 50 mg/dL (women), high blood pressure ≥130/85 mmHg (systolic blood pressure/diastolic blood pressure) and elevated fasting blood glucose >6.111 mmol/L or 110 mg/dL (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). The prevalence of the syndrome has increased in the last years, being predominant in Hispanics and South Asians (Grundy et al. 2004). Also, economic status, low mobility, lifestyle, and high body mass index have been linked with metabolic syndrome (Bahadori et al. 2019).

The prevention of not develop metabolic syndrome consist of lifestyle changes, weight loss, and exercise. However, persons that reside in disadvantaged neighborhoods have limited access to healthy food and physical activity environments (Desroches and Lamarche 2007, Martin et al. 2019). Further, in some cases, changes in lifestyle alone cannot only regulate blood cholesterol levels so that nutraceuticals can be used as adjunctive (Catapano et al. 2019). Nutraceuticals are defined as supplemental food that enhances human health and wellbeing, preventing the development of certain diseases (McClements and Xiao 2017). Those substances can be extracted from several sources, such as fruits, plants, lignocellulosic biomass, and algae (Moldes, Vecino, and Cruz 2017).

Polyphenols

Polyphenols are the second largest family of plant nutraceuticals and can be found in fruits and vegetables (Naczk and Shahidi 2004). They are secondary metabolites characterized by the presence of an aromatic ring tailored to hydroxyl groups (Fantini et al. 2015). According to their chemical structure, polyphenols are classified in: phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (flavanols, flavonols, flavones, isoflavones, flavanones, and anthocyanins), stilbenes, lignans, coumarins and tannins (Liu 2013). Plant polyphenols have been reported as compounds that ameliorate risk factor that develops the metabolic syndrome (Cherniack 2011). The main properties that have been reported to polyphenols are anti-oxidative, anti-inflammatory, anti-bacterial and anti-viral (Wang et al. 2016).

Antioxidant activity of polyphenols is attributed mainly to the phenolic structure and compounds with catechol like moieties (Wang et al. 2016). Phenolic acids exhibit antioxidant activities due to their action as free radical scavengers and metal ion chelators (Andjelković et al. 2006). The antioxidant property of these compounds depends on the number of hydroxyl groups. When polyphenols act as radical scavengers, they transfer a hydrogen atom to form antioxidant radicals which are more stables and interfere in the chain–propagation reactions (Kiokias, Varzakas, and Oreopoulou 2008). Increasing studies show evidence that phenolic compounds are responsible for protective action against several degenerative diseases. This protection is attributed to the antioxidant property of these compounds (Rojas and Buitrago 2019).

Hydroxybenzoic acids like gallic acid have improved insulin sensitivity, reduced obesity, blood pressure, and cholesterol level (Orrenius, Gogvadze, and Zhivotovsky 2007). Syringic acid is used for the treatment of bronchitis, and biological activities related to this phenolic compound include antioxidant, antiendotoxic, and anticancer (Shahzad et al. 2019). Hydroxycinnamic acids such as caffeic acid are related as a carcinogenic inhibitor and to prevent cardiovascular diseases (Spagnol et al. 2019). Another hydroxycinnamic acid of relevant biological activity is chlorogenic acid, which has shown an antidiabetic activity (Chen, Teng, and Cao 2019).

THEOBROMA CACAO

Theobroma cacao originates from tropical America and belongs to the class Magnoliopsida, order Malvales, family Malvaceae, genus Theobroma, and species Cacao. Plant measures between 5 and 8 meters of height and 4 to 6 meters of the diameter of the crown and can grow up to 20 meters high under best conditions. Leaves are large, alternate, elliptical, or oblong. The seed is the size of an almond, chocolate or purple color and bitter taste. Seeds do not have albumen and are covered by a gummy pulp of white color, of sweet and acidulated flavor. All the volume of the seed inside is practically occupied by the two cotyledons of the embryo (De Souza et al. 2018).

Cocoa is well known around the world, mainly because it is the raw material to prepare the chocolate. Cocoa is an abundant source of polyphenols, which contribute with a large list of health benefices. The consumption of cocoa is linked with the prevention of cardiovascular diseases. The main class of polyphenols that are found in cocoa are flavanols (a type of flavonoid) (Chen, Teng, and Cao 2019). Further, cocoa flavonoid has improved fatigue without effect on glycemic response (Coe et al. 2017).

Cocoa bean shell is a byproduct generated from cocoa processing. Several investigations show evidence that the cocoa bean shell is an important source of fiber and bioactive compounds that exhibits high antioxidant activity and that can be used in the formulation of a functional food (Arlorio et al. 2005).

Nutritional Composition of Cocoa Bean Shell (CBS)

CBS has different macronutrients and micronutrients that are necessary for health and contains a high content of soluble and insoluble fibers, proteins, and polyphenolic compounds. Table 1 shows the main components of CBS.

Table 1. Main components of cocoa bean shell

Parameter	(Mean ± standard deviation)
Moisture	3.73 ± 0.46
Ashes	5.96 ± 0.26
Fat	6.87 ± 0.52
Protein	16.93 ± 0.59
Insoluble Dietary Fiber%	11.08 ± 0.05
Soluble Dietary Fiber%	48.94 ± 1.21

Based on Afoakwa et al. (2013), and Panak Balentić et al. (2018).

MATERIAL AND METHODS

Plant Material

The raw material was purchased from the company PLUSTELCO S.A Exportadora de cacao, located in Guayaquil –Ecuador. Then, cocoa bean shells were dried at 60ºC in an oven using air circulation during 16 h. The dried sample was hand-milled, sieved through a 200-mesh screen and defatted with petroleum ether in a Soxhlet apparatus.

Standard and Reagents

Quercetin, chlorogenic acid, gallic acid, syringic acid, methanol were acquired from Sigma – Aldrich (St. Louis, MO, USA). Boric acid, benzoic acid, sodium hydroxide, hydrochloric acid, and ethanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). Caffeic acid was purchased from Fluka (Schnelldorf, Germany), and water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Extraction

Reflux: Dried, defatted, and powdered material was refluxed using a sample: solvent ratio (water or ethanol) of 1:10. The extraction was performed in the following time intervals: 5, 15, 30, and 60 minutes.

Ultrasound-Assisted Extraction (UAE): An ultrasonic bath VWR of 35 KHz and 180 W was employed. The extraction of polyphenols was performed by adding 2 g of dried, defatted, and powdered sample into 20 ml of solvent (water or ethanol) in a 25 ml flask. This experiment was carried out using the same time intervals of reflux.

Infusion: Freshly boiled water (200 mL) was poured onto 2 g of CBS and infused for 5 min without mixing. Infusions were filtered through a paper Whatman #1 filter and kept at -17ºC until its use.

Total Polyphenol Content

Total polyphenol content (TPC) was determinate by spectrophotometry using the Folin & Ciocalteu's method and was expressed as gallic acid equivalents (GAE) in mg/100 g dry material (Lachman, Orsák, Hejtmánková, & Kovářová, 2010). A total of 250 µl of sample and 250 µl Folin & Ciocalteu's reagents 1N were mixed, 5 minutes later 20% of sodium carbonate (750 µl) was added. Absorbance was read after 90 minutes at 765 nm.

Instrumentation

An Agilent 7100 (Agilent Technologies, Germany) capillary electrophoresis system couple to a diode array detector (CE-DAD) with a fused silica capillary of 50 µm inner diameter and 40 cm of total length (30 cm to the detector) (Polymicro TechnologiesTM, USA) was used.

CE Analysis

At the beginning of each day, the capillary was flushed with a solution of sodium hydroxide 0.1 N for 10 min, then ultra-pure water was used to wash the capillary for 20 min and buffer solution was employed to condition the capillary for 10 min. After, two blank samples were injected to stabilize the cassette temperature. Between runs, the capillary was washed with sodium hydroxide 0.1 N for 2 min, ultra-pure water for 3 min and buffer solution for 5 min. The hydrodynamic injection was applied (50 mbar for 5 s) with normal polarity with a voltage of 30 kV at a temperature of 25ºC and metabolites were identified at 210 nm.

Buffer Solution

The buffer solution was prepared using boric acid and sodium hydroxide. The concentration was adjusted to 50 mmol/L, pH 9.11.

Method Validation

The parameters evaluated for the validation were specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Linearity was determined using a calibration curve made up of six points for each compound. This analysis was carried out on three different days for measurement independence (Sanagi et al. 2010), and a lack of fit test was performed. The intra-assay precision was determined by triplicate at three different concentration levels of standard solutions. This procedure was repeated for three consecutive days to determinate inter-assay precision. Intra-assay and inter-assay precision were expressed in term of relative standard deviation (RDS). Accuracy was evaluated in triplicate using spiked samples with known concentrations of phenolic acid standards. The accuracy was reported as a percentage of the experimentally derived concentration to the nominal concentration. LOD

and LOQ were determined using a signal-to-noise ratio of 3 and 10 respectively and considering the slope of the calibration curve and the standard deviation of the blank.

RESULTS

Extraction Method

The experiment was performed in three replicates; each replicate consisted of 16 variable combinations (run). The factors studied where method (Factor A), solvent (Factor B) and time of extraction (Factor C). The factors and levels considered for the experiment are given in Table 2.

Table 2. Full factorial design experimental matrix

Run	**Design**			**Response**		
	Factor A	Factor B	Factor C	TPC (mg EAG/ 100 g)		
	(X_1)	(X_2)	(X_3)	Trial 1	Trial 2	Trial 3
1	UAE	Water	5 min	93.41	66.44	68.48
2	UAE	Ethanol	5 min	30.42	38.54	53.10
3	Reflux	Water	5 min	280,95	256.08	246.30
4	Reflux	Ethanol	5 min	59.37	38.70	42.00
5	UAE	Water	15 min	60.02	91.43	89.37
6	UAE	Ethanol	15 min	70.82	49.17	68.14
7	Reflux	Water	15 min	17.30	176.13	185.88
8	Reflux	Ethanol	15 min	72.01	46.37	42.47
9	UAE	Water	30 min	137.82	113.30	163.26
10	UAE	Ethanol	30 min	64.69	81.09	74.98
11	Reflux	Water	30 min	179.29	214.62	183.09
12	Reflux	Ethanol	30 min	47.01	45.75	41.36
13	UAE	Water	60 min	162.73	115.85	134.85
14	UAE	Ethanol	60 min	58.67	73.29	50.61
15	Reflux	Water	60 min	208.73	182.82	190.62
16	Reflux	Ethanol	60 min	31.03	41.20	20.24

Analysis of variance (ANOVA) was used to determinate the main effects of the method (X1), solvent (X2) and time of extraction (X3), and the interaction between the effects on total polyphenol content expressed as mg EAG/100 g of sample. The result is shown in Table 3.

Table 3. The ANOVA of factorial design for polyphenol extraction

	Degree of freedom (f)	**Sum of Sq.**	**Mean Sq.**	**F-ratio**	**P-value**
Method	1	20993	20993	90.94	0.00
Solvent	1	134702	134702	583.52	0.00
Time (min)	3	2082	694	3.01	0.04
Method*Solvent	1	39436	39436	170.83	0.00
Method*Time(min)	3	13974	4658	20.18	0.00
Solvent*Time (min)	3	5263	1754	7.60	0.00
Method*Solvent*Time (min)	3	4646	1549	6.71	0.00
Other (error)	32	7387	231		
Total	47	228483			

R^2= 96.77%, R^2 (adj) = 95.25%.

From Table 3, the P-value indicated a significant effect on TPC. All factors and interactions were significant at 0.05%. F-ratio meant that solvent (583.52) had a higher effect on TPC, followed by the interaction between method and solvent of extraction (170.83). On the other hand, the time had the lowest effect of TPC (3.01). The main effect plot showed in Figure 1 revealed that the water increased the TPC during the extraction. Longer extraction time decrease the TPC, and the reflux method gave a higher yield of TPC than UAE.

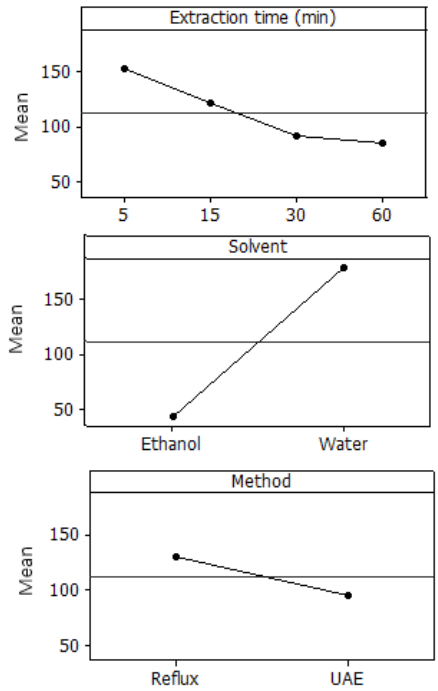

Figure 1. Main effects plot for polyphenol extraction.

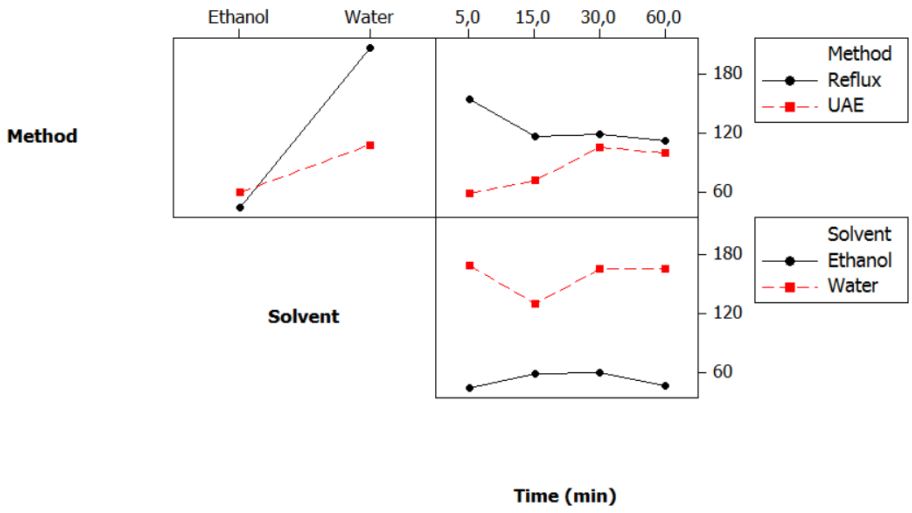

Figure 2. Interaction effects plot for polyphenol extraction.

The optimum parameters for polyphenol extraction, according to Figure 2, were reflux method, water solvent, and 5 minutes of extraction.

Full factorial design determined that reflux used with water incremented the extraction of polyphenols from cocoa bean shell. The high temperature of the process decreases water density and viscosity, which increases the mass transfer. Also, intermolecular interaction with water decreases using reflux, which allows a higher molecular motion and increments solute solubility (Wei et al. 2013). Moreover, the dielectric constant of water decreases using reflux, which makes nonpolar polyphenols to solubilize into water (Filly et al. 2016).

The values of TPC of CBS (262.73 ± 20.65 mg GAE/100 g sample) extracted with reflux using water by 5 min are higher than values reported for CBS extracted with a mixture of methanol-acetone (154.43 mg GAE/100 g sample). In contrast, extraction with UAE using ethanol by 30 min (73.59 ± 8.29 mg GAE/100 g sample) was lower than TPC reported for CBS using ethanol (82.37 mg GAE/100 g sample) (Martínez et al. 2012). A comparison of TPC value with others fibers waste indicated that CBS contain more polyphenols than tomato peels (158.10 ± 7.70 mg GAE/100 g sample) (Navarro-González et al. 2011) and orange peel (49.94 mg GAE/100 g sample) (Boukroufa et al. 2015) but less TPC than parrot peel (1380 mg GAE/100 g sample) (Chantaro, Devahastin, and Chiewchan 2008). In this context, this value-added CBS extract represents a high potential of use in the food industry, principally in the field of functional food development.

Phenolic Acid Profile of Cocoa Bean Shell Infusion

Specificity

The specificity was evaluated by calculating the peak purity and the similarity/threshold of the five phenolic acids. In all peak purity factors, the values were between 999 and 1000, and the similarity/threshold ratios were inferior to 1. These values indicated that the method is specific to determine phenolic acids in an infusion made with CBS.

Linearity, LOD, and LOQ

Linearity was evaluated from the construction of calibration curves elaborated with six concentrations. Table 2 shows the satisfactory result for linear equation, correlation coefficient, and lack of fit test ($p<0.05$) of each phenolic acid. LOD and LOQ were calculated a signal-to-noise ratio of 3 and 10, respectively and considering the slope of the calibration curve of each compound and the standard deviation of the blank.

Accurate and Precision

According to the results in Table 2, the method is accurate and precise in all concentrations for all compounds tested. Percentages of recovery were less than 120%, which shows reasonable accuracy rates.

The precision values of the method were expressed as coefficient of variation at three concentrations: high, medium, and low. Although there is no exact value indicating compliance with this parameter, it is recommended that it be less than 5%, in this case, lower values were obtained, except syringic acid, which at a high concentration showed a coefficient of variation of 7.81%.

Table 4 shows the intermediate precision. Caffeic acid was detected with precision at lower concentrations and the intermediate precision but the precision of benzoic acid was quite high (25,42%) which means that it is better to work in a medium-low than medium to high-concentrations. This helps us to see in which concentration range the method is more feasible.

Table 4. Validation parameters for phenolic acids

Parameter		**Benzoic acid**	**Gallic acid**	**Chlorogenic acid**	**Caffeic acid**	**Syringic acid**
Linear range (mg.L)		6 to 48	10 to 80	8 to 64	10 to 80	10 to 80
Calibration linear equation		Y=0.716x + 1.0909	Y=3.0307x + 18.055	Y=0.4702x – 1.1779	Y=2.1817x + 9.5667	Y=2.3582x + 3.9435
R		0.9984	0.9857	0.9983	0.9905	0.9959
Lack of fit test (p >0,05)		0.749	0.249	0.753	0.468	0.911
LOD		0.12	0.03	0.19	0.04	0.04
LOQ		0.37	0.09	0.56	0.12	0.11
RSD* (%) intraday	Low	0.14	1.92	3.98	5.13	5.15
	Middle	2.82	2.72	0.78	1.57	2.2
	High	3.14	3.5	1.97	3.15	7.82
RSD* (%) interday	Low	10.51	8.13	14.80	5.45	12.48
	Middle	25.42	9.59	6.50	4.47	5.99
	High	8.81	6.01	11.33	11.70	14.93

CONCLUSION

CBS represents a new source of polyphenols and can be used to enrich different kinds of food. Extraction is feasible since water and traditional method of extraction, reflux, works well to obtain polyphenols.

The parameters of linearity, repeatability, accuracy, the limit of detection, quantification, and range, fulfilled the requirements established by the ICH, which indicates the integrity of the analytical method, for the identification of phenolic acids by capillary electrophoresis. However, the proposed method could not detect phenolic acids in infusions of CBS.

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Chapter 12

CONSUMPTION OF *LUPINUS MUTABILIS* SWEET IN THE PREVENTION AND MANAGEMENT OF DIABETES MELLITUS TYPE-2

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ABSTRACT

The social and economic cost to treat type-2 diabetes (T2DM), one of the major public health problems in the world, is high. There is an urgent need to develop novel strategies to prevent and find new culturally friendly low-cost therapeutic alternatives to treat the disease. Several studies have shown that peptides derived from legume proteins have metabolic properties, including hypoglycemic, lipid-lowering, anti-carcinogenic, anti-inflammatory, and anti-thrombotic. People who consume diets rich in legumes significantly decrease total serum cholesterol levels compared to control groups; also, peptides derived from legumes show inhibitory properties of enzymes involved in the pathophysiology of T2DM and cardiovascular diseases such as angiotensin-converting enzyme and dipeptidyl peptidase IV inhibition. These studies demonstrate the importance of legumes in the diet and their potential as functional foods that could contribute to the prevention and treatment of metabolic diseases, including diabetes. For several years, our research group has developed studies with Andean crops to determine health beneficial properties in these foods that can be applied in the prevention and treatment of the most prevalent diseases in Ecuador, including cardio-metabolic diseases. Here we discuss clinical and molecular evidence for the use of the legume *Lupinus mutabilis* Sweet in the treatment of T2DM. Current evidence indicates that consumption of legumes including Andean *L. mutabilis* can contribute to the prevention and treatment of T2DM. It is important to educate and promote the consumption of legumes by the general population.

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Keywords: *Lupinus mutabilis*, type-2 diabetes, gamma-conglutin, glucose metabolism, cardiovascular diseases

INTRODUCTION

Type-2 diabetes mellitus (T2DM) is a major public health problem in Ecuador, the region, and worldwide (WHO 2014). In Ecuador, T2DM has been a leading cause of morbidity and mortality (INEC 2017). The social and economic cost of this disease in our country has not been measured accurately; however, it is estimated that based on regional studies in Ecuador would be high (direct annual cost per capita $ 2,112, and total cost in the country $ 2,537 million) which is a great burden for the budget allocated by the state for care of health in general (Barcelo et al. 2017). The cost for T2DM care will increase drastically in the coming years due to the high incidence of disease in the adult population, population that will continue growing shortly, particularly in low and middle-income countries.

It is possible that the great demand in resources that will be needed to attend the costs of the T2DM can ¨break¨ the health care Ecuadorian system in the coming years, currently the per capita expenditure for all diseases is US $ 1042 while the cost to treat only diabetes is US$ 2112 (Barcelo et al. 2017). On the other hand, the social price of the disease is also high due to the incapacitating nature of T2DM and its complications. It has been estimated that approximately 1 million years of productive life were lost due to T2DM throughout Latin America in 2015 (Barcelo et al. 2017, Hospedales et al. 2012). Therefore, it is critical to developing novel strategies to prevent and find new culturally friendly low-cost therapeutic alternatives for T2DM.

Several studies have shown that the peptides derived from diet proteins (from animal and plants - legumes) have metabolic properties (hypoglycemic, lipid-lowering, anti-carcinogenic, anti-inflammatory and anti-thrombotic) (Cam and de Mejia 2012). For instance, a prospective study (7.4 years of follow-up) that included 135,335 people between 35 and 70 years without cardiovascular risk in 18 countries with low, medium and high income, reported that the high consumption of fruits, vegetables, and legumes was inversely related to major cardiovascular diseases, cardiac infarction, cardiovascular mortality, non-cardiovascular mortality in models adjusted for age and sex (Miller et al. 2017). Interestingly, the consumption of legumes was inversely related to non-cardiovascular death and other causes of death. The authors recommend the consumption of fruits, vegetables and legumes (375 g/d) to reduce mortality in general (Miller et al. 2017). A meta-analysis (including ten randomized controlled trials) that analyzed the effect of consumption of legumes such as beans, peas and other seeds on cardiovascular risk factors showed that people who ate the diet with legumes decreased significantly the total cholesterol levels (-11.8% mg/dL), and LDL (-8.0 mg/dL) compared to the control groups (Bazzano et al. 2011). Studies with legumes (*Phaseolus vulgaris* L.) from Brazil and Mexico have identified peptides with potential inhibitory properties of enzymes involved in the pathophysiology of T2DM and cardiovascular diseases such as an angiotensin-converting enzyme (ACE), dipeptidyl peptidase IV (DPP-4) (Mojica and de Mejía 2015). Together, these studies demonstrate the importance of legumes in the diet and their potential as foods that could contribute to the prevention and treatment of metabolic diseases, including diabetes.

For several years, our research group has developed studies with Andean crops to determine health beneficial properties in these foods that can be applied in the prevention and treatment of the most prevalent diseases in Ecuador including cardio-metabolic diseases (Baldeón et al. 2012, Fornasini et al. 2012, Muñoz et al. 2018). Here we discuss clinical and molecular evidence for the use of the legume *Lupinus mutabilis.*

Nutritional Composition of Lupinus

Lupin is a legume of the genus *Lupinus*, whose seeds have been consumed for centuries in Europe, the Middle East, and the Andean Region (Sujak, Kotlarz, and Strobel 2006). There are more than 200 species of lupin, but only a few have been domesticated; the four main edible *Lupinus* species are *L. albus* and *L. luteus* in Europe, *L. angustifolius* in Australia, and *L. mutabilis* in South America (Cabello-Hurtado et al. 2016). The demand of lupin for human consumption is rising due to its unique nutritional value; it is a non-starchy grain legume that contains low amounts of fat (~6%), high number of essential amino acids and important dietary minerals, and high amount of protein (~40%) and dietary fiber (~28%) (Trugo, von Baer, and von Baer 2003).

In general, lupin grains are a good source of macro and microelements; although, the nutritional composition varies between species. Lupin seeds are rich sources of plant protein in the diet, and their amino acid composition is characterized by a low content of sulfur-containing amino acids (Erbaş, Certel, and Uslu 2005). Anti-nutrient compounds such as quinolizidine alkaloids are also present in lupin seeds giving the legume a bitter taste and make the raw seeds unsuitable for human and animal consumption. Consequently, lupin grains need to be debittered and cooked before consumption. Lupin seeds are debittered through boiling or fermentation, improving the nutritional value (Bartkiene et al. 2016).

Table 1. *Lupinus* spp. bromatological analysis, nutrient content per 100 g of *Lupinus* spp.

Proximates	**Unit**	**Mature, raw seeds**	**Mature seeds, cooked, boiled, without salt**	**Mature seeds, cooked, boiled, with salt**
Water	g	10.44	71.08	71.08
Energy	kcal	371	119	116
Protein	g	36.17	15.57	15.57
Total lipid (fat)	g	9.74	2.92	2.92
Carbohydrates	g	40.37	9.88	9.29
Fiber, total dietary	g	18.9	2.8	2.8
Minerals				
Calcium, Ca	mg	176	51	51
Iron, Fe	mg	4.36	1.2	1.2
Magnesium, Mg	mg	198	54	54
Phosphorus, P	mg	440	128	128
Potassium, K	mg	1013	245	245
Sodium, Na	mg	15	4	240
Zinc, Zn	mg	4.75	1.38	1.38

Table 2. Macro and micro-nutrient composition *L. mutabilis* Sweet

Proximates	**Bitter *L. mutabilis***	**Debittered cooked *L. mutabilis***	**Debittered prepared *L. mutabilis* snack**
Water (%)	10.33	77.05	2.09
Energy (kcal/100 g)	552.00		491.75
Protein (%)	47.80	54.05	45.88
Fat (%)	18.90	21.22	24.47
Total carbohydrates (%)			22.00
Ashes (%)	4.52	2.54	3.16
Fiber (%)	11.07	10.37	2.40
Reducing sugars (%)	0.42	0.61	
Total sugars (%)	0.95	0.73	0.80
Total starch (%)	4.34	2.88	
Minerals			
Sodium, Na (mg/100 g)	100		401.03
Sodium chloride, (%)			1.02
Magnesium, Mg (%)	0.24	0.07	
Phosphorus, P (%)	0.6	0.43	
Potassium, K (%)	1.22	0.02	
Calcium, Ca (%)	0.12	0.48	
Iron, Fe (ppm)	78.45	74.25	
Zinc, Zn (ppm)	42.84	63.21	
Manganese, Mn (ppm)	36.72	18.47	
Copper, Cu (ppm)	12.65	7.99	
Others			
Alkaloids (%)	3.26	0.03	
Fatty acids, total (TotFA) saturated (g/100 g)			9.19
TotFA monounsaturated (g/100 g)			10.95
TotFA polyunsaturated (g/100 g)			5.26
Cholesterol			0.00

Modified from Instituto Nacional de Investigaciones Agropecuarias – INIAP (Villacrés et al. 2006).

Table 1 shows the bromatological analysis of *Lupinus* spp. Since the grains need to be cooked, hydrated, and debittered before consumption, nutrient composition of raw mature seeds and cooked seeds is provided. Grains suitable for eating have approximately 70% of water, this has a dilution effect for the nutrients present in the legume. Like other legumes, lupins are characteristically rich in their protein content. lupins are also rich in complex carbohydrates and unsaturated fat (Cabello-Hurtado et al. 2016). Regarding micronutrients, Lupins are important sources of potassium, sodium, phosphorus, magnesium, and calcium; and vitamin C, niacin, and thiamin (Table 1).

Table 2 indicates the macro and micro-nutrient composition of Andean *L. mutabilis* grains. The table also shows the nutrient composition of a crunchy *L. mutabilis*-based snack developed by our research group. Differently than debittered cooked *L. mutabilis*, the snack has a crunchy consistency due to its low water content and shelf life of approximately a year at room

temperature. The snack has also the advantage to concentrate on the nutrient content of the legume. As shown in Table 2, *L. mutabilis* is also rich in protein, unsaturated fatty acids, and complex carbohydrate content.

Due to the important nutritional characteristics of lupins, including *L. mutabilis* and the functional properties of these legumes (see below) their consumption needs to be promoted. This is relevant when you consider the increase in growth population, current changes in weather conditions, and the reduction of fertile soil for cultivation. These global changes have led to a worldwide growing problem of malnutrition in all its forms (International Food Policy Research Institute 2016). In 2016, according to the World Health Organization (WHO 2014), 155 million children under 5 years old suffered from stunting, while 41 million were overweight or obese (IFPRI 2016, Younis, Ahmad, and Badpa 2015). All these health problems need different approaches to be solved; novel and affordable food sources that meet the distinct nutritional requirements are needed, including the consumption of legumes (Khan et al. 2015).

BIOACTIVE CONSTITUENTS AND HEALTH BENEFITS

Bioactive compounds (phytochemicals) are non-nutrient food constituents present in small amounts in cereals, legumes, fruits, and vegetables that exert metabolic effects on the human body (Singh et al. 2017). There is an important body in the scientific literature that shows pharmacologic properties of phytochemicals on human health, including antioxidant, anti-inflammatory, hepatoprotective, hypolipidemic, hypotensive, anti-atherogenic, antithrombotic, cardioprotective properties (Bouchenak and Lamri-Senhadji 2013). Importantly, studies indicate that certain types of phytochemicals like polyphenols exhibit protective actions on cardiovascular diseases and metabolic disorders (Bouchenak and Lamri-Senhadji 2013, Magalhães et al. 2017).

Polyphenols

Polyphenols are generally categorized into four major groups: flavonoids, phenolic acids, lignans, and stilbenes (Singh et al. 2017). The health benefits of polyphenols are associated with decreased oxidative stress, and low blood cholesterol levels. Total phenolic acids content in cooked dry legumes ranges from 269.1 to 727 (µg/100 g fresh wt), Table 3. Phenolic acids and flavonoids (including resveratrol) were the main polyphenols found.

Studies on the identification of phenolic compounds in *Lupinus* spp has been performed only in few species: *L. angustifolius, L. luteus, L. albus, L. mutabilis, L. hispanicus and L. exaltatus* (Khan et al. 2015). The major phenolic compounds identified in lupin species belong to subclass flavones, phenolic acids, and isoflavones, Table 4.

Studies on isoflavones suggest several mechanisms of their potential role in preventing cardiovascular diseases. Compounds like *genistein*, *daidzein* and *biochanin A* act as agonists on estrogen-related receptor α (ERR α), receptor involved in energy and homeostasis, so is a likely target for metabolic disorder treatment (Bouchenak and Lamri-Senhadji 2013). Likewise, *genistein, daidzein, biochanin A*, act on the activators of peroxisome proliferator-activated receptors (PPAR)α and (PPAR)γ. The peroxisome proliferator-activated receptors-α and

PPAR-γ active compounds are used to correct dyslipidemia and to restore glycemic balance, respectively (Bouchenak and Lamri-Senhadji 2013). Ranilla, Genovese, and Lajolo (2009) studied the isoflavone content and antioxidant capacity in six Peruvian lupin cultivars (SLP-1, SLP-4, SCG-22, H-6, Andares and Yunguyo) (*L. mutabilis Sweet*) and two Brazilian lupin cultivars (*L. albus* or white lupin, and *L. angustifolius* or blue), they found that seed coats from cultivars SLP-1 and SLP-4 *L. mutabilis* have a total content of isoflavones of 9.8 and 100mg/100g seed coat. Likewise, the genistein derivative was the major isoflavone found on raw seed coats from *L. mutabilis* cultivars by contrast samples of isoflavones were not detected on *L. albus* and *L. angustifolius* (Ranilla, Genovese, and Lajolo 2009).

Table 3. Total phenolic acids content in cooked dry legumes

Legume	Total Phenolic Acids content (µg/100 g fresh wt)
Broad beans	306.5
Chickpeas	668.1
Large lentils	269.1
Black-eyed beans	570.8
Pinto beans	727
White lupines	508.6

Modified from (Kalogeropoulos et al. 2010).

Table 4. Main Families of polyphenols in lupin seeds (*Lupinus albus* and *Lupinus angustifolius*)

Family	Compounds
Phenolic acids	a) Gallic
	b) Protocatechuic
	c) p- hydroxybenzoic
	d) Caffeic
	e) p-coumaric
Isoflavones	f) Genistein
	g) 2´-hydroxygenistein
Flavonoids	h) Apigenin-6,8-di-C-Beta-glucopyranoside
	i) Apigenin-7-O-beta-apiofuranosyl-6,8-di-C-Beta-glucopyranoside

Modified from (Khan et al. 2015, Arnoldi et al. 2015).

Phytosterols

Phytosterols have a structure like cholesterol. Studies on these compounds have shown a wide range of health benefits such as lowering cholesterol levels and reducing the intestinal absorption of dietary and endogenous cholesterol. Phytosterols bind bile acids and prevent their reabsorption, therefore decreasing serum cholesterol levels and decreasing cardiovascular risk (Kalogeropoulos et al. 2010, Singh et al. 2017). These bioactive compounds are found in seeds of many legumes like chickpeas, beans, peas, lentils and lupin (Singh et al. 2017). It has been estimated that 3 g of phytosterols intake per day significantly decreases the total cholesterol

level by 10-15% and is quite effective in reducing serum LDL-cholesterol (Singh et al. 2017). Table 5 shows the total phytosterols and squalene content of cooked dry legumes. Phytosterol concentrations ranged from 13.5 mg/100 g (freshwater) in black-eyed beans to 53.6 mg/100 g in lupines. Squalene is a compound that has important antioxidant properties and is present in considerable amounts in wheat germ oil, palm oil, amaranth oil, rice bran oil and especially in olive oil (Amarowicz 2009, Smith 2000). Legumes also contain squalene, but it is *Lupinus* spp that have higher amounts of this important compound. Furthermore, Table 6 indicates specific phytosterol concentration and indicates that ß-Sitosterol predominates in all legumes comprising 50–85% of the determined phytosterols; ß-Sitosterol highest concentration are chickpeas and lupins (Kalogeropoulos et al. 2010).

It is important to note that phytosterols concentration in cooked legumes is lower than in uncooked ones (Ryan et al. 2007). Although the phytosterols present in the cooked legumes are lower, their presence is not negligible and is considered beneficial for health (Kalogeropoulos et al. 2010).

Table 5. Total phytosterol and squalene concentrations (mg/100 g fresh weight) in selected cooked dry legumes

Legume	Total Phytosterol	Squalene
Broad beans	35.1 ± 4.0	0.32 ± 0.04
Chickpeas	48.9 ± 5.9	0.24 ± 0.03
Large lentils	31.6 ± 2.8	0.14 ± 0.01
Black-eyed beans	13.5 ± 1.4	0.12 ± 0.01
Pinto beans	21.5 ± 1.9	0.23 ± 0.02
White lupines	53.6 ± 4.8	1.74 ± 0.21

Modified from Kalogeropoulos et al. (2010). Results are the average ± standard deviation.

Table 6. Phytosterol concentrations (mg/100 g fresh weight) in selected cooked dry legumes

Legume	ß-Sitosterol	Campesterol	Stigmasterol	5-Avenasterol
Broad beans	28.6 ± 1.7	2.92 ± 0.32	1.13 ± 0.10	2.44 ± 0.38
Chickpeas	38.5 ± 3.1	4.32 ± 0.35	2.45 ± 0.27	3.58 ± 0.29
Large lentils	24.2 ± 1.0	2.18 ± 0.24	2.63 ± 0.18	2.52 ± 0.23
Black-eyed beans	6.7 ± 0.3	1.19 ± 0.11	3.84 ± 0.31	1.78 ± 0.18
Pinto beans	12.5 ± 0.9	1.75 ± 0.11	4.82 ± 0.43	2.44 ± 0.17
White lupines	30.9 ± 1.5	11.9 ± 1.1	4.98 ± 0.45	5.89 ± 0.47

Modified from Kalogeropoulos et al. (2010). Results are the average ± standard deviation.

The scientific literature provides evidence that phytochemicals present in legumes like *Lupinus* species possess beneficial properties on human health. Studies show that certain types of bioactive compounds like polyphenols exhibit protective actions on cardiovascular diseases and metabolic disorders. There are also phytosterols that lower dietary and endogenous cholesterol levels, therefore reducing the risk of cardiovascular disease. Even though phytosterols present in cooked legumes are lower than non-cooked, their presence is beneficial for health. Therefore, daily intake of legumes like lupin species should be encouraged.

Clinical Evidence

Currently, there is an important amount of evidence on the positive effects of consumption of different forms and types of lupin on human health. In this section, we present some original data from our studies which have focused mainly in the acute (Baldeón et al. 2012, Fornasini et al. 2012), and mid-term (data not published) hypoglycemic effect of consumption fixed doses of *Lupinus mutabilis* (LM) in subjects with and without hyperglycemia. In our clinical studies, we have also assessed the impact of LM consumption on blood pressure (BP), blood lipids (BL) and body mass index (BMI). Although there are some reports regarding other positive health effects of regular lupin intake, including anti-inflammatory and platelet's antiaggregant, we won't talk about them because this evidence is still scarce, and there are no studies with LM addressing these health benefits (Fechner, Kiehntopf, and Jahreis 2014).

One of the most significant health concerns around the world is type 2 diabetes mellitus (T2DM) because of its increasing incidence and the overwhelming socio-economic cost associated with its management (Barcelo et al. 2017, Hospedales et al. 2012). Currently, there are several oral hypoglycemic drugs with different mechanisms of action to treat patients with T2DM. However, they have limited efficacy, are costly, have side effects, and require continuous and incremental doses (Qaseem et al. 2018). Hence, there is a need for novel approaches to replace or complement treatments currently available. In this regard, food regularly consumed, such as debittered lupin seeds, seem to be an interesting option because there are no health concerns regarding its safety. Raw lupin seeds contain alkaloids, proteins, and peptides that have been found to have important hypoglycemic effects (Bartkiene et al. 2016). Administration of conglutin-γ to humans subjected to glucose overload has reduced significantly their plasma glucose (Bertoglio et al. 2011).

Our initial study was a phase II clinical trial conducted in young healthy individuals; volunteers with mild to moderate dysglycemia, and; a control group that received raw soybeans (SB). Eligible subjects were randomly assigned to the groups. Raw LM and SB were encapsulated in 400 mg gelatin capsules with a similar appearance. Equal amounts of LM or SB per kilogram of body weight were administered to volunteers. Since LM contained alkaloids, a safe dose of 3.1 mg/kg of body weight was used. A fasting blood sample and two additional samples were collected at 60 and 90 minutes after treatments.

In healthy volunteers, consumption of LM or soy did not induce changes in blood glucose or insulin after 60 and 90 minutes. In volunteers with newly diagnosed dysglycemia, consumption of LM decreased non-significantly blood glucose concentrations after 60 min of ingestion. However, LM ingestion produced a significant decrease in serum insulin after 90 min. SB intake did not cause changes in glucose or insulin levels.

The most significant effect in glucose and insulin concentrations was obtained in individuals whose basal glucose concentration was greater than 100 mg/dL. They decreased their glucose levels significantly at 60 and at 90 minutes whereas in patients consuming SB there were not important changes in blood glucose. Regarding insulin, after 60 min of treatments, there was a significant decrease in insulin concentration in the LM compared with the SB group. Only in individuals with dysglycemia that consumed LM there was a decrease of HOMA-IR 60 and 90 min after its intake. About 30% of patients that received the raw LM experienced dizziness, hypotension or blurred vision. It was attributed to the alkaloids of raw LM (Table 7).

Table 7. Comparison of blood glucose and insulin concentrations in dysglycemic volunteers treated with *Lupinus mutabilis* or soy, with basal glucose levels ≥100 mg/dL

Treatment	**0 min**	**60 min**	**90 min**	**P-value**	**P-value**
	μ ± SD	μ ± SD	μ ± SD	0vs.60 min	0vs.90 min
	(mg/dL)	(mg/dL)	(mg/dL)		
	Glucose				
Lupine (n = 12)	114.2 ± 11.6	105.4 ± 5.6	107.8 ± 5.6	0.00	0.03
Soy (n = 5)	105.2 ± 5.0	102.4 ± 4.3	104.2 ± 6.7	0.28	0.46
	Insulin				
Lupine (n = 12)	15.1 ± 18.6	11.0 ± 9.6	10.0 ± 8.9	0.15	0.00
Soy (n = 5)	12.2 ± 5.5	14.4 ± 5.2	8.4 ± 4.8	0.23	0.23

Modified from Fornasini et al. 2012.

Table 8. Comparison of blood glucose and insulin concentrations in volunteers with type 2 diabetes treated with cooked Lupinus mutabilis or its purified alkaloids

	Glucose		**Insulin**	
Treatment	Alkaloid	Cooked *L. mutabilis*	Alkaloid	Cooked *L. mutabilis*
0	112.8 ± 18.2	114.4 ± 27.2	9.5 ± 5.0	11.7 ± 6.5
60	112.9 ± 16.4	106.6 ± 25.1	7.8 ± 3.4	11.8 ± 6.5
90	101.6 ± 12.6	98.1 ± 21.6	9.1 ± 5.4	11.4 ± 6.3
Difference 0 vs. 60	+0.1 (+0.09%)	-7.8 (-6.82%)	-1.7 (-8.0%)	+0.1 (+0.85%)
P-value 0 vs. 60	0.796	0.000	0.083	0.731
Difference 0 vs. 90	-11.2 (-9.9%)	-16.3 (-14.2%)	-0.4 (-4.21%)	-0.3 (-2.5%)
P-value 0 vs. 90	0.015	0.000	0.8	0.876

Modified from (Baldeón et al. 2012).

Our second study was an experimental- phase-II random clinical trial conducted with volunteers recently diagnosed with type-2 diabetes to assess the acute effect of cooked LM (n = 20) and its purified alkaloids (n = 10) on blood glucose and insulin. Preparation of cooked LM and its purified alkaloids is described (Baldeón et al. 2012, Fornasini et al. 2012). Three blood samples at 0, 60 and 90 minutes were obtained as previously described (Fornasini et al. 2012).

We found that the consumption of cooked LM or its purified alkaloids decreased blood glucose and insulin levels. The decreases in serum glucose concentrations from baseline to 90 minutes were statistically significant within both treatment groups; however, there were no differences between groups. Serum insulin levels were also decreased in both groups; however, the differences were not statistically significant. The percentage of decrease in glucose concentration from baseline to 90 min was higher in the cooked LM group compared with the alkaloid group, 14.25%, and 9.93% respectively. On the other hand, the percentage of insulin decreased in the alkaloid treatment group at 90 min was higher than the decrease observed in the cooked Lupinus treatment group, 4.21% versus 2.56% respectively (Table 8).

These results are consistent with those from our initial report in which the consumption of raw LM by individuals with glucose abnormalities significantly decreased blood glucose and

insulin levels. This study complemented our previous observations since it assessed the effect of purified alkaloid and cooked LM without alkaloids and remains clear that the eatable part of LM has important positive effect on glucose metabolism. Considering the previous report on raw LM along with this study with volunteers with T2DM, it is evident that the most considerable decrease in serum glucose was observed in those individuals that consumed cooked LM (14.25%) while the reduction after alkaloid and raw LM consumption was 9.93% and 5.6% respectively, although this comparison has limitations because both studies were carried out with different doses of LM or its alkaloids, different populations of volunteers and were done at different times.

To assess the mid-term effect (3-months) of LM intake we conducted a single-blinded random clinical trial in patients (n = 81) with T2DM. Patients on oral hypoglycemic drugs with inappropriate control of their glycemia were recruited and randomized into two groups. Patients in the first group (G1) n = 44, received an upgrade of their oral hypoglycemic medication as judged by their physician; usually consisted in an increase of the dose of metformin from 500 mg to 1000 mg or from 850 mg to 1700 mg. The second group (G2) n = 37, continued with the same treatment (dose) of hypoglycemic drugs and additionally received a daily 10 g snack of LM which was about 52% protein and was also rich in fiber and omega-3 fatty acids. The primary endpoint was the change in A1C, and secondary endpoints were changes in BMI, BP, and lipid profile (LP).

After 3-months of intervention, compared with baseline, patients in G1, presented significant improvements in A1C and a non-significant decrease in glucose and insulin. Besides, patients who consumed LM (G2), showed also a significant reduction in BMI. The A1C decrease in G1 was -0.74, whereas in G2 was -0.32. However, when the analysis was restricted to patients that maintained A1C values ≤8, the absolute reduction in the two groups tended to be more similar; G1 -0.70 and G2 -0.51. This finding suggests that LM achieves a more significant effect in patients who maintain tight glucose control. A reduction of A1C of 0.3-0.5% triggered by LM consumption could be associated with a decrease in blood glucose between 9-15 mg/dl, whereas the glucose decrease related to oral hypoglycemic drugs dose adjustment could be around -21.0 mg/dl.

Also, subjects from the LM group showed a non-significant improvement in their LP and a non-significant tendency to decrease in the insulin resistance index (HOMA). There was no adverse effect when consuming LM, unlike the group that consumed Metformin who manifested some gastrointestinal complaints.

Since our initial mid-term study in patients with T2DM showed an important hypoglycemic effect, we wanted to strengthen this evidence and designed a quasi-experimental-28-week crossover-study that assessed the impact of daily consumption of LM on glucose control on regular T2DM patients under their usual oral hypoglycemic treatment. We initially recruited and followed (during-14-weeks) 79 adult patients that were taking their regular oral hypoglycemic medication only (without LM consumption). After that period, in addition to their usual oral hypoglycemic treatment, they started to consume daily doses of 10 and 15 g of an LM-based-snack (which was the same we used in our previous study), for other 14-weeks. Only patients with serum A1C concentrations ≤8.0% reduced their A1C significantly after the intervention with the LM based snack, and 71% achieved an A1C target concentration of 6.5%. The magnitude of A1C decrease in this study was -0.2 to -0.4 which corresponds to a reduction of about 10 mg/dl of glucose. Besides, after consumption of LM, patients showed a significant

decrease in blood pressure; both systolic and diastolic, and a substantial increase in HDL-cholesterol.

In conclusion, the acute effect (up to 90 min) of consumption of LM by normal-weight healthy young individuals does not modify importantly blood glucose or insulin levels. However, the use of LM by individuals with dysglycemia (fasting glucose > 100 mg/dL) decreased significantly blood glucose and insulin levels. *L. mutabilis* effect was greater in those subjects with higher basal glucose levels.

Regarding mid-term (3-months) effects of daily LM intake of a snack of 10-15 g, combined with the usual hypoglycemic treatment with oral drugs combined in patients with T2DM produces a significant reduction in A1C values. This effect is of greater impact in patients that maintain tight control of their glucose ≤8%. In these patients, the reduction of A1C ranged from -0.2 to -0.5 which corresponds to an average reduction of about 10 mg/dl of their regular glucose values. Besides, a greater percentage of patients can reach the ideal target values of A1C.

Other positive health effects of the LM snack were observed in blood pressure, BMI, and LP. Only the study with a higher dose of the snack of LM (a mean daily consumption of 15g) showed a significant decrease of systolic and diastolic blood pressure. This same study also indicated a substantial increase in HDL. Only one study demonstrated a significant reduction in BMI after the addition of the LM snack. Finally, both clinical studies showed a non-significant tendency to improve lipid profile and a non-significant trend to increase insulin levels.

Patients with T2DM could benefit from the addition of LM-snack to their conventional treatment. Previous *in vitro* and *in vivo* work indicates that gamma-conglutin (γC) and alkaloids from *Lupinus* spp. cause critical metabolic effects, which agree with the presented evidence. It is necessary to carry out new research combining biologically active foods with drugs commonly used for the treatment of T2DM. In this way, the data obtained in these studies with LM can be contrasted with other foods with active ingredients. All this information suggests that LM by itself or its derivates may become a source of ingredients of innovative functional foods.

Molecular Studies

A growing body of evidence regarding the cellular and molecular mechanisms responsible for the beneficial effects of Lupin consumption on cardio-metabolic diseases has accumulated in the last decades (Gonzalez-Santiago et al. 2017, Lovati et al. 2012). Among the potential bioactive components present in lupin grains, the protein fraction and within it, γC, has been extensively studied (Cabello-Hurtado et al. 2016). It has been estimated that γC constitutes approximately 5% of the protein content of Lupin seeds (Czubinski et al. 2015). The quaternary structure of the molecule indicates that γC is a glycosylated tetramer of approximately 200 kDa; each subunit of the tetramer is formed by two subunits of 29 and 15 kDa (Czubinski et al. 2015, Duranti et al. 2008). We have demonstrated γC expression at the mRNA and protein levels in the Andean *L. mutabilis* (Muñoz et al. 2018).

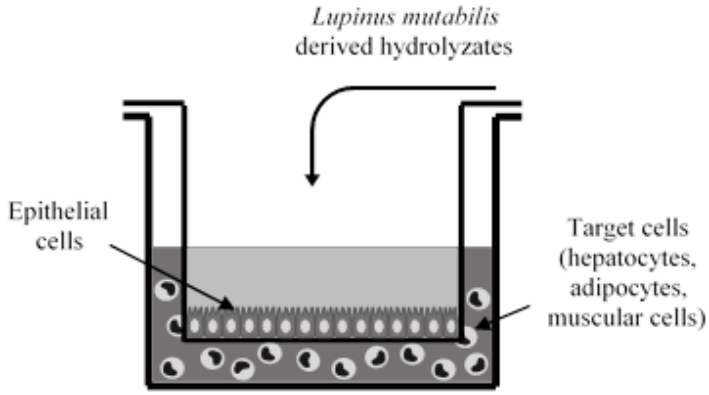

Figure 1. Dual culture system to evaluate lupin-derived hydrolysates on insulin-responsive cells.

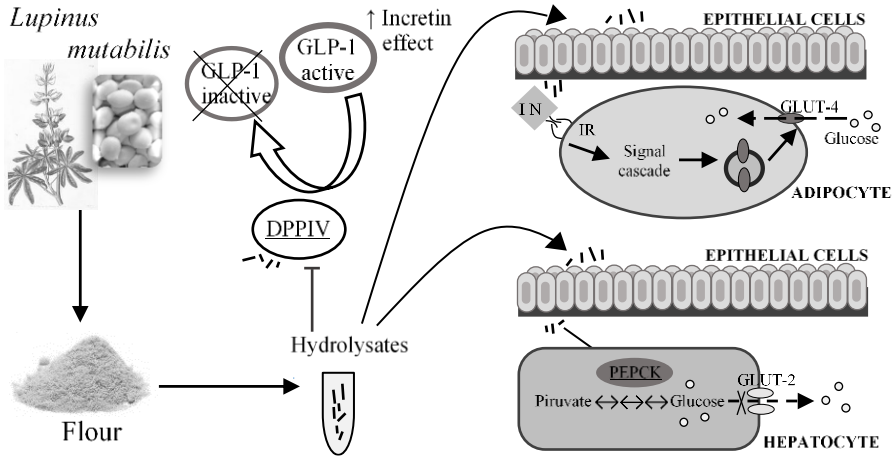

Figure 2. Cellular and molecular evidence on the beneficial effects of *L. mutabilis* on glucose metabolism.

Previous work has shown the hypoglycemic effect of γC preparations isolated from *L. Albus* in rats and humans (Bertoglio et al. 2011). That study demonstrated the hypoglycemic effect of γC during a glucose tolerance test of 2g/kg bodyweight in rats that was dose-dependent. Thus, rats received increasing doses of γC between 50 to 200 mg/kg of weight, the effect of the highest dose of γC was similar to the hypoglycemic effect of metformin (50 mg/kg of weight), a common drug used in the treatment of T2DM. Similarly, a progressive hypoglycemic effect was observed in 15 individuals who had a glucose tolerance test (volunteers consumed 75g of glucose) and were treated with increasing doses of γC (from 750 to 3000 mg) (Bertoglio et al. 2011). In addition to this short time observations on the effect of γC on hyperglycemia, the long-term use of γC treatment in rats with hyperglycemia also showed a positive effect on serum glucose and insulin concentrations (Lovati et al. 2012). Concomitant glucose treatment (average intake 2-3 g/d) and γC (28 mg/kg) attenuated the increase in plasma concentrations of glucose and insulin in these animals (Lovati et al. 2012).

The beneficial effects of Lupin consumption on glucose metabolism imply that grain digestion in the gastrointestinal tract is necessary. As indicated above γC molecular weight (200

kDa) would not allow its intestinal absorption. Although data shows that γC is resistant to the proteolytic action of intestinal enzymes at pH 4, under more physiologic gastric conditions, γC digestion generates hydrolysates that can be absorbed in the intestinal epithelium (Capraro et al. 2009, Muñoz et al. 2018). Absorbed hydrolysates will then act on target cells affecting internal metabolism. In this regard, there is evidence that γC administration to diabetic rats increases mRNA and protein insulin expression (Vargas-Guerrero et al. 2014). As expected, the increase in insulin was accompanied by reductions in serum glucose concentrations. Also, treatment of insulin-resistance or type-2 diabetes rat models with 150mg/kg of γC from *L. albus* decreased the expression of hepatic neoglucogenic - glucose-6-phosphatase (G6pc) gene; reduction of G6pc expression was also accompanied of a decrease in serum glucose concentrations (Gonzalez-Santiago et al. 2017).

Moreover, Terruzzi et al. (2011) have shown that the activation of C2C12 muscle cells with γC activates intracellular pathways similar to those that result from insulin stimulation active during glucose homeostasis (Terruzzi et al. 2011). Stimulation of C2C12 myocytes with γC turned on the IRS/PI-3-kinase intracellular pathway involved in glucose homeostasis. In that study, stimulation with γC increased flottilin-2 and caveolin-3 concentrations and the phosphorylation of CBL involved in the translocation of glucose transporter GLUT4 from the cytosol to the plasma membrane. Increased GLUT4 levels in the plasma membrane of insulin-responsive cells could explain the lowering plasma glucose concentration in animals and humans with diabetes that consumes lupin grains. Together, these studies show that the intake of *L. albus* components has important effects on the metabolism of glucose and insulin (Terruzzi et al. 2011).

Until recently, the cellular and molecular evidence on the beneficial effects of lupin on glucose metabolism was carried out with species of the legume from Europe and Australia and very little was known about the components of Andean *L. mutabilis*. We have established a dual culture system that allows the evaluation of legume hydrolysates, including those from *L. mutabilis*, that is obtained by enzymatic digestion similar to what would occur in the intestine, Figure 1.

This dual culture system can be used as a simplified model of the intestine in which the absorption of peptides or other substances produced by digestion and their potential action in cells of the organism can be tested. Thus, our research group has shown *in vitro* that hydrolysates of γC isolated from *L. mutabilis* affect glucose metabolism by inhibiting the enzymatic activity of DPP4 (between 80 and 100% inhibition compared to the positive control-sitagliptin). The increase in the sensitivity of the insulin receptor in adipocytes with the consequent increase in glucose consumption (between 8 and 13 mM glucose/mg protein versus 15mM glucose/mg with the positive control-insulin) and the presence of the GLUT-4 transporter on the cell membrane; and the inhibition of gluconeogenesis in liver cells (50% inhibition that is similar to the effect of the positive control - metformin) (Muñoz et al. 2018). The sequences of these hydrolysates derived from *L. mutabilis* predict important bio-active properties, including inhibition of DPP4 and ECA enzymes, antioxidant, and anti-thrombotic effects to improve the metabolism of glucose and the cardiovascular systems. These data show that *L. mutabilis* and its hydrolysates exert important clinical effects that are explained by their cellular and molecular mechanisms of action *in vitro*, Figure 2.

CONCLUSION

Current epidemiological, clinical, molecular, and cellular evidence indicates that consumption of legumes, including Andean *L. mutabilis* has important beneficial effects on cardio-metabolic metabolism. It is necessary to promote legume consumption as part of a healthy diet among the population. Nutritional guidelines should include legume consumption to prevent and treat metabolic diseases which are major public health problems worldwide. Besides, further research with legumes is necessary to fully understand the potential beneficial effects of these crops on human health.

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Chapter 13

ALLIUM: CASE STUDIES

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ABSTRACT

Genus *Allium* belongs to a group of species of Liliaceae Family. This valuable genus has widely been used in the traditional medicine of several cultures to treat some types of human diseases. Recent investigations performed with genus *Allium* showed several pharmacological activities such as antidiabetic, antihypertensive, anti-inflammatory, antiobesity, antioxidant, antihypercholesterolemic, anticancer (prostate, colorectal, breast and esophageal), immune system activator, antifungal and bactericide. Organosulfur and polyphenols compounds are considered secondary metabolites that are responsible for several pharmacological activities. Therefore, the main purpose of the present chapter is to

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show current research concerning successful, useful, and representative studies that demonstrate the pharmacological actions of the genus *Allium*.

Keywords: allicin, cysteine, organosulfur compounds

INTRODUCTION

In developed countries, public health represents one of the main priorities of governments. The main goal of public health is the development of community medicine, which aims to get health better and prevent diseases. Health promotion consists of providing people the necessary means to improve their health and exercise greater control over it (Singleton et al. 2016).

The use of medicinal plants by some human groups began in the early stages of humanity. People always had tried to cure their diseases with the use of plants that are found in their environment. Nowadays, there are several medicinal plants used for this purpose, and that has absolute validity (Sharma et al. 2017).

Recently, studies which have been conducted with medicinal plants by several research groups in the medical field are those related to the genus *Allium*; this is due to their specific biological properties.

The genus *Allium* includes approximately 600 species (Putnik et al. 2019). Genus *Allium* belongs to the Liliaceae Family. There are several species such as *Allium sativum* L. (Garlic), *Allium cepa* (Onion), *Allium porrum* (Leek), among others, whose fragrant properties determined the name since *Allium* which means odorous in Latin. In general, these plants have a strong and solid bulb; their stem is erect and cylindrical. The leaves are smooth, narrow, keeled, and their flowers whitish or reddish. For many years, these plants have coexisted as a fundamental part of human culture, being used by various civilizations in the preparation of food and multiple medicinal preparations (Lanzotti, Scala, and Bonanomi 2014).

The annual world production of garlic is around 11.79 million tons, highlighting China with 74% of the whole production, followed by Korea, India, and the United States. Mexico has only 0.55% of such production with 65200 tons and sown area of around 5654 ha (Macías-Duarte, Grijalva-Contreras, and Robles-Contreras 2010).

The representative vegetables of the genus *Allium* are commonly used in the kitchen recipes: garlic, onion, leeks, chives, and scallions (Sengupta, Ghosh, and Bhattacharjee 2004). Garlic and onion have also been used for millennia in the traditional medical practices of many cultures to treat cardiovascular diseases (Kendler 1987) and because it has anti-tumor, lipid-lowering, anti-arthritic, hypoglycemic and antithrombotic activities, additionally is commonly used as antimicrobial agents. Organosulphurs and polyphenols compounds, which are present in vegetables of the genus *Allium* are considered responsible for beneficial effects on human health. It is well known that the species that belong to the genus *Allium* can synthesize polyphenols. Garlic (*Allium sativum*) contains some acid compounds as ferulic acid, gallic acid, p-coumaric acid, 4-hidroxybenzoic acid, caffeic acid and chlorogenic acid; whereas onion contains quercetin, epicatechin-3-gallate, kaempferol, myricetin, epigallocatechin gallate and catechin. Around 70-80% of garlic odor is caused by the active molecule: allicin (Ramirez et al. 2017) that also serves as a quality indicator in the garlic marketing. Other compounds that are present in the garlic are methiin, propiin and isoalliinin. Alliin is an odorless compound that is found at concentrations from 5 to 14 mg/g in the garlic bulbs.

High concentrations of reactive oxygen species are responsible for oxidative stress in human cells. This is mainly related to several pathologies such as the Alzheimer´s disease (brain disorder which trigger dementia in older adults), Parkinson (movement disorder provoked by the insufficient dopamine amount synthesized by the neurons of the brain), hypertensive brain injury, muscular dystrophy (group of diseases that cause progressive weakness and loss of muscle mass), multiple sclerosis (disease of the nervous system that affect the brain and spinal cord), cancer (disease in which abnormal cells are divided without control and destroy healthy body tissues), retinal degeneration, retrolental fibroplasia (disease which is observed a week after of birth in preterm infants or children with lower birth weights who have been exposed to high oxygen concentrations), auto-immune diseases, rheumatoid arthritis (a form of arthritis that causes pain, inflammation, stiffness loss of function in the joints), diabetes mellitus (pancreas does not produce enough insulin or produces too little), metabolic syndrome (group of conditions that put a person at risk to develop a heart disease and diabetes type 2), cardiovascular anomalies, hypertension (chronic pathology that consisted in the increase of blood pressure), nephrology disorders, pulmonary emphysema, heart attack, anemia (enough healthy red blood cells in order to transport an adequate oxygen level), hepatitis (liver inflammation caused by one of the five types of hepatitis viruses named type A, B, C, D, and E), pancreatitis (inflammation in the pancreas), Werener disease (premature aging), premature wrinkles and skin dehydration, endothelial dysfunction (first signs of vascular disease and arteriosclerosis), among others (Sainz, Lombo, and Mayo 2012).

In garlic, are found some hormones that act similarly to the male and female sex hormones, besides other macromolecules are present such as ferments, choline, hydrorodonic acid and iodine; 17 amino acids have also been isolated, among theme are included aspartic acid, asparaginase, alanine, arginine, histidine, methionine, phenylalanine, leucine, serine, threonine, proline, tryptophan, and valine. On the other hand, the common onion (*Allium cepa* L.) biosynthesize a great variety of metabolites such as flavonoids, phytosterols, anthocyanins, and saponins (Nishimura et al. 2016). Onions have antioxidant, antimicrobial, and antidiabetic properties (Marrelli et al. 2018). Onion is an excellent source of quercetin, which can provide the recommended daily intake of flavonoids; approximately 20-35 mg. The skins of red onion contain high concentrations of flavonoids such as quercetin as well as glycosides, flavonols, anthocyanin and dihyflavonol (Singh et al. 2009). This chapter will focus on the current knowledge and investigations to support the properties of genus *Allium* as preventive medicine against certain diseases in experimental models.

GENERALITIES OF GENUS *ALLIUM*

The most representative species of the genus *Allium* is *sativum* L., commonly known as garlic. Its characteristic odor is due to the enzymatic reaction that is carried out when the bublets are cut, crushed or milled. When a garlic clove is intact, it contains mainly alliin (S-methyl cysteine sulfoxide). Alliin is a sulfoxide which is biosynthesized in the fresh garlic and is derived from the cysteine amino acid. When it is cut and crushed, the enzyme is known as alliinase, or s-alkyl cysteine sulfoxide that is present in the garlic acts converting alliin to allicin (a molecule responsible of the characteristic aroma of garlic) and when oxidized allicin

becomes a diallyl disulfide which is the basic molecule responsible for the odor and flavor of the garlic (Figure 1).

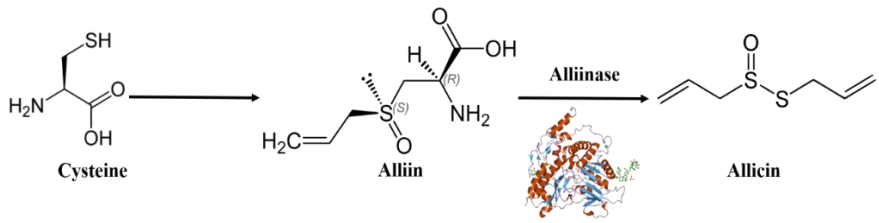


Figure 1. Synthesis mechanism of allicin (Source: Barrales-Cureño H.J.).

Recently, another species of the genus of *Allium* has been found. Several ethnobotanical reports said that it has similar properties. This plant is commonly known as bear garlic, and its scientific name is *Allium ursinum*.

This plant is native in Europe and Central Asia but is not extensively cultivated like garlic, it has been subjected to several studies where were found that its chemical composition and pharmacological activity are closely compared with *A. sativum* L.

CASE STUDIES

Allium as Antidiabetic Treatment

Diabetes mellitus is a heterogeneous group of disorders characterized by abnormalities in the metabolism of carbohydrates, proteins, and lipids. Diabetes mellitus type 1 is an endogenous autoimmune disease provoked by the destruction of beta-pancreatic cells and is characterized by a defect in insulin secretion, while diabetes mellitus type 2 is due to the failure of insulin secretion, insulin action, or both (Ashraf, Khan, and Ashraf 2011).

Garlic is a medical treatment option that enables improvements when it is used in the treatment of patients with diabetes and hyperlipidemia.

Ashraf, Khan, and Ashraf (2011) reported the hypoglycemic effect of the garlic in patients with type 2 diabetes. The study was based on two treatments: the treatment one consisted of 30 patients to whom were administered a single tablet with 30 mg of garlic (three times a day) + 500 mg of metformin (twice a day); the treatment two consisted of 30 patients to whom were administered placebo + 500 mg of metformin (twice a day) during 24 weeks. The treatment one showed a significant decrease in fasting blood glucose levels within the 24 weeks when was compared to treatment 2.

Taj Eldin, Ahmed, and Elwahab (2010) investigated the clinical hypoglycemic effect of *Allium* in patients with type 1 and type 2 diabetes. The consumption of 100 g of uncooked *Allium* caused a significant reduction of the fasting blood glucose levels at approximately 89 mg/dl respect to insulin (145 mg/dl) in diabetes type 1 patients and reduced the fasting blood glucose levels at 40 mg/dl in comparison with glibenclamide (81 mg/dl) in patients with type 2 diabetes four hours later administration. In patients with type 1 diabetes, the same dose of *Allium* produces a significant reduction of induced hyperglycemia at approximately 120 mg/dL with respect to the water (77 mg/dl) and insulin (153 ng/dl).

In patients with type 2 diabetes, considerably reduced the induced hyperglycemia at 159 mg/dl with respect to the water (55 mg/dl) and glibenclamide (114 mg/dl) after about 4 hours.

Eidi, Eidi, and Esmaeili (2006) investigated the antidiabetic effect of garlic ethanol extracts (*Allium sativum* L.) on common and diabetic rats induced with streptozotocin. The oral administration of garlic ethanol extracts decreased the level of serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate aminotransferase, and levels of alanine aminotransferase significantly, whereas the level of serum insulin was increased in diabetic rats but not in normal rats. The antidiabetic effect of the garlic extract was more effective than the observed with glibenclamide at 600 mg/Kg.

Allium as Antiobesity Treatment

Yoshinari, Shiojima, and Igarashi (2012) analyzed the anti-obesity effect of onion extracts in fatty diabetic Zucker rats (with obesity and diabetes) based on effective corporal measurements related to diabetes and obesity. Body and adipose tissue weights in 5% of the onion extract-fed group were found to be significantly lower than the control group without onion extract. Fasting blood glucose and levels of resistance to the insulin HOMA-IR (Homeostatic Model Assessment) also have been improved, although the serum levels of insulin and leptin showed no differences. The levels of triglycerides in serum and free fatty acids in the group fed with 3% and 5% of onion were significantly reduced compared to the control group.

Yang et al. (2018), explored the effect of garlic and onion oil against the serum lipids level in model rats with hyperlipidemia. The authors work with a population of 96 Sprague-Dawley rats (male) which were randomly divided into eight groups according to body weight, serum triglyceride levels, and total cholesterol. Rats received a repeated oral dose of volatile oils extracted from garlic and onion for 60 days. Volatile oils eliminated the weight body gain induced by HFD, and the rats tended to diminish the weight of fatty tissue. The oils decreased the levels of TG, TC, and LDL-C and also allow the increase of serum level of HDL-C in comparison with the control group. Oils were also effective to alleviate the hepatic steatosis

Allium as an Antihypercholesterolemic Agent

Aouadi et al. (2000) evaluated four experimental treatments in rats: the first treatment received a standard diet, the second treatment received supplementation with 10% of fresh garlic, the third treatment consisted of 2% of cholesterol and the fourth treatment received 2% of cholesterol and 10% of fresh garlic. The level of blood cholesterol was significantly lower in the second treatment than the treatment one. The hypercholesterolemia induced by the ingest of cholesterol (third treatment) was reduced significantly by the effect of fourth treatment. The results showed that the garlic has a hypercholesterolemia effect in normal rats.

Sun, Wang, and Qin (2018) examined the garlic impact in the increase of the blood lipid level through a meta-analysis. These authors concluded that garlic could reduce the level of total cholesterol and low-density lipoproteins and therefore, to indicate their antihyperlipidemic capacity.

Allium as an Anticancer Treatment

Onion biosynthesizes allyl sulfide, flavonoids such as the quercetin, are related to the anticancer effects (Sengupta, Ghosh, and Bhattacharjee 2004). Epidemiological studies are correlated with laboratory investigations; the results demonstrated a close relationship between the high ingestion of *Allium* products with the reduction of suffering certain types of cancer. Several mechanisms have been proposed to explain the preventive effects of cancer by the organic sulfur compounds present in the genus *Allium*.

This included the mutagenesis inhibition, modulation of the enzymatic activities and inhibition of DNA adduct formation, elimination of dangerous free radicals and the effects over the cell proliferation and tumoral cell growth (Sainz, Lombo, and Mayo 2012).

Some researches support the protective effect of vegetables of the genus *Allium* against cancer, specifically in the small intestine. The anticancer effects of vegetables of genus *Allium* are attributed to several organosulfur compounds (Challier, Perarnau, and Viel 1998). Also, there are studies related to the chemopreventive capacity of the genus against cancer (Le Bon and Siess 2000, Fleischauer, Poole, and Arab 2000, Fleischauer and Arab 2001). Below are presented some representative and successful cases of genus *Allium* as an anticancer model.

Rose et al. (2005) described a direct relationship between *Allium* consumption and the risk reduction of cardiovascular diseases and cancer; likely, many of these beneficial effects could be to the anti-inflammatory properties.

Park et al. (2013) evaluated the anticancer, antiobesity, and anti-inflammatory effects of nine vegetables of the genus *Allium*. Many of the extracts evaluated were able to reduce the cell viability of breast cancer. The order to see the cellular growth effects of the extracts have ministered 100 µg/ml during 72 hours and the effects were as follows: *Allium tuberosume* ≥ *Allium macrostemon* > *Allium thumbergii.*

Prostate Cancer

Hsing et al. (2002) investigated a population of individuals from Shangai, a case study and control that proved that men with the highest dietary intake of vegetables of the genus *Allium* showed 53% less likely to suffer prostate cancer in comparison to the others with lower consumption.

Colorectal Cancer

Millen et al. (2007), through a detection assay of prostate, lung, colorectal, and ovarian cancer, evaluated 562 cancer cases where 5932 individuals were used as controls, the protection against colorectal adenomas was associated with the ingestion of garlic and onion.

Fleischauer, Poole, and Arab (2000) performed a data meta-analysis that consisted of the revision and prevalence of several types of cancer grouped in colorectal and stomach about the garlic consumption (raw, cooked or both type garlic). The study suggested that the highest ingestion of garlic provoked a protective effect against some types of cancer as: stomach, colon, rectum, breast, prostate, and larynx.

Breast Cancer

Pourzand et al. (2016) reported a case-control study in a hospital to explore the association between *Allium* consumption in the daily diet and the risk of suffering breast cancer in a group of women in northwestern Iran. Such researchers mention that the highest use of a particular type of vegetables of the genus *Allium*, such as the garlic and leek, aid in the reduction of the risk of breast cancer, whereas the highest consumption of cooked onions, is associated with an increase the incidence cases of this type of cancer.

Esophageal Cancer

Chen et al. (2009) reported that in men from Taiwan, the fresh garlic or onion consumption once time a week is associated with protective effects against esophageal cancer. An Italian and Swiss case-control study also reported that the highest consumption of seven portions of onions by week has a protective effect against esophageal cancer as well as the garlic consumption (Galeone et al. 2006).

ANTICANCER EFFECTS OF SEVERAL ORGANOSULFUR COMPOUNDS FROM THE GENUS *ALLIUM*

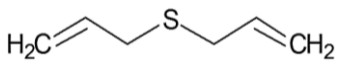
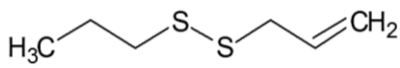
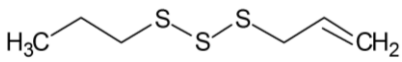
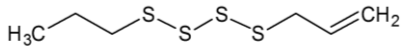
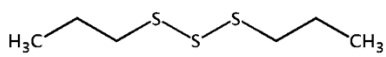
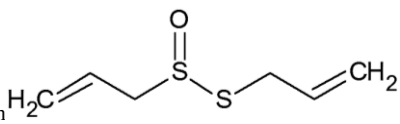
Hereafter are presented several investigations that concern on the anticancer effects of several types of organosulfur compounds biosynthesized by the genus *Allium* and the molecular interactions performed with the reactive oxygen species (ROS). Table 1 shows the increase or decrease of ROS depending on the type of molecule.

Several authors have reported an increase in ROS, which could be attributed to different molecules present in de genus *Allium*. Das, Banik, and Ray (2007) found that DAS showed a biological effect, a type of chemotherapeutic agent in human cancer cells, increasing the levels of ROS. Shukla and Kalra (2007) reported that DADS has a biological effect as a chemotherapeutic agent in human cancer cells, increasing the ROS level. Herman-Antosiewicz, Powolny, and Singh (2007) and Xu et al. (2006) concluded that DATS has an anti-angiogenic effect in normal human cells, enhancing the expression of ROS. Patya et al. (2004)indicated that allicin has a biological impact as an immune system stimulator in mice; therefore, the ROS levels were increased.

On the other hand, some molecules found in *Allium*, present a decrement in the ROS concentration. Chen et al. (2004) demonstrated that DAS has a biological effect as a cytoprotective agent on human cancer cells, therefore, reducing the levels of ROS. Devrim and Durak (2007) and Prasad et al. (2008) discovered that DAS has two biological effects as chemopreventive and cytoprotective agents in mice, declining the ROS level. Dashwood and Ho (2007) and Chen et al. (2004) demonstrated that DADS showed two biological effects as a cytoprotective and chemotherapeutic agent, declining the ROS levels in human cancer cells. Also, Chen et al. (2004) demonstrated that DATS showed a biological effect as a cytoprotective agent in human cancer cells, decreasing the levels of the reactive oxygen species. Liu et al. (2006) demonstrated that DATS has two biological effects: iNOS (Inducible nitric oxide synthase) reduction and activation of NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells) in macrophages, which decrease the ROS levels. Ariga and Seki (2006) and Fukao et al. (2004) showed that DATS has two biological effects in rats; a chemopreventive

and cytoprotective agent, and it can reduce the ROS levels. Murugavel and Pari (2007) demonstrated that DATTS has a biological effect as a cytoprotective agent in rats, decreasing the ROS levels. Chen et al. (2004) and Nishimura et al. (2006) indicated that DPTS has a biological effect as a cytoprotective agent in human cancer cells in mice, decreasing the ROS expression. Oommen et al. (2004) demonstrated that allicin showed two biological effects as a chemopreventive and chemotherapeutic agent in human cancer cells, increasing and diminishing the ROS level, respectively.

Table 1. Changes in ROS concentration attributed to several molecules present in *Allium*

Molecule	**Increment of ROS**	**Decrement of ROS**
Diallyl sulfide (DAS)  Molecular weight: 146.28 g/mol Chemical formula: $C_6H_{10}S_2$	(Das, Banik, and Ray 2007)	(Chen et al. 2004) (Devrim and Durak 2007)
Diallyl disulfide (DADS)  Molecular weight: 146.28 g/mol Chemical formula: $C_6H_{10}S_2$	(Shukla and Kalra 2007)	(Dashwood and Ho 2007)
Diallyl trisulfide (DATS)  Molecular weight: 178.33 g/mol Chemical formula: $C_6H_{10}S_3$	(Herman-Antosiewicz, Powolny, and Singh 2007) (Xu et al. 2006)	(Chen et al. 2004) (Liu et al. 2006) (Ariga and Seki 2006) (Fukao et al. 2004)
Diallyl tetrasulfide (DATTS)  Molecular weight: 210.386 g/mol Chemical formula: $C_6H_{10}S_4$		(Murugavel and Pari 2007)
Dipropyl trisulfide (DPTS)  Molecular weight: 182.358 g/mol Chemical formula: $C_6H_{14}S_3$		(Chen et al. 2004) (Nishimura et al. 2006)
Allicin  Molecular weight: 162.26 g/mol Chemical formula:$C_6H_{10}OS_2$	(Patya et al. 2004)	(Oommen et al. 2004)

Allium as an Antihypertensive Treatment

The species of genus *Allium*, as well as their extracts and chemical components of these plants, have been investigated by their potential effects against risk factors that causes cardiovascular diseases such as hyperlipidemia (elevated levels of lipids such as cholesterol and triglycerides), hypertension (chronic condition where the blood pressure is increased when the heart pumps blood into the arteries to circulate through the whole body) and hyperglycemia (abnormal increase of glucose levels in the blood) as suspects: platelet aggregation (platelets clump together and causes blood clotting) and fibrinolytic blood activity (a pathway of blood clots disintegration, where the major component is the plasminogen).

The use of certain garlic and onion formulations is accompanied by beneficial effects against the risk factors in normal persons and patients with atherosclerotic disease. The garlic supplements could be a promising treatment against uncontrolled hypertension, decreasing the blood pressure of 10 mmHg systolic, approximately, which is similar to the standard therapies used to treat blood pressure. The next examples show some representative and successful cases of the genus *Allium* as an antihypertensive treatment.

Ushijima et al. (2018) investigated the antihypertensive effect of the S-1-propenylcysteine (a sulfur compound present in aged garlic extracts) using as a model hypertensive rats. S-1-propenylcysteine is responsible for the antihypertensive effect and involves an improvement in the peripheral circulation. The treatment with S-1-propenylcysteine (6.5 mg/kg of body weight) significantly decreased the systolic blood pressure. On the other hand, S-allyl cysteine and S-allymercaptocysteine, which are present in the aged garlic extracts, were ineffective.

Ried and Fakler (2014) reported that the aged garlic extracts contain S-allyl cysteine, the sulfur content of this molecule acts as a bioactive compound, which can be S-allyl cysteine, but it does not have any possible harmful interaction when is taken with other medicines to reduce blood pressure.

Ashraf, Khan, and Ashraf (2011) evaluated the effect of garlic on blood pressure in patients with essential hypertension. A population of 210 patients with essential hypertension on stage 1 was divided into 7 groups named A, B, C, D, E, F, and G. Every group was composed of 30 patients. Each patient of the groups A, B, C, D, and E received garlic tablets of 300/mg, 600/mg, 900/mg, 1200/mg and 1500/mg at several daily doses, respectively, during 24 weeks, while the group G and G received doses of atenolol and placebo tablets, respectively. The blood pressure readings were recorded at 0, 12, and 24 weeks. The study demonstrated a significant decrease in the systolic and diastolic blood pressure, and the specific value and duration were dose-dependent.

Al-Qattan et al. (2003) investigated the effect of one dose of raw garlic over the expression of the exchanger Na/H^+ (NHE): NHE-1 and NHE-3 as well as the activity of the sodium pump in a model of hypertension 2K-1C in rats. The treatment with garlic significantly reduced the induction of NHE-1 in the kidneys 2K-1C, but increased the activity of the sodium pump in both kidneys 2K-1C. The activation of the sodium pump by the garlic extract in the kidneys should reduce the intracellular Na^+ concentration and normalizing blood pressure.

More recently, several studies have been conducted in order to compare the effect of two species (*A. sativum* L. and *A. ursinum*) in hypertensive rats. The results showed higher antihypertensive activity by *A. ursinum* at lower doses: 1%, than *A. sativum* L., therefore it becomes a promise in the pursuit of natural medicine with higher efficiencies (Preuss et al. 2001).

Allium as Antiinflammatory and Immune System Activator

There is strong evidence that some phytochemicals such as phenolic, alkaloids, nitrogenated and organosulfur compounds can improve the immune response in chronic infectious processes or immunodeficiency diseases (Jantan, Ahmad, and Bukhari 2015), these compounds can inhibit the production of proinflammatory cytokines or increase of antibodies production of immunoglobulins (Zhu, Du, and Xu 2018).

Below is presented a preliminary outline of studies related to the organosulfur compounds which are essential components of garlic and onion as beneficial substances on the immune system and inflammatory response.

Ajoene. Ajoene inhibits the production of NO, PGE-2, TNF-α, IL-1β, and IL-6, also inhibits the enzymatic activity of COX-2 and the release of PGE-2, besides increases the level of intestinal IgA (Lee et al. 2012).

Diallyl sulfide. DAS is a selective inhibitor of cytochrome P450 2E1, which is known to metabolize many xenobiotics, including alcohol and analgesic drugs in the liver (Rao et al. 2015). Diallyl sulfide inhibits the production of NO and reduces the expression of iNOS. It also exerts an inhibitory effect on the production of TNF-α, IL-1β, IL-6, IL-10 and inhibits the release of NO and PGE-2 (Santhosha, Jamuna, and Prabhavathi 2013, Hall et al. 2017, Ho and Su 2014).

Diallyl disulfide is one of the major volatile degradative compounds of garlic formed from allicin. Diallyl disulfide reduces the levels of IP-10, IL-6, IL-1β, and TNF-α and decreases the levels of the production of NO. It also inhibits the activation of NF-kB (Santhosha, Jamuna, and Prabhavathi 2013, Arreola et al. 2015, Ho and Su 2014, Fasolino et al. 2015).

Diallyl trisulfide (DATS) was investigated for its anticancer property as well as the molecular basis of the activity. DATS binds to specific cysteine residues in a β-tubulin molecule to build S-allylmercaptocystein, and that this could be the cause of cell cycle arrest and successive apoptosis with activation of caspase-3 (Seki et al. 2008). DATS inhibited significantly the growth of human colon carcinoma (Hosono et al. 2005). Diallyl trisulfide reduces the levels of IL-6, IL-10, IL-12 y TNF-α, also reduces the expression of iNOS and the production of NO. Besides exerts an inhibitory effect over the activation of NF-kB (Santhosha, Jamuna, and Prabhavathi 2013, You et al. 2013).

Allyl methyl sulfide increases the number of white blood cells and antibody titers (Schafer and Kaschula 2014). Allyl methyl sulfide also reduces the expression of iNOS and the production of NO; also inhibits the activation of NF-kB, leads to a reduction of the production of TNF-α and increases of IL-10.

Allicin reduces the expression of iNOS and favors the accumulation of NO. It also decreases the expression of TNF-α and NF-kB, IL-1β and IL-6 (Santhosha et al. 2013; Arreola et al. 2015; El-Sheakh et al. 2015; Panyod et al. 2016).

Alliin reduces the production of MCP-1 and IL-6, and increases the levels of IL-1β and TNF-α (Arreola et al. 2015).

S-allyl Cysteine prevents cell membrane damage, loss of cell viability and lipid peroxidation through avoiding the GSH degradation and by the activation of NF-kB (Santhosha, Jamuna, and Prabhavathi 2013, Colín-González et al. 2015).

Propyl disulfide inhibits the production of NO and PGE-2 (Chu et al. 2017).

Propyl propane thiosulfonate reduces the levels of TNF-α, IL-1β, IL-6, and IL-17 and increases the production of antibodies (Kim et al. 2013, Liu et al. 2013) (Liu et al. 2013; Kim et al. 2013; Trouerbach-Martínez 2017).

CONCLUSION

Genus Allium emerges as a model to prevent and treat diseases of global importance. It was experimentally found that the garlic helps to decrease the atherosclerosis process and antibacterial activity. Additionally, allium decreases platelet aggregation (one of the main causes of senile diseases such as Alzheimer). Moreover, allium was clinically proved for its antihypertensive, anticancer, and vasodilator effects. Organosulfur compounds that are present in the extracts of the genus Allium are secondary metabolites that interact to diminish the reactive oxygen species, activate the immune system or exhibit anti-inflammatory responses. All the case studies showed here demonstrated that the species of the genus *Allium* have the potential to be used as pharmaceuticals and could be used in medicine against certain human diseases to reduce the actual mortality rates.

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Chapter 14

STEVIA REBAUDIANA: AQUEOUS EXTRACTION OF DITERPENOID GLYCOSIDES AS A POTENTIAL REPLACEMENT FOR ARTIFICIAL SWEETENERS

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ABSTRACT

The use of plant products and derivatives for prevention and treatment of metabolic syndromes has increased in recent years. In this context, the effect of extraction temperature, particle size, and solid/solvent ratio on aqueous extraction of diterpenoid glycosides from *Stevia rebaudiana* Bertoni leaves is discussed in this chapter, in order to establish ideal conditions that provide further applications to the aqueous extract such as nutraceuticals products focused on dealing with health problems. A 2^3 full factorial design of 8 experimental runs was applied to describe the concentration of stevioside as a function of assessed extraction parameters. The extraction was carried out in stainless steel digestor equipment. Organic solvents were used for clarification of raw extracts. UV-Vis screening of raw and clarified extracts was performed in a Synergy HTX multi-mode microplate reader. Rebaudioside A, and stevioside identification, and quantification were performed in a High-Performance Liquid Chromatography (HPLC) Perkin Elmer Series 200. The data

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analysis and graph plotting were performed in MINITAB 17.0 statistical software. The result obtained indicates that particle size and solid/solvent ratio mainly influence on aqueous extraction of stevioside. Additionally, the highest achieved stevioside concentration (3.30 g/100 g dried leaves) showed that natural sweeteners could be obtained at 60°C, 250µm, 1/10 kg/L by digestion, and the extraction conditions found possibly could be applicable on an industrial scale with the aim of substitute artificial sweeteners.

Keywords: asteraceae, digestion, HPLC, rebaudioside A, stevioside

INTRODUCTION

Sugar is conventionally used in the food industry as a sweetening agent. Nonetheless, there is an increasing demand for other sweeteners due to the health problems caused by its excessive intake (Khan and Abourashed 2011). This fact contributes directly to the increase of energy density of food, its effect on weight gain, and its straight relationship in some human diseases (Laville and Nazare 2009). Obesity, hypertension, cardiovascular diseases, health problems related to type 2 diabetes mellitus, and certain types of cancer have been associated with diets high in fat, and sucrose (Bermudez Menendez de la Granda and Sinclair 2009). Consequently, new alternatives have appeared, and the consumption of foods and beverages containing artificial sweeteners has increased dramatically in the last years (Spencer et al. 2016).

Substituting sugar for low-calorie sweeteners can be an effective strategy for weight control, and to avoid various health problems. However, the use of artificial sweeteners has been controversial concerning its long-term safety or excessive use (Alonso 2010). Since its discovery and introduction into the market, there has been a great debate about the health advantages and disadvantages of these. The first evidence of health problems related to artificial sweeteners was observed by the Food and Drug Administration (FDA) in 1970. The substitute for commonly used sugar known as cyclamate was deemed inappropriate for the consumption due to its carcinogenic effects evidenced through many animal studies (Patra, Tomar, and Arora 2009). After this, researchers have been attracted to obtained sweeteners from natural sources as an excellent alternative to replace sucrose.

In this perspective, the extract obtained from *S. rebaudiana* Bertoni leaves known as stevia has been given much attention since it is natural, sweet-tasting, and calorie-free. So, it could be used as a substitute for sugar or as an alternative to artificial sweeteners, since obesity has been related to a broad spectrum of co-morbidities, such as cardiovascular diseases, reproductive disorders, particular cancer types, type 2 diabetes, and hypertension (Kyrou et al. 2018). Stevia has increased insulin sensitivity in rodent models (Chang et al. 2005), it has been shown beneficial effects on blood glucose, it has been maintained insulin levels in human studies (Curi et al. 1986, Gregersen et al. 2004), it has shown improvement in chronic kidney disease patients (Rizwan et al. 2018), and it has no adverse secondary effects in humans (Barriocanal et al. 2008). These studies suggest that this extract containing diterpenoid glycosides may have an important role in the regulation of food intake, and consequently in diabetic people with hyperglycemia. Nevertheless, there are no studies about optimum conditions for extraction of compounds in a laboratory-scale digestor.

Thus, the aim of this study was to assess the effect of extraction temperature, particle size, and solid/solvent ratio on aqueous extraction of diterpenoid glycosides from *S. rebaudiana*

Bertoni leaves, in order to establish ideal extraction conditions, and to support the use of aqueous stevia extract as a plant-derived alternative source against metabolic syndromes.

ARTIFICIAL AND NATURAL SWEETENERS

Sweeteners are additives that confer sweet flavor to food. Some of them are natural, while others are synthetic. So, the latter are called artificial sweeteners.

Artificial Sweeteners

Artificial sweeteners are substances that do not provide calories when ingested and maintain a degree of sweetness higher than sucrose. Although most artificial sweeteners have side effects, they are widely used in the manufacture of diet foods.

A well-known sweetener is saccharin, it is 200 to 700 times sweeter than sugar, but it has a slightly unpleasant metallic taste. Its glycemic index is zero, and the acceptable daily intake (ADI) is 5 mg/kg of body weight. Saccharin has been investigated extensively to determine its carcinogenic potential; due to studies have been reported bladder cancer in rats exposed to high doses of saccharin. Despite the history of this sweetener, people continue to consume foods that contain non-nutritive sweeteners to lose weight (Sánchez-Medina and Vargas 2013).

On the other side, a sweetener produced from the amino acids: aspartic acid and phenylalanine is aspartame. This compound provides 4 kcal/g, and it is 200 times sweeter than sucrose with an ADI of 50 mg/Kg of body weight. Aspartame is metabolized to phenylalanine, aspartic acid, and methanol in the gastrointestinal tract, which produces effects such as dizziness, headaches, digestive problems, and mood swings (Tandel 2011). Also, the consumption of aspartame causes diseases such as Alzheimer's, attention deficit disorders, congenital disabilities, cancer, diabetes, and lupus. It is one of the most controversial artificial sweeteners widely used today.

Similarly, acesulfame-K is approximately 200 times sweeter than sugar with an ADI of 15 mg/ Kg of body weight. Nevertheless, one of the products of the breakdown in the body after metabolization is the acetoacetamide, which is toxic in high doses (Mukhopadhyay, Mukherjee, and Chakrabarti 2000).

Another substance is sodium cyclamate. It is the second oldest artificial sweetener in use currently. It is the least powerful of this group because it is between 35, and 50 times stronger than sucrose with an ADI of 11 mg/Kg of body weight, it has a glycemic index (GI) of zero, and it doesn’t provide calories (Carocho, Morales, and Ferreira 2017).

Among other artificial sweeteners, sucralose is 600 times sweeter than sugar and has no unpleasant taste. It is made by the selective substitution of chlorine by hydroxyl groups on a sucrose core (Grotz and Munro 2009).

Finally, it is important to highlight neotame, which is a dipeptide derived from aspartame. It is permitted as a sweetener, and flavor enhancer in foods, and it has sweetening power 10,000 times greater than sucrose (Roberts 2016).

Natural Sweeteners

Natural sweeteners are extracted mainly from some plants. They are used in the beverage, food, dental, and hygiene industries for their pleasant taste. Among natural sweeteners, the best known is the sucrose (sugar). It is constituted by a molecule of fructose and another glucose, linked by a glycosidic bond. It provides excellent sweetness characteristics, so it imparts a palatable flavor for consumers despite being caloric.

The sweetener that exceeds sucrose in sweetening power is honey produced by bees. It is also rich in fructose and glucose, but it has a sweetening power is twice as high as cane sugar (Alonso 2010). Glucose is present in most fruits and many vegetables, and it has a sweetening power of 60%, while fructose is the sugar with the most significant sweetening power and is abundant in fruits. Besides, there are both natural and artificial fructose polymers, but these compounds do not contribute to the sweetness of foods in a significant way. Moreover, there are sweeteners such as those found in milk, whose main sugars are lactose, and tagatose. The first is a disaccharide composed of glucose and galactose. It has low solubility in water and sweetness of 40% compared to sucrose, while tagatose produces a lower glycemic response. Another sweetener constituted by glucose, and a glycosidic bond is maltose. This reducing sugar has a sweetness 50% high compared with sucrose.

There are sweeteners obtained by complete depolymerization of corn starch, such as dextrose, which is a monosaccharide with a sweetening power of 60% to 70% on a sugar basis. Also, sweeteners derived from saccharides such as sugar alcohols. They are classified as monosaccharides, and hydrogenated disaccharides such as sorbitol, mannitol, xylitol, isomaltose, maltitol, lactitol, among others.

A widely used sweetener is the high fructose corn syrup obtained by the isomerization of dextrose. It has a sweetening power of 1, and it synergizes the sweetening power of sucrose and other non-nutritive sweeteners. Nonetheless, its consumption can cause liver damage and increases in uric acid.

Other sweeteners are thaumatin and glycyrrhizin. Thaumatin represents a set of proteins (polypeptides) extracted from *Thaumatococcus daniellii* Benth, and it is considered the sweetest substance on the planet. Thaumatin is 1,600 times stronger than a 10% sucrose solution. Glycyrrhizin is obtained from the licorice species *Glycyrrhiza glabra*, and it has a sweetening power 60 times greater than sucrose (Alonso 2010).

As a final point, there are steviol glycosides extracted from *S. rebaudiana* Bertoni leaves. This species is known as sweet yerba. Its most abundant sweet components are stevioside, and rebaudioside A. The leaves have a glycemic index of zero and they are 15-30 times sweeter than sugar, and the stevioside in pure form is 300 times sweeter than a 0,4% sucrose solution.

STEVIA REBAUDIANA BERTONI

Stevia rebaudiana Bertoni native from Paraguay is one of the 950 plant species belonging to the Asteraceae family, and it has been used extensively by the Guarani Indians for more than 1500 years. The plant was botanically classified by Moisés Santiago Bertoni in 1899 and initially was called *Eupatorium rebaudianum* (Lemus-Mondaca et al. 2012). Stevia has a long history of ancestral use in Paraguay and Brazil, and its generational importance considers it within the therapeutic area. Currently, more than 300 species of Stevia are known, but Bertoni's

is the only one with important sweetening properties. The leaves contain approximately 4-15% stevioside, which have a sweetening power 100-300 times greater than sugar. They are low in calories, and they are neither toxic, nor mutagenic (Goyal, Samsher, and Goyal 2010).

Stevia leaves contain several natural nutrients that are important in the medical area, including vitamin B3 (Niacin), and minerals such as chromium, magnesium, manganese, potassium, selenium, and zinc, which are beneficial for human health (Kumari and Chandra 2014). The percentage of the main glycosides and other biochemical constituents of *Stevia* leaves are shown in Table 1.

Table 1. Main glycosides and other biochemical constituents of stevia leaves

Chemical constituents	**% (mg/100 g dry leaves) (Yadav et al. 2011)**	**% (mg/100 g dry leaves) (Razo 2011)**
Stevioside	4 – 14	–
Rebaudioside A	2 – 4	–
Rebaudioside C	1 – 2	–
Dulcoside A	0.4 – 0.7	–
Rebaudioside B,C,E, F	> 0.4	–
Carbohydrates	35.2	56.33
Proteins	12.0 – 20.42	12.09
Lipids	2.7 – 4.34	1.81
Ash	13.12	6.11

The plant has been propagated mainly by seeds that result from cross-pollination. This method of reproduction implies genetic recombination, and therefore, high heterogeneity in the growth of plants, and the production of steviosides (Madan et al. 2010). Moreover, it has resorted to asexual reproduction using cuttings, which are stems or buds that are separated from a plant to introduce it into the earth to be born a new one. However, with this form of reproduction, a low number of individuals per plant have been obtained (Ferreira et al. 2006). Another alternative is the cultivation of tissues using micropropagation since this procedure offers the possibility of getting many plants of a selected genotype, with more consistent results in terms of their growth, and production of steviosides. Likewise, it has allowed greater control over the health of the material, facilitating its transportation from one country to (Sivaram and Mukundan 2003).

This species has been reported that possess antitumor, antimicrobial (Gamboa and Chaves 2012), antioxidant (Shivanna et al. 2013), hepatoprotective (Jayaraman, Manoharan, and Illanchezian 2008), hypoglycemic (Chen et al. 2005), anti-rotavirus (Takahashi et al. 2001), anti-hypertension (Lee et al. 2001), and antiviral activity (Kedik, Yartsev, and Stanishevskaya 2009). In this context, some studies about the comparative effects of leaves, and stevioside on glycemic, and hepatic gluconeogenesis have been described (Ferreira et al. 2006). Also, other studies about the use of this compound for the treatment of diabetes have been informed. The hypoglycemic action focuses on the presence of steviosides extracted by chemical affinity with methanol and water (Chen et al. 2005).

Due to the properties of this species, the Food and Drug Administration (FDA) and the European Food Safety Authority recognized the use of diterpenoid glycosides as a natural non-caloric safe product for food and beverages. The stevia market is estimated to grow from US$

347 million in 2014 to US$ 565 million by 2020 worldwide (Sanchez-Aceves et al. 2017). Therefore, it is important to look for new alternatives to produce this sweetener.

MATERIAL AND METHODS

Experimental analysis was done at Centro de Investigaciones Biotecnológicas del Ecuador (CIBE) of Escuela Superior Politécnica del Litoral (ESPOL). The flow process of the experimental setup is presented in Figure 1.

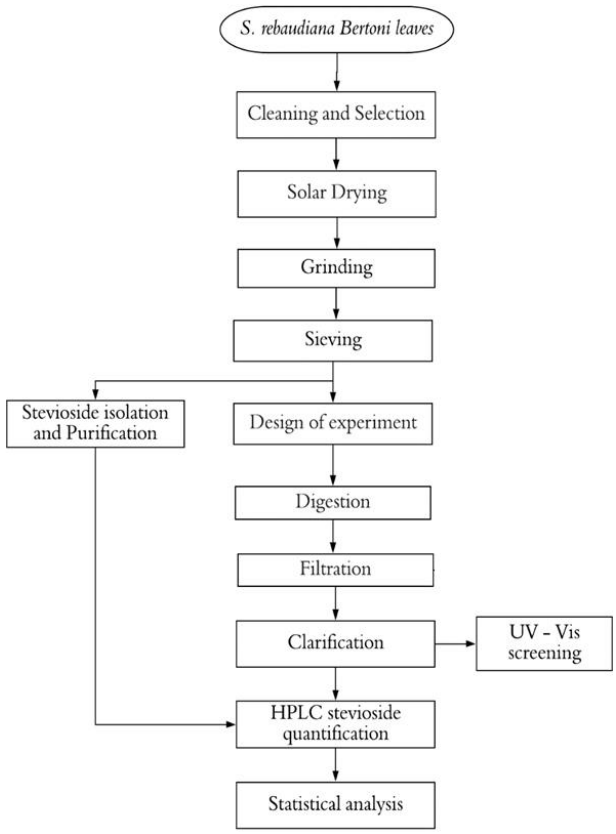

Figure 1. Stevioside extraction and quantification flow process.

Plant Material

S. rebaudiana Bertoni leaves were collected in Santa Elena, Ecuador (2°23'70.58" S, 80°82'74.32" W). The collected plant material was authenticated by the National Herbarium of Ecuador (QCNE) with voucher CIBE038. Then, green leaves were separated from brown, and yellow leaves, later green leaves were dried for 48 hours by solar drying at 20-35 °C, they were ground to a fine homogenous powder, and they were stored at room temperature (25°C) in a heavy-duty kraft paper drum with high strength paperboard lid and lever ring with sealing before the extraction process.

Standard and Reagents

Rebaudioside A standard was acquired from Wako Chemical (Richmond, USA). Methanol, methanol HPLC grade, acetonitrile HPLC grade, tricarboxylic acid, and calcium hydroxide were purchased from Merck (Darmstadt, Germany). Hexane and chloroform were obtained from Fisher Scientific (New Hampshire, USA). Phosphoric acid was purchased from Sigma-Aldrich (Missouri, USA), and Milli-Q grade water was prepared using a Direct-Q 3 with UV system Millipore (Massachusetts, USA).

Extraction Procedure

The conditions used in each experiment were established according to the 2^3 full factorial design presented in Table 2. The independent variables or factors defined were extraction temperature (X_1), particle size (X_2), and solid/solvent ratio (X_3). The response variable was the stevioside concentration (Y). As shown in Table 3 a total of 8 experimental runs at two different levels of factors were performed. All experiments were carried out three times.

The digestion process was employed for the extraction of steviosides from *S. rebaudiana* Bertoni leaves. The extraction was carried out in stainless steel digestor equipment of 30 liters of process capacity, 40 psi of maximum pressure, with central agitation, cambered lid, staple system, and drainage valve. The digestion process was done by batch for 1 hour, and 30 minutes with constant agitation, constant pressure, and water was used as solvent extraction. Next, the raw extracts were filtered through Whatman paper No.1 for measuring pH, and °Brix before instrumental analysis.

Table 2. Factors and levels using in the 2^3 factorial design study

Factors	**Levels**	
	(-)	(+)
X1 = Extraction temperature (°C)	50	60
X2 = Particle size (µm)	250	500
X3 = Solid/solvent ratio (kg/L)	10/1	13/1

Table 3. Experimental design used for steviosides extraction

Run	**Independent Variables***		
	X1 (°C)	**X2 (µm)**	**X3 (Kg/L)**
1	60	250	1/10
2	50	500	1/13
3	60	500	1/10
4	50	500	1/10
5	50	250	1/10
6	60	500	1/13
7	60	250	1/13
8	50	250	1/13

X_1 = extraction temperature, X_2 = particle size, X_3 = solid/solvent ratio.

Stevioside Isolation

A sample of fine homogenous powder previously obtained was used. First, 50 g leaves were macerated with hexane for 24 hours. Next, the hexane was recovered by rotary evaporation, and the maceration process was repeated with chloroform for 2 hours. Then, the vegetal matrix without organic solvents was dried for 1 hour at 55°C. The powder obtained was extracted by a reflux procedure with 100 mL of water for 20 minutes. The extract was filtered, it was adjusted to pH = 4 with tricarboxylic acid, was adjusted to pH=8 with calcium hydroxide, and was neutralized with citric acid. Later, the neutralized aqueous extract was mixed with hexane for 2 hours, and separation was done in a decanting funnel. The aqueous extract was mixed with activated charcoal, they were subjected to reflux procedure again for 20 minutes to remove interfering components, and they were filtered through 0.22 µm millipore filter. Next, the aqueous extract was purified with methanol HPLC for precipitation of contaminants. Finally, steviosides were concentrated by rotary evaporation, and they were crystalized at 50°C.

Clarification and UV-Vis Screening

Colorimetric analysis of clarified extracts was performed in order to know the percentage of clarification of extracted samples. First, clarification was performed according to (Inamake et al. 2010). Raw extracts were treated with organic solvents. A mixture with hexane for 24 hours was carried out to lipids removal. Then, the aqueous phase was separated and was adjusted with tricarboxylic acid, calcium hydroxide, and citric acid, as mentioned before. Subsequently, a second mixture with hexane was done for 4 hours, the separation was performed in a decanting funnel, aqueous phase was treated with methanol HPLC grade to pigments precipitation, and was centrifuged at 5000 rpm for 20 minutes, and 20°C to obtain clarified extract. Afterward, Ultraviolet-visible (UV-Vis) screening of raw and clarified extracts were performed in a Synergy HTX multi-mode microplate reader with UV-VIS detector (Biotek, Winooski, Vermont) at different wavelengths 450 500, 550, 600, and 645 ηm.

Diterpenoid Glycosides Quantification

Rebaudioside A, isolated stevioside, and clarified extracts quantification were performed according to Inamake et al. (2010) and Halim, Sarijan, and Shaha (2013) using a High-Performance Liquid Chromatography (HPLC) Perkin Elmer Series 200 with Diode Array Detector (DAD). A Jupiter C18 (250 mm x 4.6 mm) 300 Å, 5 µm of internal diameter with fully porous silica was used as stationary phase, and HPLC grade acetonitrile-water (80:20) adjusted to pH = 3 with phosphoric acid (85%) was used as mobile phase. The column temperature was set at 28°C. The samples were filtered through a 0.22 µm millipore filter, the injection volume was 10 µL at a flow rate of 1 mL/min with linear-gradient, and samples were analyzed to 210 ηm. The data obtained were analyzed in TotalChrom Navigator Software (Connecticut, USA). A calibration curve of isolated stevioside (1000-5000 mg/L) was used for sample quantification. Afterward, an area vs. concentration plot was generated, and it was adjusted by linearization analysis.

Statistical analysis

The influence of defined independent variables extraction temperature, particle size, and solid/solvent ratio on response variable stevioside concentration was analyzed in MINITAB 17.0 statistical software (Pennsylvania, USA). The software was also used for graph plotting.

RESULTS AND DISCUSSION

In this section, ideal parameters, and conditions for *S. rebaudiana* Bertoni leaves extraction in digestor equipment are presented and discussed.

Digestion

The digestion of the sweetening principles was chosen as the extraction method because it is a process that works in temperature ranges between room temperature, and 60°C. According to previous reports, temperatures higher than 60°C may produce the oxidation of glycosides, and the presence of unwanted compounds which increase the bitter taste of steviosides (Halim, Sarijan, and Shaha 2013). After the extraction process, the pH, and °Brix of extracts (50 mL) were measured at different temperatures. Table 4 shows the experimental values at 10°C and 20°C. These results indicate that there are no changes of pH, and °Brix depending on measurement temperature. Additionally, some studies have been revealed that aqueous solutions prepared with stevia leaves show diterpenoid glycosides stability in a wide range of pH (1-10) and temperatures close to 60°C after 2 hours of thermal treatment (Lemus-Mondaca et al. 2012). Hence, the results of this study agree with the reference values and confirm the high content of steviosides after digestion.

On the other hand, it is important to mention that the grinding process of plant material previous extraction was significant since the particle size reduces the apparent density of the matrix, improves the kinetics of digestion, and implies smaller storage conditions. In fact, previous studies on stevia leaves indicate that smaller particle size increments the glycosides content and reports values between 0.5-1.6° Brix for particle size among 2340–660 µm, respectively (Sabreena et al. 2017).

Table 4. Parameters variation as a function of temperature

Run	**pH**		**°Brix**	
	(10°C)	(20°C)	(10°C)	(20°C)
1	5.44	5.36	5.00	5.00
2	5.39	5.42	4.00	4.20
3	5.03	4.93	4.00	4.05
4	4.82	4.73	5.00	4.95
5	5.07	5.17	4.80	4.90
6	5.33	5.27	4.20	4.10
7	5.34	5.33	3.80	3.95
8	5.40	5.43	4.60	4.20

UV-Vis Screening

A clarification process at a laboratory scale with organic solvents: hexane and methanol HPLC grade were carried out to define an ideal measurement wavelength before clarification analysis. Table 5 shows the absorbances values of the crude extract obtained at 50°C, 250µm, and solid/solvent ratio 1/10 at different wavelengths (450–645 ηm). This sample was randomly selected after clarification, and it is evident that the highest value of absorbance corresponds to the crude extract measured at 450 ηm.

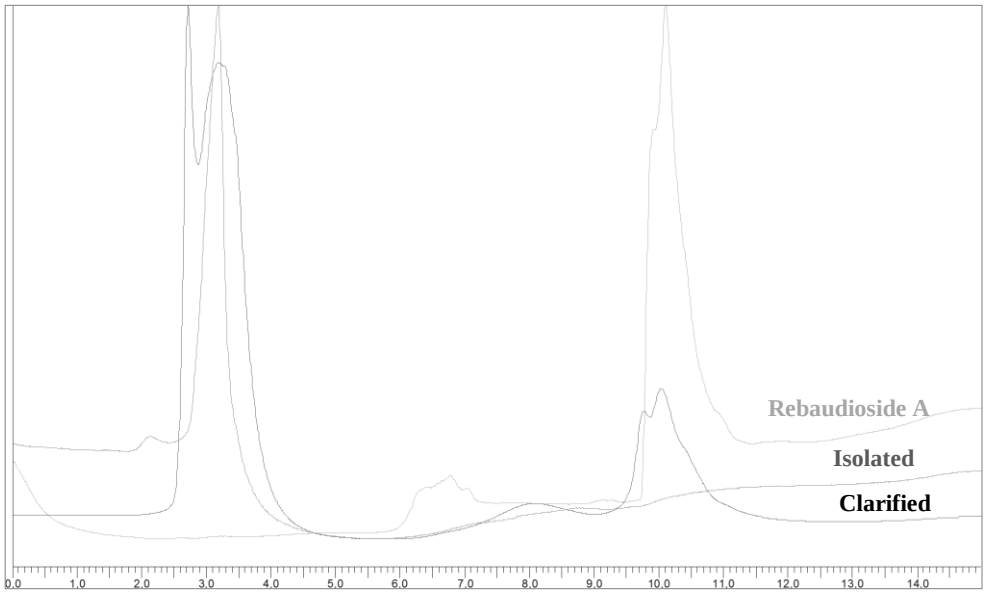

Figure 2. Overlay Chromatograms of Rebaudioside A, Isolated Stevioside, and Clarified Extract.

Table 5. UV-Vis screening of raw and clarified extract

	Wavelength (ηm)	**Absorbance**	
		Raw extract	Clarified extract
	450	3.529	0.183
	500	1.670	0.113
	550	0.966	0.087
	600	0.576	0.079
	645	0.440	0.079

This phenomenon indicates the presence of color pigments such as chlorophyll, and other compounds as protein, lipids, and carbohydrates therein (Chhaya, Majumdar, and De 2013). Therefore, the exploration of wavelengths ranges assessed was a useful tool to follow the course of the removal of unwanted compounds from the raw extracts.

Clarification

The absorbance of the 8 raws and clarified extracts obtained in the digestor equipment were measured at 450 ηm and later clarified with solvents (Table 6). The raw extracts exposed a dark green color, and its absorbance values were higher than 2.588, while the clarified extract treated with organic solvents showed a yellow color, and absorbance values lower than 0.230. The employed clarification procedure revealed percentages values from 92.53 to 95.47%. The results obtained are comparable with values described by Rao et al. (2012), who reported values removal between 80-90% using ultrafiltration as a procedure for clarification process.

Table 6. Corresponding clarification values of the dependent variables to extraction conditions

Run	**Absorbance**		**Clarification**
	Raw extract	Clarified extract	(%)
1	3.768	0.199	94.72
2	2.658	0.172	93.53
3	3.024	0.137	95.47
4	3.266	0.225	93.11
5	3.529	0.183	94.81
6	2.588	0.143	94.47
7	3.009	0.191	93.65
8	3.078 extract	0.230	92.53

Stevioside Isolation Yield

After the isolation procedure, 1.73 g of stevioside was obtained, and 50 g of stevia dry leaves were used before extraction and isolation. Thus, the stevioside yield was 3.46% (w/w). This result accords with stevioside content values between 1.98–3.75% (w/w) reported for stevia leaves from eleven different regions (Chester et al. 2012).

HPLC Stevioside Quantification

HPLC identification and quantification of diterpenoid glycosides of clarified extracts were made by retention time comparison, and the calibration curve area vs. concentration, respectively. Figure 2 shows the overlay chromatograms of standard Rebaudioside A, isolated stevioside, and clarified extract. The chromatogram illustrates the presence of Rebaudioside A, and stevioside in the clarified extract assessed. The retention time (Rt) of isolated stevioside was 3.27 minutes, while the Rt of standard Rebaudioside A was 10.09 minutes at the same chromatographic conditions. As revealed by the chromatograms, the isolated stevioside showed one peak compare with the clarified extract which indicated the existence of impurities at the beginning of elution. This phenomenon could indicate that the isolation stevioside procedure previously used was suitable. The results obtained agree with Inamake et al. (2010), who reported stevioside quantification after stevioside extraction, and isolation from stevia leaves using similar chromatographic conditions and comparing with analytical standard.

In the same way, stevioside (Rt = 3.30 minutes) and Rebaudioside A (Rt = 4.10 minutes) have been detected in stevia extracts prepared with different solid/solvent ratios (Halim, Sarijan, and Shaha 2013). Alternatively, the concentration of stevioside has been informed for dried stevia leaves employing a validated isocratic solvent system (Jadhao, Katekhaye, and Thorat 2011). Also, both compounds: stevioside (Rt = 7.20 minutes), and Rebaudioside A (Rt = 6.90 minutes) have been reported as main glycosides in two different varieties of stevia using isocratic (Aranda-González, Moguel-Ordonez, and Betancur 2015). Hence, the use of gradient elution for glycosides separation could explain the differences in retention times between this work and reference studies. The choice of an appropriate eluent system contributes a suitable separation in terms of speed, retention, and quantification (Schellinger and Carr 2006). In the present work, Rebaudioside A is not reported quantitatively because the impurities of clarified samples interfered with the separation of the compounds. Consequently, the isolated stevioside was used for quantification of clarified extracts.

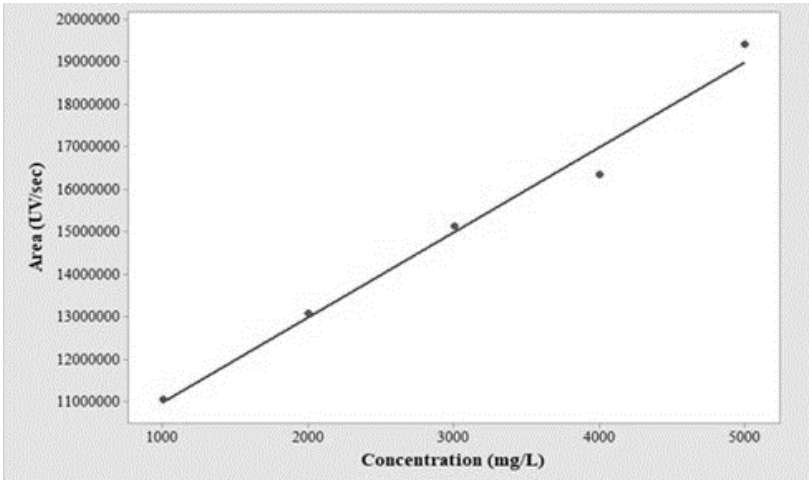

Figure 3. Calibration Curve of Isolated Stevioside.

In the present work, Rebaudioside A is not reported quantitatively because the impurities of clarified samples interfered with the separation of the compounds. Consequently, the isolated stevioside was used for the quantification of clarified extracts. Figure 3 illustrated the area vs. concentration plot obtained for quantification. The linearization analysis indicated a correlation coefficient $R^2 = 0.98$ in the glycoside concentrations range assessed.

Effect of Independent Variables on Stevioside Concentration

The data for the influence of extraction temperature, particle size, and solid/solvent ratio on response variable stevioside concentration is shown in Table 7. The stevioside concentration of 8 clarified extracts evaluated according to the design of the experiment was calculated by interpolation analysis in the previous calibration curve (1000-5000 mg/L).

Ideal parameters of extraction were selected based on the concentration of stevioside (mg/L). First, the main factors were determined using a Pareto diagram. According to Figure 4, the factor that exposed the most relevant effect on the concentration of stevioside extracted

was the interaction between particle size, and solid/solvent ratio (X_2X_3), followed by the extraction temperature (X_1), and the interaction of all factors ($X_1X_2X_3$). Particle size had a negative effect on stevioside concentration. Meanwhile, the solid/solvent ratio presented a positive impact on the concentration of stevioside extracted. In general, the statistical analysis indicated that diterpenoid glycoside stevioside (3303.61 mg/L) could be obtained at 60°C, 250 µm, and 1/10 Kg/L by digestion of *S. rebaudiana* Bertoni leaves. The stevioside concentration found in this study (3.3 g/100 g dried leaves) agrees with stevioside content (3.97–8.80 g/100 g dried leaves) previously informed for two varieties of stevia obtained after aqueous extraction at 100°C for 30 min (Aranda-González, Moguel-Ordonez, and Betancur 2015).

Table 7. Experimental design and corresponding response values of the dependent variables to extraction conditions

Run	Independent Variables*			Response Variable**	
	X1 (°C)	X2 (µm)	X3 (kg/L)	A (uV/sec)	Y (mg/L)
1	60	250	1/10	15741347.21	3303.61
2	50	500	1/13	12479484.72	1705.13
3	60	500	1/10	11091389.99	1024.89
4	50	500	1/10	11834056.66	1388.83
5	50	250	1/10	12522411.44	1726.16
6	60	500	1/13	15701969.75	3284.31
7	60	250	1/13	13517717.76	2213.92
8	50	250	1/13	12521802.34	1725.87

* X1 = extraction temperature, X2 = particle size, X3 = solid/solvent ratio.
**A = peak area (uV/sec), Y = stevioside concentration.

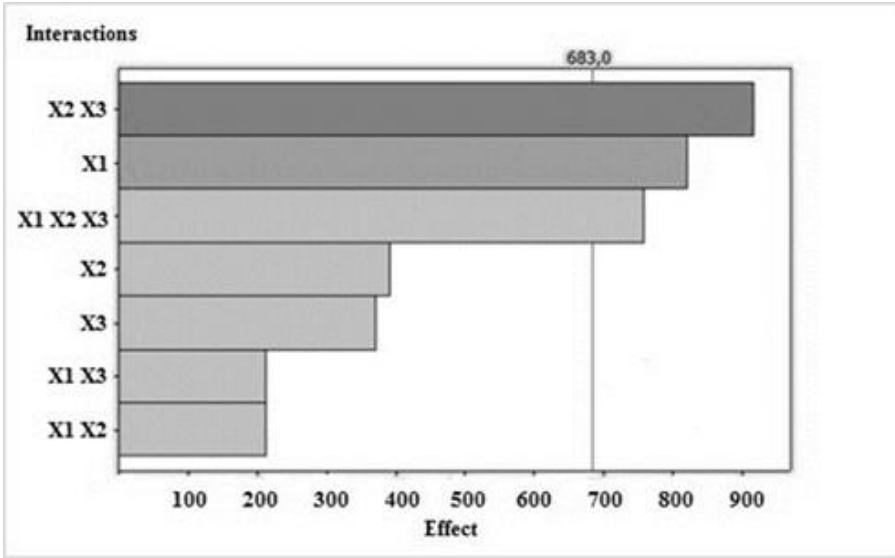

Figure 4. Pareto diagram of significance, X_1 = extraction temperature, X_2 = particle size, X_3 = solid/solvent ratio.

Although Rebaudioside A quantification was not achieved, this compound probably appears as the second main glycoside as shown in Figure 4. The Rebaudioside A concentration (2.24 g/100 g dried leaves) has been reported for stevia and the results also demonstrate the chromatographic peak of Rebaudioside A after stevioside (Halim, Sarijan, and Shaha 2013).

CONCLUSION

This study indicates that particle size and solid/solvent ratio directly influence aqueous extraction of stevioside, and Rebaudioside A obtained by digestion from *S. rebaudiana* Bertoni leaves. The achieved results show that natural sweeteners could be obtained using water as an extraction solvent, and the procedure describes in this work possibly could be applicable on an industrial scale to produce a natural sweetener for human consumption.

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Chapter 15

PHARMACOGNOSIS AND PHYTOCHEMICAL STUDY OF LEAVES, FLOWERS, FRUIT SHELLS, AND SEEDS OF TWO VARIETIES OF *NEPHELIUM LAPPACEUM* L. CULTIVATED IN ECUADOR

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ABSTRACT

Nephelium lappaceum L., commonly known as achotillo, rambutan, or Chinese sucker is a fruit native to Asia whose cultivation has spread in many countries. In addition to its use as food due to the chemical components it possesses, various therapeutic properties are attributed to it. Among these are its use to combat obesity and diabetes is, which is why it is also considered a source of nutraceutical products. The objective of this work was to study two varieties of rambutan grown in Ecuador and to evaluate their pharmacognostic and phytochemical characteristics and their use as a source of anti-obesity nutraceutical products. The aerial parts of two varieties of adult plants in the flowering-fruiting state from the province of Los Rios were used. The organoleptic characteristics and dimensions of the organs; histological analysis of leaves, and fruit bark, physicochemical parameters, phytochemical screening and content of phenols and flavonoids were evaluated. Macroscopically the varieties presented slight differences in the dimensions and coloration; however, histologically there were no differences. The physicochemical parameters showed a discrepancy between the varieties and different plant organs.

The content of phenols and flavonoids established the difference between the two varieties. Differences among the organs of the two varieties that underwent qualitive and

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quantitive analysis were found and thus it was established that the bitter type had the highest concentrations of the active ingredients responsible for battling obesity.

Keywords: Achotillo, pharmacognosy, physicochemical parameters, phenols and flavonoids

INTRODUCTION

Functional, nutraceutical or design foods are foods that have two main characteristics. The first and more common characteristic being its nutritional properties, therefore usually used in diets. The second and less known but not less significant, its focus on the biological-phytochemical products they possess. These properties which give them therapeutic properties of unmeasurable value and that promise to be the axis of the new treatment approach of many diseases that are currently addressed with overly artificial methods.

These products have been scientifically proven to contain compounds with functional and nutraceutical characteristics and with various properties that can be used to control, amongst others, the excess amounts of free radicals and the destructive actions of the cells that cause infections. Within this group is the Achotillo or Rambutan, which presents phytochemical components and vitamins, which allow it to be considered nutraceutical.

Nephelium lappaceum L., commonly known as achotillo, rambutan or Chinese sucker is a fruit native to the Asian continent particularly from Malaysia and Indonesia and which has been cultivated in other countries such as Thailand, Vietnam, India, and Sri Lanka since 1912. Rambutan term comes from Malay "rambut" which means hair, which refers to the long soft spines that cover the fruit (Sukasih and Setyadjit 2015, Arias-Cruz et al. 2016). This fruit has a characteristic sweet flavor, juicy pulp, and considerable content of ascorbic acid and riboflavin.

Within the genus Nephelium there are other tree species that produce edible fruits known in the Southeast Asian countries among which are the pulasan (*N. mutabile* Blume), the bulala (*N. intermedium* Radlk), the aluao (*N. xerospermoides* Radlk) and the Kuching (*N. malaiense* Griff.) (Arias-Cruz et al. 2016).

In America, it is a well-known fruit and Mexico was one of the first countries that cultivated it in the 1950's. Nowadays you can find plantations of this species in many tropical and subtropical areas around the world; this is the case from Colombia, Ecuador, Honduras, Mexico, and Cuba. The achotillo is a fruit with an important content of vitamins A, B1, B2, B3, B5, B6, B9 and C and the minerals it contains are calcium, iron, magnesium, manganese, phosphorus, potassium and sodium; It also includes sugars and water that can complement the nutritional needs of people. Table 1 describes the nutrient composition of rambutan (Wall 2006, Fila et al. 2013).

There are more than 100 varieties worldwide; which are characterized by the quality of the fruit, maturation, and climatic requirements, among others. The most important varieties are illustrated in Figure 1 and are described in Table 2.

Little is known about the chemical components of the leaves and flowers; the studies have concentrated on the fruit, mainly its shell and seed. One of the most important elements in rambutan is its seed since it's considered the reproduction organ. The proximal percentage of compounds that are located in the seed are: ash (7.80%), fiber (2.8% - 11.6%), carbohydrates (28.7% - 48.1%), protein (7.8% - 14.1%) and fat (37.1% and 38.9%). The main fatty acids found in the seed were 40.3% oleic, 34.5% arachidic acid, 6.1% palmitic, 7.1% stearic, 6.3%

gondoic (11-eicosanoic) and 2.9% behenic (docosanoic acid); the values of the refractive index, saponification and iodine were 1.468; 186 and 47.0, respectively (Solís-Fuentes et al. 2010, Sirisompong 2011, Mahisanunt et al. 2017).

Table 1. Nutritional composition of rambutan

COMPONENTS (mg in 100 g)			
Carbohydrates	20.87	Magnesium	7.000
Dietary fiber	900.0	phosphorus	0.343
Fat	210.0	Potassium	9.000
Proteins	650.0	β-carotene	42.00
Thiamin	13.00	Sodium	0.002
Riboflavin	22.00	Zinc	11.00
Niacin	1352		
Vitamin B6	20.00		
Folic acid	0.008		
Vitamin C	4.900		
Calcium	22.00		
Iron	0.350		
Water	82.1	Niacin	5.00
Protein	0.90	Carotene	0.00
Fat	0.30	phosphorus	0.00
Ashes	0.30	Calcium	15.0
Glucose	2.80	Iron	0.10
Fructose	3.00	Vitamin C	70.0
Saccharose	9.90	Thiamin	0.01
Starch	0.00	Riboflavin	0.07
Dietary fiber	2.80	Potassium	140
Malic acid	0.005	Sodium	2.00
Citric acid	0.31	Magnesium	10.0
Energy	297		

Figure 1. Different types of fruits of rambutan.

Table 2. Characteristics of the fruit of the main crops of *Nephelium lappaceum* L.

Cultivars	Shape and size of the fruit	Color	Texture of the aril
Bangyeekhan	Large, oval	Red	Above average
Binjai	Large, oval	Red	Above average
Jittlee	Medium	Red	Above average
Leebakbulus	Large, oval	Oranged Red	Average
Rapiah	Half, round	Yellowish green	Above average
Rongrien	Big	Red	Above average
R3 (Guiabatu)	Half, round	Red	Above average
R 134	Half, round	Red	Above average
R 156 (Muar Goding)	Large, round	Yellow	Average
R 160 (Khaw Tow Bok)	Half, round	Red	Average
R 161 (Lee Long)	Large, oval	Red	Average
R 162 (Doun Hijau)	Large, oval	Red-orange	Above average
R 163	Large, oval	Yellow	Average
R 170 (Deh Cheng)	Large, oval	Red	Average
Seechompo	Large, oval	Pinkish	Above average
Seenjonja	Small	Red	Average
Simancen	Large, round	Red	Average

According to Mariod, Mirghani, and Hussein (2017), the plant contains a great variety of substances; such as polyphenols, carotene, tocopherol, vitamin C, vitamin E, xanthophyll and tannins; the seed constitutes about 5.6% - 7.4% of the whole fruit and contains 11.9% - 4.1% protein, 37.1% - 38.9% crude fat, 2.8% - 6.6% crude fiber, 2.6 - 2.9 dry weight ash. The oils contain mainly palmitic acids (7.39% - 10.33%), stearic (12.21% -16.58%), arachidic (12.34% - 16.22%) and behenic (6.53% - 8.91%) as saturated fatty acids (SFA), oleic (50.17% - 52.18%) as monounsaturated fatty acid (MUFA) and linolenic acid (2.02% - 3.04%), such as polyunsaturated fatty acid (PUFA).

The methanolic extract of the fruit shell exhibited strong antioxidant properties. Sephadex LH-20 chromatography has been used in the isolation of its components, and the antioxidant properties of each of them have been studied. The isolated metabolites were identified as ellagic acid, corilagina, and geraniin. These compounds represented 69.3% of the methanolic extract, with geraniin (56.8%) as the main one and exhibited antioxidant activities higher than BHT both in lipid peroxidation (77-186 times) and in the DPPH assay (42-87 times) (Thitilertdecha et al. 2010).

Regarding the pharmacological activity, several studies have been performed for the bark and seeds of the fruit. The hypoglycemic activity has been demonstrated by Rahayu, Zakir, and Keban (2013) and Primarianti and Sujono (2015), who indicate that the geraniin metabolite present in the fruit's shell shows effectiveness to inhibit the carbohydrate hydrolyzing enzyme in a much more significant level than the drug acarbose.

Another activity demonstrated by the aqueous extracts of the seeds was their antibacterial activity by the disc diffusion method and the protein profile. These extracts showed moderate inhibition against pathogenic bacteria, both Gram-positive as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtillis* and Gram-negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* (Alvarez-Padilla, Alvarez-Parrilla, and Gonzalez-Aguilar 2009).

MATERIALS AND METHODS

Harvest: the different plant organs (leaves, flowers, shell and seed of the fruits), of the two varieties used (sweet and bitter) were collected between the months of June and August of 2017 in a farm located in the Province of Los Ríos district Quevedo, situated at 1° 1'49 "S and 79° 24'48" E; adult plants of about 15 to 25 m height were chosen in the flowering-fruiting state.

Macro-morphological characterization was done with the help of a magnifying glass. The organoleptic characteristics, the shape, and dimensions of the different organs (leaves, flowers, fruits, and seeds) were evaluated.

Micro-morphological characterization was performed on the leaves and bark of the fruit. For the histological analysis of the leaves, cross-sections were made at the fresh state, by manual method, which was hydrated and rinsed with 1% sodium hypochlorite. They were stained with 1% safranin in water, fixed with glycerinated gelatin, according to the procedures described by Peacock (1935).

To observe the anatomical details at the level of the epidermis of the leaf the diaphanising technique was performed. The sample was rinsed with sodium hypochlorite solution, washed with distilled water and colored with 1% safranin in water (Gattuso and Gattuso 1999).

To view the different internal anatomical characters of the plant, a NOVEL microscope (10X lens) with coupled camera model HDCE-50B was used.

The crust of the fruit was analyzed in the dry and crushed state.

- *Selection, washing, drying, and grinding:* leaves of the two varieties were selected. Those that were not attacked by insects were washed with running water, and dried in an oven. The flowers were washed using the same process and dried. The rinds were separated from the pulp of the fruit and dried, while the seeds were extracted from the fruit, washed to eliminate pericarp waste, and dried in an air recirculation oven. The drying temperature for all plant organs was 50°C, and once dried, they were subjected to milling in a knife mill independently, ensuring a particle size of 2 mm.
- *Quality control parameters:* the different tests were performed on raw drugs according to the methodology established by WHO (2011). The tests carried out were: humidity, total ash, water-soluble ashes, insoluble ashes in HCl, and soluble substances.
- *Qualitative chemical study:* Phytochemical screening was performed on raw drugs, from different organs, according to the procedure described by Miranda and Cuéllar (2000). An extraction system with a battery of solvents was used, from lower to higher polarity, on the same plant material, to ensure that each metabolite was extracted correctly according to its selectivity for the solvent used. The samples were extracted successively with ethyl ether, ethanol, and water, to obtain the corresponding extracts, which were subjected to the different tests.
- *Quantification of total phenols:* the content of total phenols was determined by the Folin-Ciocalteu method (Pourmorad, Hosseinimehr, and Shahabimajd 2006, Sengul et al. 2009, Chlopicka et al. 2012).
- *Quantification of total flavonoids:* It was carried out by the colorimetric method of aluminum trichloride (Chang et al. 2002, Pourmorad, Hosseinimehr, and Shahabimajd 2006).

RESULTS AND DISCUSSION

Macromorphological Characterization

The macromorphological evaluation of the Achotillo leaves is presented in Table 3.

Table 3. Macromorphological characteristics of the leaves of the two varieties of Achotillo

Parameters	Sweet Variety	Bitter Variety
Shape	Stalked leaf with mucronate obtuse apex and base entire margins elliptical and palminervia venation. With a glabrous surface and a membranous texture.	Stalked leaf with mucronate obtuse apex and base entire margins elliptical and palminervia venation. With a glabrous surface and a membranous texture.
Odor	Characteristic	Characteristic
Color	Green	Green
Length (cm) $\bar{X} \pm DS$	13.44 ± 0.75	13.66 ± 0.80
Width (cm) $\bar{X} \pm DS$	6.24 ± 0.38	6.77 ± 0.42

Table 4. Macromorphological examination of the flowers of the two varieties

Parameters	Sweet Variety	Bitter Variety
Color	Yellowish coffee	Greenish coffee
Odor	Characteristic pronounced	Characteristic little pronounced
Type of inflorescence	Hermaphrodite female	Hermaphrodite female
Lenght (cm) $\bar{X} \pm DS$	0.56 ± 0.04	0.63 ± 0.04
Width (cm) $\bar{X} \pm DS$	0.26 ± 0.05	0.28 ± 0.03

It was observed that the two varieties have similar dimensions; the bitter variety having a slightly higher dimension than the sweet variety.

In the analysis of the flowers, it was observed that both had two weeks of inflorescences, but each had a characteristic color. As for their smell they both had the characteristic smell but the flowers of the sweet variety had a more intense aroma (Table 4).

The macromorphological analysis of the fruits is reported in Table 5.

Differences were observed in the appearance and dimensions of the varieties studied.

The macromorphological analysis of the seeds of the fruits of both varieties showed that neither have significant differences concerning their morphology. However, it should be noted that the seeds of the sweet variety are broader and smaller in lenght than those of the bitter variety (Table 6).

Table 5. Macromorphological analysis of the fruits

Parameters	**Sweet Variety**	**Bitter Variety**
Appearance	 Schizocarpus fruit. deeply split in 2-3 mericarpios. is large rounded. red. its thin rind. fleshy aril seed. translucent white and very juicy.	 Schizocarpus fruit. deeply split in 2-3 mericarpios. is small oval. yellowish-greenish red. its bark is thick. fleshy aril seed. juicy white-translucent.
Odor	Characteristic	Characteristic
Largo (mm) $\bar{X} \pm DS$	38.61 ± 0.08	33.21 ± 0.05
Ancho (mm) $\bar{X} \pm DS$	32.90 ± 0.06	25.63 ± 0.04

Table 6. Macro-morphological examination of the seeds of the two varieties

Parameters	**Sweet Variety**	**Bitter Variety**
Color	Light brown	Light brown
Odor	Characteristic	Characteristic
Shape	Oval	Oval
Appearance	Oily to the touch	Oily to the touch
Lenght (mm) $\bar{X} \pm DS$	22.496 ± 3.165	22.678 ± 6.294
Width (mm) $\bar{X} \pm DS$	12.884 ± 1.157	12.290 ± 5.750

Micromorphological Characterization

This study was conducted in leaves and shells of the fruit.

The micromorphological analysis of the leaf of both species (bitter and sweet variety), it is exposed in Figure 2.

A cross-section at the level of the central nerve of the leaf (bitter variety) (K) allowed the observation on the adaxial surface the presence of a cuticle with some unicellular trichomes, followed by protuberances occupied by the collenchymatous tissue, which can also be seen in the abaxial zone. The topographic sequence of structures continues with a ring of perivascular fibers (sclerenchyma supporting tissue) covering the vascular tissue. In the central zone are the elements of vascular tissue xylem and phloem as well as the medullary parenchyma (Figure 3).

From the micromorphological point of view, there were no structures that could make the difference between the two varieties of *Nephelium lappaceum*.

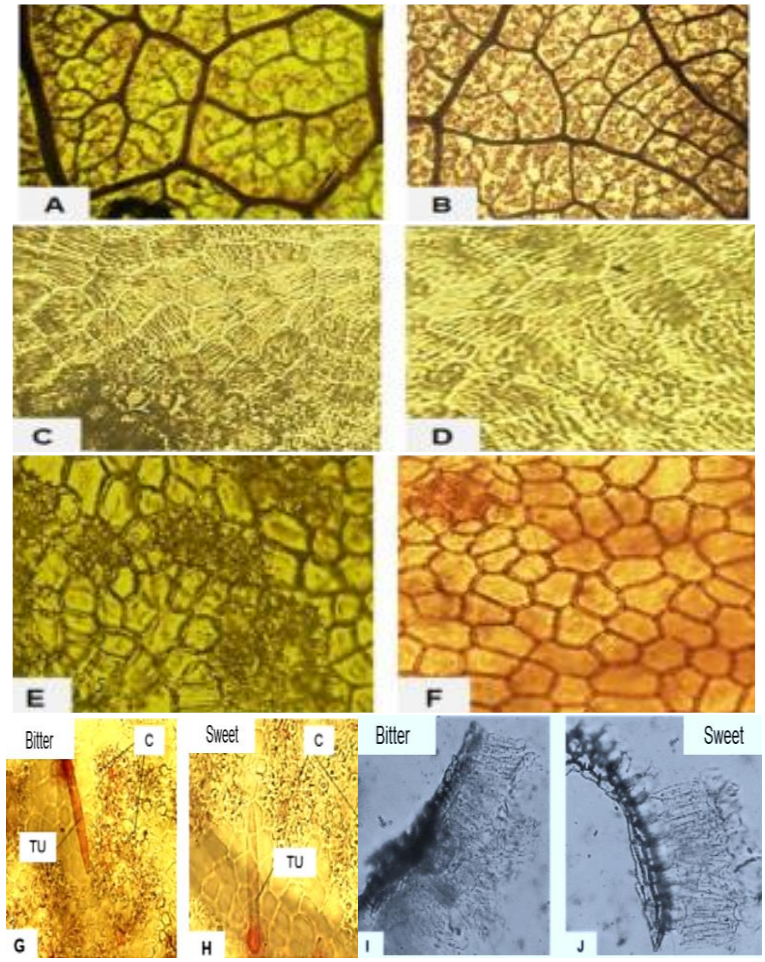

Figure 2. Micromorphological analysis of the leaf of both species. A: sweet B: bitter Closed venation system. C (bitter) and D (sweet) hexagonal cells with cuticular striae at the adaxial level. E (bitter) F (sweet), cells of the abaxial epidermis of varied form and with a thick wall. G and H, unicellular trichomes in both species (TU), groups of crystals in the form of drusen (C). I and J, fragment of radial collenchyma.

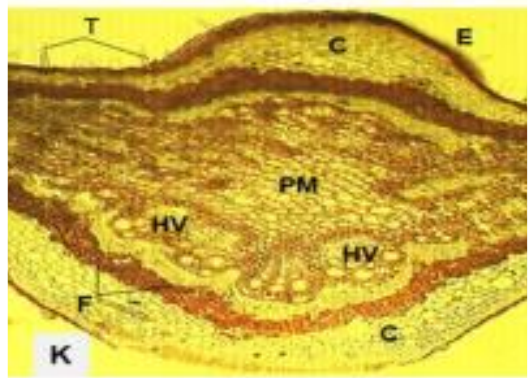

Figure 3. Central venation of the bitter variety. T: trichome, E: epidermis, C: collenchyma, F: perivascular fibers, HV: vascular bundles, PM: medullary parenchyma.

Shell Micromorphology

Figure 4 shows the micromorphological study carried out on the shell of the fruits of the two varieties.

Due to the characteristics found under the used parameters and conditions, it can be affirmed that no differences were found between the two varieties, at least from the micro-morphological point of view.

Physico-Chemical Parameters

Generally, the norms and pharmacopeias establish a residual moisture content between 8% and 14%, depending on the plant material (Zhi-cen 1980, Miranda and Cuéllar 2012). In the study, there were significant differences between the sweet and bitter variety, for the flowers and seeds, and the average of the determinations was within the required range, except for the flowers, where the percentages were very high.

The amount of soluble ash in water and insoluble ash in 10% hydrochloric acid are also parameters that help to assess the purity of the drug (Evans 2009, Miranda and Cuéllar 2012). In both determinations, the values were small and in particular insoluble ashes in acid were around 2% limit established by the literature.

Some Pharmacopoeias have a total ash index of up to 5% and another such as the Chinese Pharmacopoeia, which refers to 15% (Zhi-cen 1980). In this research, the values obtained were framed in the established intervals.

Regarding the content of soluble substances, the lower extractive power for shell and seeds was achieved with hexane and ethyl acetate; no significant differences were found in the values obtained for the same solvent when the two varieties were compared. When analyzing the behavior of the rest of the tested solvents, it was observed that the bitter variety had a higher metabolite content than the sweet variety.

Qualitative Chemical Analysis: Phytochemical Screening

It is considered of interest in the study of a drug, to know its general chemical composition initially. These are obtained using phytochemical screening methods. Tables 8 and 9 show the results of the three extracts tested for the two varieties in all the organs tested.

As components of these organs, oils and fats, triterpenes and/or steroids, phenolic compounds, saponins and reducing substances stand out, with some differences in the intensity of reaction coloring, attributable to the concentration of metabolites.

Components of these organs also include oils and fats, triterpenes and/or steroids, lactones, phenolic compounds, saponins, and reducing substances. Unlike extracts of leaves and flowers, lactones and coumarins were positive for extracts of the shell and seeds. For the seeds, a high concentration of saponins was detected. Despite differences between plant organs, no differences were detected between the varieties studied.

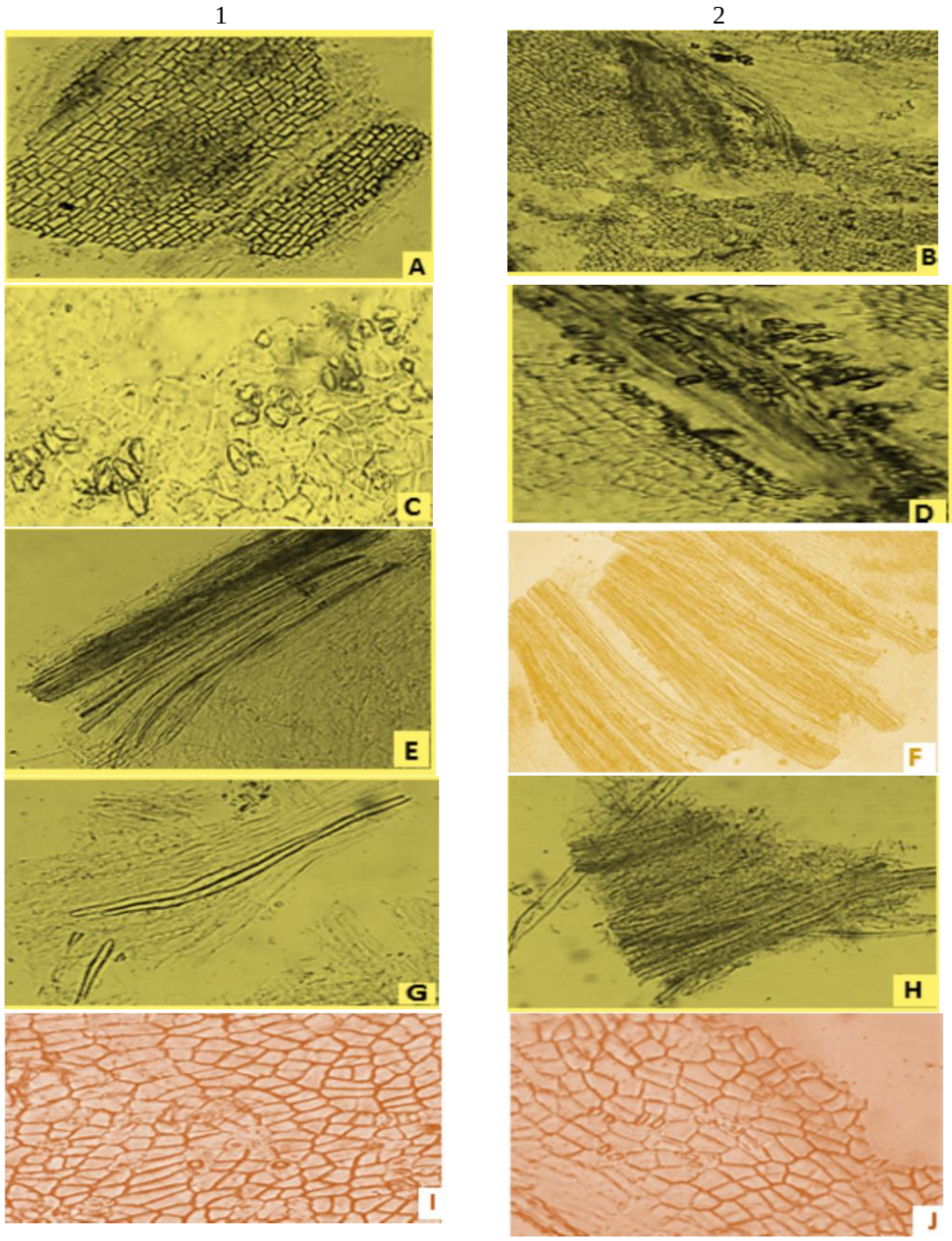

Figure 4. Micromorphological characteristics of the shell of the fruits of the two varieties 1 (Bitter variety), 2 (Sweet variety). A y B (suberous or cork tissue, corresponding to the Peridermis). C y D (crystals of calcium oxalate). E y F (longitudinal series of elements of the conductive tissue formed by fibers and vessels that make up the vascular system). G y H (detail of an isolated, lignified, non-septate fiber with thick walls that sometimes appear in small groups in both varieties). I y J (a superficial view of a set of fundamental or epidermal cells of the polygonal outline, although sometimes they become elongated) (Essau 1989, Khandelwal 2004).

Table 7. Physical-chemical parameters of the different organs of the two varieties

Parameters (%)	Leaves $\bar{x} \pm DS$		Flowers $\bar{x} \pm DS$		Shell $\bar{x} \pm DS$		Seeds $\bar{x} \pm DS$	
	Sweet V.	Bitter V	Sweet V.	Bitter V	Sweet V.	Bitter V	Sweet V.	Bitter V
Residual humidity	6.5 ± 0. 2a	6.3 ± 0. 5a	20.33 ± 0. 23a	17.0 ± 0.0^{b}	10.00 ± 1. 41a	9.5 ± 0. 70a	3.997±0.005^{a}	2.640 ± 0.01^{b}
Total ashes	7.2 ± 0.7^{c}	10.0 ± 0.2^{d}	3.4 ± 0.28^{c}	5.19 ± 0.14^{d}	2.93 ± 0.05^{n}	2.83 ± 0.04^{o}	1.469±0.002^{c}	1.498±0.001^{d}
Water soluble ashes	1.2 ± 0.4^{e}	1.4 ± 0.3^{e}	0.26^{e}	1.02^{f}	1.46 ± 0.05^{p}	1.91 ± 0.05^{q}		
Insoluble ash in 10% HCl			1.22 ± 0.00^{g}	2.24 ± 0.10^{h}	1.75 ± 0.31^{r}	1.68 ± 0.17^{r}	1.240 ± 0.00^{e}	1.34 ± 0.001^{f}
Water-soluble substances			48.10 ± 1.06^{i}	48.96 ± 0.59^{i}	29.01 ± 0.49^{b}	32.98 ± 0.87^{c}		
98% soluble substances in ethanol	24.1 ± 0.2^{f}	24.0 ± 0.7^{f}	27.16 ± 0.58^{j}	37.68 ± 1.17^{k}	10.46 ± 0.86^{j}	12.96 ± 0.27^{k}	3.414 ± 0.001^{g}	3.528 ± 0.001^{g}
Soluble substances in ethyl acetate					2.53 ± 0.23^{l}	2.87± 0.15^{l}	8.660 ± 0.001^{h}	7.513 ± 0.001^{i}
Soluble substances in hexane					0.38 ± 0.11^{m}	0.30 ± 0.04^{m}	14.680 ±0.001^{j}	3.960 ± 0.001^{k}

$\bar{x} \pm DS$: mean value of the determinations ± standard deviation.

Equal letters in a row for a plant organ show that there are no significant differences ($p > 0.05$) and different letters if there are significant differences ($p < 0.05$) for a 95% confidence. according to t-student

Table 8. Compounds detected in the extracts of leaves and flowers of two varieties of *N. lappaceum L.*

Essays	Leaves						Flowers					
	EE		AE		WE		EE		AE		WE	
	S	B	S	B	S	B	S	B	S	B	S	B
Oils and fatty compounds	+	+					+	+				
lactones / coumarins	-	-	-	-			-	-	-	-		
triterpenes /steroids	+	+	+	+			+	++	+++	+		
resin			-	-					-	-		
tanin / phenols			++	++	++	++			++	++	++	++
saponins			+++	+++	++	++			++	++	++	++
quinones			-	-					++	+		
flavonoids			+++	+++	+	+			±	+	±	+
anthocyanidins			+++	+++					-	-		
Reducing substances			++	+++	++	+++			++	++	++	++
mucilage					-	-					-	-
bitter compounds					+	+					-	-

Legend: EE = Ethereal extract; AE = Alcoholic extract; WE = Water extract.
S = Sweet; B = Bitter.

Table 9. Compounds detected in shell extracts and seeds of two varieties of *N. lappaceum* L.

Essays	Fruit shell						Seeds					
	EE		AE		WE		EE		AE		WE	
	S	B	S	B	S	B	S	B	S	B	S	B
Oils and fatty compounds	+	+					+	+				
lactones/coumarins	+	+	+++	+++			++	++	-	-		
triterpenes/steroids	+	+	+	+			+	+	+	+		
resin			-	-					+	+		
tanin/phenols			++	++	++	++			+	+	+	+
saponins			++	++	++	++			+++	+++	+++	+++
quinones			++	++					-	-		
flavonoids			+	++	+	+			+	+	+	+
anthocyanidins			++	++					-	-		
Reducing substances			++	++	++	++			+++	++	+++	++
mucilage					-	-					+	+
bitter compounds					+	+					+	+

Legend: EE = Ethereal extract; AE = Alcoholic extract; WE= Water extract.
S = Sweet; B = Bitter.

Quantification of Phenols and Flavonoid

The content of total phenols was determined by the Folin-Ciocalteu method which measures the amount of extract necessary to inhibit the oxidation of said reagent (Kale et al. 2010). The phenols react with the phosphomolybdic acid in an alkaline medium, forming the

phosphotungstic-molybdenum blue chromophore. This colored complex can be quantified spectrophotometrically, allowing the content of total phenols to be determined (Pękal and Pyrzynska 2014).

During the test, it was observed that the reaction mixture with the extracts was blue. This observation indicated the formation of the complex above and the possible presence of phenolic compounds in the extracts analyzed (Pękal and Pyrzynska 2014).

The total flavonoids were determined by the colorimetric method of aluminum trichloride. In this test, the flavonoids react with aluminum trichloride in ethanol, producing a complex of yellow color that has a light absorption peak at 415 nm. The concentration is expressed in quercetin equivalents.

This study was performed on the leaves, flowers, and shells. The results are presented in Table 10.

Table 10. Content of total phenols in the extracts of the leaves flowers and rinds of the two varieties of N. l*apaceum* L.

Extract	Total phenols mg/mL			Total Flavonoids µg/mL		
	Leaves	Flowers	Shell	Leaves	Flowers	Shell
Sweet Variety	0.75	0.91	22.42	2.93	8.92	3600
Bitter Variety	0.81	0.86	23.98	3.65	6.74	4070

It was observed that the highest content of total phenols and total flavonoids was presented by the shell of the bitter variety, in agreement with the behavior obtained from the leaves. However, in the case of flowers, these metabolites were higher in the sweet variety.

CONCLUSION

Macroscopically the varieties exhibited slight differences in the dimensions and coloration; however, histologically, there were no differences.

The physicochemical parameters showed differences between the varieties and among the vegetal organs, credited in the latter to its nature. Qualitatively, in some trials, changes in intensity were observed due to differences in the concentrations of the metabolites.

The quantification of phenols and flavonoids established the difference between both varieties by obtaining different values for the varieties studied.

Due to the higher concentrations of phenolic compounds, the bitter variety must have the highest activity to combat obesity and diabetes.

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Chapter 16

CALAHUALA (*POLYPODIUM* SPP)

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ABSTRACT

Several species of ferns from the *Polypodium* genus are known under the generic name "Calahuala." These ferns grow in many tropical places all over the world and the rhizomes or leaves are empirically used in traditional medicine for the treatment of many diseases. *Polypodium* genus has been the object of scientific interest especially for the properties shown by its extracts in preventing the harmful effects of UV radiation and the effectiveness in the treatment of psoriasis and skin problems; however, many of other biological properties remain scientifically undocumented. The objective of this chapter is to provide a general overview of the *Polypodium* genus and its reported beneficial properties to human health. We also highlight some of its biological properties such as the tumor inhibition that needs to be supported with scientific evidence.

Keywords: *Polypodium* genus, ferns, psoriasis

INTRODUCTION

Several ferns from the *Polypodium* genus are used in countries like Guatemala, Honduras, and México as a part of their traditional ethnobotanical medicine. The ferns proliferate in tropical areas of Latin America and other parts of the world where climatic conditions allow their reproduction. The rhizomes and leaves of the plant are used under different common names, in Mexico and some Latin-American countries, it is known as Calahuala, Canahuala, or Calaguala, while in Brazil it is named Samambaia. In this chapter, the authors will refer as Calahuala for the vegetable material used in traditional herbal medicine.

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Classification

The term Calahuala includes several tropical ferns, a terrestrial or epiphytic rhizomatous plant. *Polypodium* genus includes many species, and because of their close botanical relationship, different ferns are used under the common name Calahuala. Depending on the region, the genus commonly used are *Phlebodium* or *Polypodium*. Among *Phlebodium* species, we can found *Phlebodium decumanum* (Will) J. Sm., *Phlebodium pseudoaureum* (Cav.) Lellinger, *Phlebodium aureum* (L.) J. Sm (Gattuso, Cortadi and Gattuso 2008) and *Phlebodium areolatum* (H.&B. ex Willd) J. S. Smith (Fernández Nava, Ramos Zamora and Carranza González 2001). Among *Polypodium* species, these are the most relevant: *Polypodium decumanum*, *Polypodium aureum*, *Polypodium triseriale*, *Polypodium lowery*, *Polypodium loriceum*, *Polypodium californicum* Kaulf and *Polypodium hastatum* Thunb (Liu et al. 1998, Reyes-Silva, Villavicencio-Nieto and Pérez-Escandón 2008, Yao et al. 2012).

Due to some author includes *Phlebodium* as a section subgenus or synonym of *Polypodium*, for the information to be reviewed within this chapter, the authors will refer to the genus *Polypodium*.

Ethnobotanical Use

A mixture of rhizomes and leaves are used in folk medicine as an analgesic, expectorant, febrifuge, tranquilizer, depurative, diuretic, anti-inflammatory, emmenagogue and spasmolytic (Gattuso, Cortadi and Gattuso 2008, Fernández Nava, Ramos Zamora and Carranza González 2001). Other uses include treating peptic ulcers, kidney problems, arthritis, pain in joints and tendons, psoriasis, atopic dermatitis, and vitiligo (Liu et al. 1998). In Mexico, rhizome decoctions or infusions are used in some respiratory disorders like cough, flu and pulmonary disease (Reyes-Silva, Villavicencio-Nieto and Pérez-Escandón 2008) meanwhile in other Latin American countries they are used for treating malignant tumors and cancer, kidney problems, hypoglycemia, cardiac problems, and genitourinary affections (Cruz 2010, Taylor 2003). The genus has been extensively studied in order to identify the metabolites involved in the therapeutic properties attributed to the plan. For example, a phytoextract known as Anapsos® is obtained from the rhizomes of *Polypodium leucotomos* and has been used for more specific uses like antipsoriatic or a modulator of the immune system (Sánchez-Rodríguez et al. 2018)

Phytochemistry and Biological Properties

Several groups of secondary metabolites have been described in Calahuala, among these, alkaloids, saponins, flavonoids, lipids and fatty acids, volatile oils, coumarins, anthraquinones, alkaloids, unsaturated steroids as well as cyanogenic and cardiotonic glycosides were found in the rhizomes or the whole plant (Cruz 2010).

Table 1. Reported biological activity for extracts or metabolites obtained from some *Polypodium* species

Scientific name	Extract or metabolite	Biological activity	Reference
Polypodium leucotomos	Hydrophilic extract	Photoprotection against UVA light; Immunosuppressive agent; inhibition of proinflammatory cytokines: TNFα and IL-6	(Alonso-Lebrero et al. 2003, Brieva, Guerrero, and Pivel 2002
	Aqueous extract	Decrease histological damage associated with photoaging and prevalence of UVB-induced tumors in mice.	(Alcaraz et al. 1999)
	Fraction of low molecular weight from methanolic extract	Reactive oxygen species (ROS) scavenger, including superoxide anion	(Gomes et al. 2001)
	Hydroalcoholic extract	Antioxidant, anti-inflammatory, photoprotective against photo-oxidation	(González and Pathak 1996)
	Hydroalcoholic extract	Control of colitis	(Galvez et al. 2000)
	Aqueous extract (Phenolic acids: Protocatechuic, Chlorogenic, p-coumaric, vanillic, caffeic and ferulic acids)	Antioxidant, anti-inflammatory, immunomodulatory	(Berman, Ellis, and Elmets 2016, García et al. 2006)
Polypodium leucotomos	Calagualine	Antitumoral	(Horvath et al. 1967)
Polypodium decumanum; Polypodium triseriale	Methanolic extract	Elastase inhibitor, anti-inflammatory (Inhibitory activity for the phospholipid derived mediator platelet activating factor (PAF) and leukotriene B_4.)	(Vasänge et al. 1997, Liu et al. 1998)
Polypodium triseriale	Selligueain; *O*-coumaric acid glucoside (Melilotoside)	Elastase inhibitor, anti-inflammatory	(Vasänge et al. 1997)

Table 1. (Continued)

Scientific name	Extract or metabolite	Biological activity	Reference
Polypodium decumanum	Kaempferol 3-*O*-*β*-D-xylopyranosyl-(1 -2)- *β*-D-arabinopyranoside; Kaempferol 3-*O*-*α*-D-arabinopyranoside; Quercetin 3-*O*-*α*-D-rhamnopyranosyl- (1,6)-*β*-D-glucopyranoside (Rutin)	Elastase inhibitor, anti-inflammatory	(Vasänge et al. 1997)
	Linoleic, linolenic, arachidonic acids	Anti-inflammatory	(Liu et al. 1998)
	1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol	Elastase inhibitor, anti-inflammatory	(Vasänge, Rolfsen, and Bohlin 1997)
Polypodium aureum	Calagualine	Hypoglycemic Hypocholesterolemic	(Duke 2017)
	Ecdysone, Ecdysterone,	Hypoglycemic Hypocholesterolemic	(Duke 2017)
	Polypodaureine		(Jizba, Dolejš, and Herout 1974)
Polypodium vulgare	Inokosterone; pterosterone; abutasterone; 24-hydroxyecdysone; 5-hidroxyabustasterone	Hypoglycemic Hypocholesterolemic	(Coll et al. 1994)
Polypodium hastatum Thunb	β-sitosterol, coumarin, juglanin, kaempferol-7-*O*-*α*-L-rhamnopyranoside, (+)-afzelechin-5-O- *β*-D-apiofuranoside	Urinary disease	(Yao et al. 2012)

Reported biological activity for different hydrophilic (aqueous or hydroalcoholic) extracts (Table 1) includes beneficial effects like antioxidant, photoprotective, anti-inflammatory, immunoregulator, antipsoriatic, hypoglycemic, hypocholesterolemic and antitumoral (Alonso-Lebrero et al. 2003, Horvath et al. 1967, Alcaraz et al. 1999, Brieva, Guerrero and Pivel 2002, Liu et al. 1998, García et al. 2006).

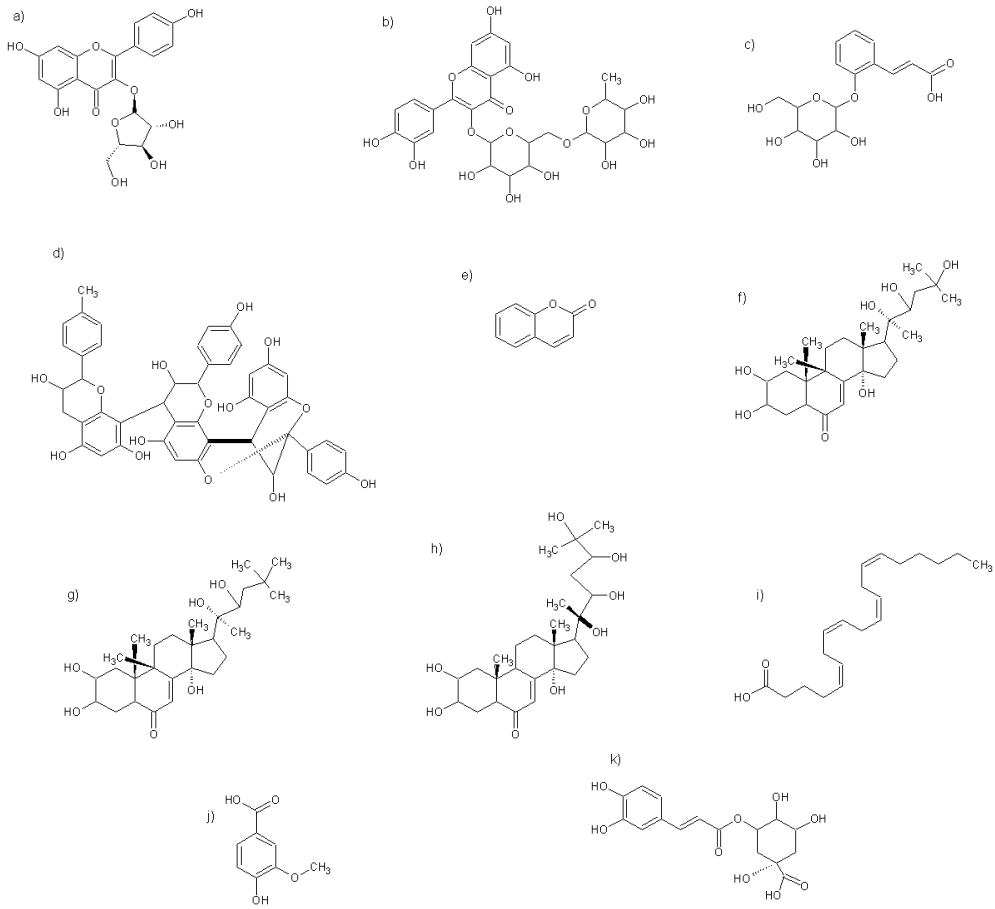

Figure 1. Example of metabolites identified in some species of *Polypodium* genus. a) Juglanin; b) Rutin; c) Melilotoside; d) Selligueain; e) Coumarin; f) Ecdysterone; g) Polypodaureine; h) Abutasterone; i) Arachidonic acid; j) Vanillic acid; k) Chlorogenic acid.

Among specific metabolites identified in the genus, we can find flavonoids (rutin, selligueain, and melilotoside), fatty acids (linoleic, linolenic, arachidonic), sulphoquinovosyl dipalmitic glycerol, saponins (calagualine), phenolic acids (ferulic, chlorogenic, vanillic) and ecdysteroids (ecdysone, polypodaureine). Structures of some of these metabolites are shown in figure 1 (Vasänge et al. 1997, Vasänge, Rolfsen and Bohlin 1997, Jizba, Dolejš and Herout 1974, Yao et al. 2012).

Clinical Case Studies

Formulations based on *Polypodium leucotomos* extracts are commercialized in some countries like Spain and the United States, and a large quantity of studies about the safety and beneficial properties of these extracts is available. There are several reviews that compile the scientific evidence of using *Polypodium leucotomos* extracts orally or topically (Parrado et al. 2018, Del Rosso 2016, Jeter et al. 2019).

Table 2. Reported biological activities and clinical cases for *Polypodium leucotomos* extracts

Assay	Route of administration/Dose	Biological activity	Reference
In vivo animal studies			
Hairless albino mice	Topical: NS	Photoprotective against UVB radiation: decrease sunburn response, photoaging damage, development of skin tumors.	(Alcaraz et al. 1999, Zattra et al. 2009, Rodríguez-Yanes et al. 2012, Siscovick et al. 2008, Mulero et al. 2008, Murbach et al. 2017)
Hairless rats	Oral*: ~300 mg/Kg/day, 10 days	*Downregulation of Cox-2 Reduce immunosuppression induced by UVB radiation Reduce UV mediated depletion of Langerhans cells	
Hairless rats	Oral**: ~300 mg/Kg/day	**Antioxidant: Reduces oxidative DNA damage by reducing ROS species and reduces glutathione oxidation	
Hsd. Han Wistar rats	Oral+: 0, 2000, 3500 and 500 mg/kg/day 28 days	+ No mortality or toxic effects were observed and no target organs were identified	
In vitro human cell cultures			
Human keratinocytes, Human fibroblasts	NS	Anticarcinogenic: Inhibits tumor necrosis factor-alpha (TNFα), Nuclear factor-kappa B (NFκB), and activator protein-1 (AP-1) Inhibits nitric oxide production (NOs) Cytoprotective and photoprotective against UV-induced damage Inhibited lipid membrane peroxidation Prevents cell damage caused by visible light and infrared light. Reduces the expression of metalloproteinase-1 (MMP-1) and cathepsin K	(Jańczyk et al. 2007, Alonso-Lebrero et al. 2003, Capote et al. 2006, Zamarrón et al. 2018, Philips et al. 2003)

Assay	**Route of administration/Dose**	**Biological activity**	**Reference**
Human neutrophils, Human leukocytes	NS	Reduce immunosuppression induced by UVB radiation inhibiting the effect on platelet activating factor (PAF). Anti-inflammatory by inhibiting the inflammatory leukotriene B_4.	(Vasänge et al. 1997, Vasänge, Rolfsen, and Bohlin 1997)
Clinical studies.			
Healthy humans (n=9). Exposition to UV radiation: 305-400 nm	Oral: 2 capsules (7.5 mg/kg each)	Photoprotective: decrease erythema response, sunburn cell numbers, cyclobutane pyrimidine dimers, proliferating epidermal cells and dermal mast cell infiltration	(Middelkamp-Hup et al. 2004)
Healthy humans (n=10). Exposition to UVA light: 10-35 J/m^2	Oral: 240 mg given 8 and 2 h prior UVA exposure	Decrease 42% the mitochondrial deletion known as "common deletion", a marker of chronic UVA radiation exposure	(Villa et al. 2010)
Humans with polymorphic light eruption (PLE) (n=25). Humans with solar urticaria (n=2)	Oral: 480 mg daily	Of the 25 subjects 80% improved, 30% normalized their response to sunlight, 13% had a clear improvement and 36% a slight improvement For solar urticarial patients, the dose was not effective.	(Caccialanza et al. 2007)
Humans with long-standing polymorphic light eruption (PLE) (n=30).	Oral (according to body weight/daily): ≤ 55 kg: 720 mg 56-70 kg: 960 mg >70 kg: 1200 mg 2 weeks	Of the 30 subjects, PLE lesions were completely blocked in 30%, after UVA photoprovocation and 28% after UVB provocation. The other patients were not completely protected	(Tanew et al. 2012)
Children and adolescents with atopic dermatitis using topical steroids (n=105).	Oral in addition to their standard treatment: extract capsules of Anapsos ® 120 mg	Reduces not significantly the days on which topical steroids were used respect to placebo Reduces significantly the use of oral antihistamines	(Ramírez-Bosca et al. 2012)

Table 2. (Continued)

Assay	Route of administration/Dose	Biological activity	Reference
Healthy humans (n=40).	Oral: Extract capsules of Heliocare® 240 mg twice daily 2 months (n=20) Placebo, n=20	No adverse effects, changes in physical, clinical laboratory parameters, or vital signals were reported. For subjects treated with the capsules; 4 reported mild episodic fatigue, bloating and headaches. One placebo-treated subjects reported fatigue. There were no significant differences between groups after sun exposure.	(Berman, Ellis, and Elmets 2016, Nestor, Berman, and Swenson 2015)
Healthy humans (n=22).	Oral: 480 mg of extract	Photoprotective properties, decrease UVB induced changes in 17 of the 22 subjects Decrease histological findings related with biomarkers of UV damage in all patients: DNA damage and apoptosis (sunburn cells, cyclobutane pyrimidine dimers), inflammation (Cox-2) and proliferation (Cyclin D1, Ki67 and proliferating cell nuclear antigen)	(Kohli et al. 2017)
Healthy humans with melasma (n=40).	Oral: 480 mg of extract/daily plus topical 4% hydroquinone cream at night and sunscreen SPF 50 during daytime 2 months	40% of the treated subjects achieved 60% of improvement in their melasma area severity index (mMASI) The extract is safe and is useful for the treatment of melasma.	(Goh et al. 2018, Grimes et al. 2019)

NS = Not specified.

For this reason, a deep analysis of the clinical cases that evaluates the effectiveness and safety of *Polypodium* extracts is out of the scope of this chapter. We summarized the available information in Table 2.

CONCLUSION

Despite that *Polypodium* genus has been extensively studied for its biological properties related to the treatment of skin affections, more studies are needed for supporting the additional

reported properties like antitumor and cancer treatment. Also, pharmacokinetic and LD_{50} are necessary in order to understand their systemic effect and toxicity. On the other hand, only hydrophilic (aqueous or alcoholic) extracts have been tested or studied for metabolites characterization, however, the extraction of low polar compounds with non-polar solvents is necessary in order to have the whole vision of the nutraceutical potential of this plant.

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Chapter 17

TURMERIC OIL: POTENTIAL ALTERNATIVE FOR THE TREATMENT OF METABOLIC EPILEPSY

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ABSTRACT

Epilepsy is a life-shortening brain disorder that currently affects ~ 1% of the worldwide population. Despite the availability of several antiepileptic drugs (AEDs), severe side effects such as cognitive and affective disorders, teratogenicity, hepatotoxicity, among others, have been reported after chronic administration. Also, some patients remain refractory to the available AEDs. Such is the case of metabolic epilepsy. Hence, there is a current need for the discovery of novel active principles with minimal or no adverse side effects. Nature is an exciting source of potential drug candidates for the treatment of pharmacoresistant epilepsy (PRE) due to the highly diverse and complex chemical structures of bioactive vegetal compounds. In this context, we analyzed the rhizome powder of *Curcuma longa*, commonly known as turmeric. Until our study, the anticonvulsant properties of turmeric were exclusively attributed to its curcuminoids. For the first time, we revealed the anticonvulsant properties of turmeric oil and its main bisabolene sesquiterpenoids, ar-turmerone, α-, β-turmerone, and α-atlantone. Thus, the present chapter discusses the botanical aspects of turmeric, the chemical composition and phytopharmacological aspects of turmeric, curcumin and turmeric oil as well as our results obtained from the anticonvulsant activity characterization of turmeric oil and ar-turmerone. Our findings support further characterization of the anticonvulsant properties of these active compounds and demonstrate the usefulness of the zebrafish and mouse models for searching novel AEDs. Also, the potential therapeutic application of turmeric oil and ar-turmerone for the treatment of metabolic epilepsy is discussed.

Keywords: turmeric oil, metabolic epilepsy, curcumin, ar-turmerone, anticonvulsant

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INTRODUCTION

Epilepsy: General Definitions

Epilepsy is a health chronic condition characterized by the presence of more than one unprovoked recurrent "epileptic seizure" within two years (Alarcón and Valentín 2012). The term "epileptic seizure" refers to the transient occurrence of signs or symptoms originated from a hyper-synchronization of neuronal networks in the absence of underlying acute conditions that can trigger seizure onset such as hypoglycemia, alcohol withdrawal, hypercalcemia, encephalitis, among others (Alarcón and Valentín 2012, Berg et al. 2010). An exception for this definition is "reflex epilepsy", in which case seizures could be triggered by specific stimuli (i.e., flashing lights, visual patterns, music, intellectual activity, etc.) (Alarcón and Valentín 2012).

Epidemiology and Therapeutic Limitations

Currently, around 70 million people are affected by this neurological disorder worldwide (Singh and Trevick 2016). From these, 70% of cases can be treated pharmacologically with satisfactory results. However, 30% of patients present problems concerning the currently available pharmacological therapy. Among the main therapeutic limitations are those related to pharmaco-resistance and adverse reactions. Therefore, it becomes evident the need to search for new effective and safe alternatives for the treatment of epilepsy.

Classification of Epileptic Seizures

(a) *Generalized epileptic seizures* originate simultaneously in bilaterally distributed neuronal networks of cortical or subcortical structures. Generalized seizures do not necessarily spread into the entire cortex (Berg et al. 2010, Berg and Millichap 2013).

(b) *Focal epileptic seizures* initiate in networks located in one hemisphere, mainly at the level of the subcortical structure. Occasionally, focal seizures may evolve to bilateral convulsive seizures (Berg et al. 2010). The spread of focal seizures to both hemispheres causes impairment of consciousness since each hemisphere can maintain consciousness independently (Alarcón and Valentín 2012).

(c) *Status epilepticus (SE)* is a condition characterized by repeated and prolonged epileptic seizures occurring in the middle of brief intervals producing an unvarying and enduring epileptic condition (Berg et al. 2010). SE affects 10-60 per 100,000 patients/year, and half of the cases occur in people with no previous clinical history of epilepsy. Infections with fever in children and cerebrovascular accidents, hypoxia, metabolic disorders, and alcohol intake in adults are among the most commonly known causes of this condition. SE presents a high mortality rate (50%) when it does not respond to first-line antiepileptic drugs (AEDs). After presenting SE, spontaneous seizure occurrence is present in 10% of the cases.

Classification of Electro-Clinical Syndromes and Other Epilepsies

(a) *Electro-clinical syndromes* are a group of clinical entities with a cluster of signs, symptoms as well as electrophysiological, neuroimaging, developmental and genetic features that define a recognizable and specific epilepsy disorder as the case of Dravet syndrome, Lennox-Gastaut syndrome, among others (Berg et al. 2010, Berg and Millichap 2013).

(b) *Distinctive constellations/surgical syndromes* are diagnostically meaningful forms of epilepsy based on specific lesions such as the case of mesial temporal lobe epilepsy with hippocampal sclerosis, gelastic seizures with hypothalamic hamartoma, hemi-convulsion-hemiplegia-epilepsy, and others. Therefore, they may have implications for clinical treatment, particularly surgery (Berg et al. 2010, Berg and Millichap 2013).

(c) *Non-syndromic epilepsies* are a group that comprises a group of epilepsies originated secondarily to structural or metabolic lesions or conditions that do not correlate with any specific electro-clinical pattern. Thus, epilepsies that were cited in the past as "cryptogenic" (of unknown cause) are included in this group (Berg et al. 2010, Berg and Millichap 2013).

Diagnosis of Epilepsy

There are other clinical conditions distinct from epilepsy, such as psychogenic non-epileptic attacks (PNEAs) in adults and non-epileptic staring spells, breath-holding spells, and shudder attacks in children, that include non-epileptic seizure episodes among their symptoms (Benbadis 2009). Therefore, it is essential to perform a differential diagnosis of epilepsy based on the neurological clinical history of the patient, physical exam, electroencephalography (EEG), computed tomography (CT) and magnetic resonance imaging (MRI) analyses as well as blood tests.

Clinical history is relevant for association with any seizure event in the past, as well as for a description of the seizure type, behavioral changes and potential triggering stimuli (e.g., flashing lights, striped visual patterns, etc.) (Alarcón and Valentín 2012, Hauser et al. 1998). It has been stated that there is a relatively low risk of recurrence (33% in five years) after a first unprovoked seizure (Hauser et al. 1998). Nevertheless, this risk increases considerably (75%) after the second or third unprovoked seizure within four years (Hauser et al. 1998). A physical examination for epilepsy diagnosis allows identifying any possible cause of seizures such as abnormal cardiac examination (cardiac syncope), obstructive airway disease (cough syncope), among others (Alarcón and Valentín 2012). It can also detect motor impairment, the loss of balance, and reflexes as indicators of brain malfunction.

Electroencephalographic (EEG) analysis constitutes a reliable diagnostic tool for epilepsy. In a standard awake interictal EEG, epileptiform discharges are present in 55% of cases of patients with epilepsy (Pillai and Sperling 2006) (Alarcón and Valentín 2012). Noteworthy, EEG interpretation should always be accompanied by a detailed clinical history of the patient, including age at onset, administered medication, state of awareness, and others, to help to understand this clinical condition. More recently, continuous video-EEG monitoring – when carried out for several hours, or even days - allows seizures recordings with simultaneous EEGs for a most accurate correlation between clinical and electroencephalographic manifestations

(Alarcón and Valentín 2012). Additionally, magnetic resonance imaging (MRI) and computed tomography (CT) are diagnostic neuroimaging tests capable of revealing physical changes in the brain, thus suggesting potential causes of epilepsy.

Blood exams are needed to identify conditions such as infections, anemia, and metabolic disorders, such as diabetes, abnormal electrolyte levels and even genetic alterations that could be associated to seizure (Alarcón and Valentín 2012, Berg et al. 2010). Correspondingly, evidence suggests that *SCN1A* plays a role in the etiology of Dravet syndrome thus constituting the basis of its molecular diagnosis (Baraban, Dinday, and Hortopan 2013, Depienne et al. 2009). In the case of structural/metabolic epilepsies, they are related to brain lesions caused by stroke, trauma, tumor, infections or metabolic causes such as drug intoxication, drug withdrawal, hypoxia, liver failure, uremia, among others.

As emphasized previously, epilepsy is a term that does not refer only to a particular neurological disorder. Epilepsies constitute several syndromes with a multitude of different causes, manifestations, treatments, and prognosis. Precise identification of the involved seizure type allows determining its association to a recognized syndrome. Since treatment differs markedly among epilepsy syndromes, its effectiveness relies on the accurate diagnosis.

TREATMENT OF EPILEPSY

Pharmacological Treatment

Historical Aspects of Antiepileptic Drug Discovery and Development

The pharmacological approach comprises the initial modality of treatment for patients with epilepsy. It began around 156 years ago with the use of potassium bromide as a treatment for "hysterical" epilepsy in young women (Brodie 2010). Later, phenobarbital (PB) was introduced in the middle of the nineteenth century as a sedative drug. The anticonvulsant properties of PB were discovered perchance, and soon it became preferred over bromides due to lower toxicity. To date, PB is still prescribed in developing countries, mainly due to its modest cost (Kwan et al. 2010). In 1938, phenytoin (PHT) was launched as the result of screening for potentially less sedative AED candidates in a cat model of electrical seizures (Merritt and Putnam 1938). Thus, the discovery of PHT underscored the fact that sedative and anticonvulsant properties could be separated (Alarcón and Valentín 2012, Merritt and Putnam 1938). The following major AEDs to be approved were carbamazepine (CBZ) and valproic acid (VPA). CBZ was first licensed as an antidepressant drug, but it was predicted to have antiepileptic properties because of its chemical structure (Alarcón and Valentín 2012). VPA was used as an organic solvent before the unexpected discovery of its anticonvulsant properties in a rodent model of PTZ-induced seizures (Henry 2003). Thus, the first generation of AEDs included primidone, ethosuximide, carbamazepine, and valproic acid. Also, scientific evidence revealed the efficacy of diazepam (DZP) for the management of status epilepticus (Gastaut et al. 1965).

Later, fourteen new compounds constituting the second and third generations of AEDs were licensed from 1989 to 2009 (Johannessen Landmark and Patsalos 2010). Thus, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, rufinamide, stiripentol, tiagabine, topiramate, vigabatrin, and zonisamide comprise the second-generation and eslicarbazepine acetate and lacosamide, the third-generation.

Mechanisms of Action of Antiepileptic Drugs

The main mechanisms whereby AEDs exert their pharmacological properties are commonly categorized in four groups (Rogawski and Löscher 2004, Meldrum and Rogawski 2007, Bialer and White 2010, Loscher et al. 2013).

Modulation of Voltage-Gated Ion Channels

(a) Voltage-Gated Sodium Channels

Nav1.1, Nav1.2, and Nav1.6 are three out of nine isoforms of the voltage-gated (VG) Na^+ channels expressed in the mature mammalian brain. They generate transient and persistent Na^+ currents responsible for potential action generation and burst discharges, respectively. Mutations in alpha or beta subunits (*SCN1B*, *SCN1A*, and *SCN2A*) of the VG Na^+ channels have been associated to forms of generalized epilepsy such as Dravet syndrome and epilepsy with febrile seizures plus (GEFS+) (Wallace et al. 2002, Scheffer et al. 2009, Escayg and Goldin 2010). AEDs acting through VG Na+ channels such as phenytoin and lamotrigine exert their activity by binding to a site in the alpha subunit (White 1999, Rogawski and Löscher 2004). As a result, the refractory period is extended, and the maximal firing rate is reduced, thus lessening high-frequency burst firing.

(b) Voltage-Gated Calcium Channels

These channels are present in all excitable membranes and are categorized as L-, P/Q-, N-, R- and T-type according to their biophysical properties. L-type Ca^{2+} channels control hormone secretion in endocrine cells and excitation/contraction of skeletal, smooth and cardiac muscles also. In neurons, P/Q-, N-, R- type channels are located at presynaptic terminals where they diminish entry of Ca^{2+} decreasing synaptic release of glutamate (Zamponi, Lory, and Perez-Reyes 2010). Since T-type VG Ca^{2+} channels can be activated at hyperpolarized membrane potentials, they are known as low voltage-activated (LVA). T-type channels are involved in oscillatory potentials including those occurring in thalamic and cortical neurons that correlate to “spike and wave discharges” (SWDs), which are typical of absence-type seizures (Alarcón and Valentín 2012, Merritt and Putnam 1938). Moreover, mutations in the genes encoding for the CaV3.1 and CaV3.2 T-type calcium channels have been identified in patients with generalized epilepsies (Cain and Snutch 2013, Khosravani et al. 2005).

T-type current in thalamic neurons is discretely reduced by ethosuximide and zonisamide. This action appears capable of controlling the oscillatory potentials associated with SWDs in absence-like seizure syndromes (Tringham et al. 2012). There is evidence of gabapentin and pregabalin acting through the α2-δ1 and α2-δ2 subunits of VG Ca^{2+} channels, thus decreasing the N-, P/Q-type currents (Löscher and Schmidt 2006, Dolphin 2012).

Excitability in numerous types of neurons is regulated by the native muscarinic-sensitive K^+ current (M current), where K^+ channels are involved. Indeed, K^+ channels are critical for establishing and stabilizing the resting potential of neurons, thus decreasing neuronal hyperexcitability. Mutations in the genes encoding for KCNQ2 or KCNQ3 K^+ channels underlie benign familial neonatal convulsions, which characterizes autosomal dominant epilepsy of infancy (Singh et al. 1998, Jentsch 2000). Hence, KCNQ (Kv7) channel activators such as retigabine, appear as good candidates for the treatment of epilepsies whose etiology is associated with K^+ channels (Gunthorpe, Large, and Sankar 2012).

Enhancement of Synaptic Inhibition Mediated by GABA

Gamma-aminobutyric acid (GABA) is the primary inhibitory transmitter in the mammalian central nervous system. Only a small part of neurons localized in the neocortex, hippocampus and amygdala use GABA as a neurotransmitter. Although small in number, they are crucial to inhibit excitatory inputs in these key brain locations (Rogawski and Löscher 2004).

$GABA_A$ and $GABA_B$ are two types of receptors in neuronal membranes. Activation of $GABA_A$ receptors results in two inhibitory responses, phasic and tonic inhibition. Phasic inhibition is a fast response caused by GABA or GABA-agonists such as muscimol, diazepam, clobazam, and clonazepam in postsynaptic receptors containing γ subunits with a response to benzodiazepines (Alarcón and Valentín 2012, Rogawski and Löscher 2004). Tonic inhibition produces an extra-synaptically sustained Cl^- current involving α4 or α6 and δ subunits that are not activated by benzodiazepines, but they do respond to neurosteroids such as progesterone metabolites or ganaloxone (Alarcón and Valentín 2012, Rogawski and Löscher 2004).

$GABA_B$ are G-protein-coupled to ion channels (metabotropic receptors) whose activation enhances the negative intracellular potential (K^+ currents) while decreasing channel opening and pre-synaptic release of glutamate (Ca^{+2} currents) (Alarcón and Valentín 2012, Rogawski and Löscher 2004). Baclofen is a $GABA_B$ agonist with anti-spastic properties, but it is also involved in spike-and-wave cortical and thalamic discharges similar to those observed in absence epilepsy syndromes (Terrence, Fromm, and Roussan 1983).

In humans and rats, four GABA transporters have been identified as GAT-1, GAT-2, GAT-3, and BGT-1. GAT-1 and GAT-3 are involved in phasic and tonic inhibition, respectively. The AED, tiagabine, selectively inhibits GAT-1 (Borden et al. 1994).

Following re-uptake into neurons and astrocytes, GABA undergoes further metabolism by the mitochondrial enzyme GABA-transaminase (GABA-T) transforming it to succinic semi-aldehyde. Vigabatrin is a GABA-T inhibitor commonly prescribed for the treatment of epilepsy until association with irreversible constriction of the visual field (Hemming et al. 2008) (Wang et al. 2008). Despite this, vigabatrin is still prescribed for infantile spasms and refractory epilepsy cases (Hemming et al. 2008). Moreover, mutations in the $GABA_A$ receptor gamma-2 subunit (GABRG2), resulting in reduced inhibitory function, have been observed in various epilepsy syndromes such as GEFS+ type 3 and childhood absence epilepsy (Wallace et al. 2001, Harkin et al. 2002).

Inhibition of Glutamatergic Excitatory Transmission

Glutamate, the principal excitatory brain neurotransmitter, exerts its activity through three ionotropic receptors known as AMPA [2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid], kainate and NMDA (N-methyl-d-aspartic acid). Local delivery of AMPA and kainate agonists in cortical structures triggers focal seizures, whereas systemic administration induces limbic seizures (Alarcón and Valentín 2012). Evidence from animal models suggests AMPA/kainate receptor antagonists as potential anticonvulsants as in the case of topiramate (Meldrum and Rogawski 2007, Rawls et al. 2009). Likewise, NMDA receptors have a crucial role in seizure generation, excitatory synaptic transmission and excitotoxic neuronal damage (Frasca et al. 2011). Hence, NMDA receptor antagonists appear to be interesting pharmacological alternatives for the treatment of epilepsy. Indeed, it has been verified that felbamate, an NMDA receptor antagonist with selectivity for NR1a/NR2B subunits, has the capability of effectively regulating glutamatergic excitatory transmission (Kleckner et al. 1999, Meldrum and Rogawski 2007).

Other Actions

The AEDs levetiracetam and its analogs brivacetam and seletracetam specifically bind to the synaptic vesicle protein 2A (SV2A). Although the precise mechanism by which they control neuronal excitability is not entirely understood, SV2A constitutes a new target different from other known AEDs (Ulloa, Towfigh, and Safdieh 2009). Synaptic vesicle protein 2 (SV2) is a membrane glycoprotein explicitly found in secretory vesicles of neural and endocrine cells (Custer et al. 2006). Development of severe seizures was observed in SV2A-knockout mice, thus implying the role of SV2A in the modulation of neuronal excitability (Crowder et al. 1999). It has been suggested that SV2A prepares vesicles for fusion, thereby assisting in synaptic vesicle exocytosis and neurotransmitter release.

Epilepsy Surgery

Surgery is suggested in case of pharmacoresistant epilepsies (PRE) exhibiting a single localized epileptic focus in the brain. Indeed, resective epilepsy surgery has achieved important seizure remission rates in patients with MTLE and neocortical focal epilepsy when exhibiting correlated MRI and EEG abnormalities (Wiebe et al. 2001). Corpus callosotomy, lobar and multi-lobar resections, and hemispherectomy constitute other surgical alternatives also useful in cases of intractable epilepsy.

Vagus Nerve Stimulation

Vagus nerve stimulation (VNS) is an adjunctive treatment well suited for patients from 12 years of age, with PRE that do not qualify for intracranial epilepsy surgery. A reduction to 50% seizure frequency has been reported for 45% of patients (DeGiorgio et al. 2000).

Ketogenic Diet

The ketogenic diet (KD) is a high-fat and low-protein diet with verified effectiveness in children with PRE (Lefevre and Aronson 2000). KD was conceived to mimic the effects of starvation based on the correlation observed between this condition and seizure alleviation. Some of the metabolic changes triggered by KD include ketosis, reduced glucose, elevated fatty acid levels, and enhanced bioenergetic reserves (80). In addition to its anticonvulsant properties, KD seems to exert neuroprotective properties and anti-epileptogenic effects (Masino and Rho 2012).

PROBLEMS RELATED TO THE PHARMACOLOGICAL TREATMENT

Pharmacoresistant Epilepsies

Despite the continuous efforts devoted to the discovery and development of novel AEDs over the past two decades, around 30-40% of patients with epilepsy remain resistant to currently available drugs (Kwan and Brodie 2000, Remy and Beck 2005, Alexopoulos 2013). The International League Against Epilepsy (ILAE) defines PRE as the clinical condition characterized by the failure to achieve seizure freedom following adequate drug trials of two tolerated and appropriately chosen and used AEDs regimens (mono- or polytherapy) (Kwan et

al. 2010). Based on the variable nature of PRE, the minimum interval of sustained seizure freedom has been defined as a period of twelve months, or, at minimum, three times longer than the longest pre-intervention inter-seizure interval in the preceding twelve months (Alexopoulos 2013, Kwan et al. 2010). Remarkable, this proposed definition of PRE will continue to have an indeterminate status until achieving a better understanding of its molecular and cellular mechanisms.

Occurrence of PRE seems to be more common (~ 60%) in patients with focal epilepsies, mainly undergoing temporal lobe epilepsy, than in primary generalized epilepsies (20-40%) (Remy and Beck 2005, Pati and Alexopoulos 2010) and mortality incidence in PRE is 2-10 times higher than in the general population (Alexopoulos 2013). Certainly, sudden unexpected death in epilepsy (SUDEP) is the most frequent cause of death, followed by status epilepticus in patients with PRE (Ryvlin, Cucherat, and Rheims 2011). Additionally, PRE has been strongly associated with comorbid depression and anxiety (Alexopoulos 2013).

Three patterns of PRE have been suggested based on epidemiological studies (Alexopoulos 2013):

(a) *De novo pharmacoresistance* recognized in patients newly diagnosed with epilepsy that exhibit AED resistance from the first seizure event. When the treatment fails with the first AED prescribed according to the specific epilepsy syndrome, there is only 11% of success probability with the second (Kwan and Brodie 2000).
(b) *Progressive pharmacoresistance* occurs in patients with epilepsy that become refractory over time. This pattern of pharmacoresistance has been observed in childhood epilepsies, as well as in mesial temporal lobe epilepsies (Berg et al. 2010, Berg and Millichap 2013).
(c) *Waxing and waning resistance* is identified in patients with alternating pharmacoresistant and pharmacoresponsive periods. The exact causes underlying this response are not completely understood. There is evidence of relapse after the first year of seizure remission, and therefore, permanent seizure freedom is unlikely to happen in this group of patients (Callaghan et al. 2011).

Consequently, two theories have been suggested to explain the development of PRE: a) the transporter and, b) the target hypotheses (Remy and Beck 2005).

In the *transporter hypothesis*, PRE is a result of an increased expression or function of multidrug transporters in the brain. P-glycoprotein (PGP) and multi-drug resistant associated proteins (MRPs) are multidrug transporter proteins expressed in endothelial cells of the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier where they transport lipophilic substances across membranes. Thus, it is implied that their overexpression decreases the effective concentration of AEDs at their brain targets (Sills et al. 2002, Borst et al. 2000). This theory is supported by the upregulation of PGP1 (also known as MDR1), MRP1, and MRP2 observed in different forms of PRE (Lee et al. 2001).

On the other hand, the *target hypothesis* states that epilepsy-related changes in drug targets account for reduced drug sensitivity at this level. Indeed, seizures appear to trigger changes in the transcription of ion channels subunits in human epilepsies (Brooks-Kayal et al. 1999) and also induce post-translational modifications in ion channels (e.g., phosphorylation, glycosylation, etc.) that affect their responsiveness to AEDs (Bernard et al. 2004). Epilepsy-related changes are not only referred to ion channels, but transcriptional modifications have

also been observed in the α-subunit composition of $GABA_A$ receptors in neurons of epileptic rats (Brooks-Kayal et al. 1998). Moreover, alteration in the normal function of neurotransmitters seems to contribute to the development of pharmacoresistance. An excellent example of this action shift is GABA, which exhibits excitatory activity in the immature brain and in the epileptic brain (Ben-Ari et al. 2007; Ben-Ari et al. 2012). The depolarizing activity of $GABA_A$ results from a net outward flux of Cl^- through the $GABA_A$ receptor ionophore caused by an initially higher intracellular Cl^- concentration (Ben-Ari et al. 2007, Ben-Ari et al. 2012).

The fact that patients with epilepsy who are resistant to one first-line AED usually are also resistant to other AEDs with diverse mechanisms of action suggests that PRE may be explained by alterations occurring at the level of drug target, transporter or perhaps a combination of both. For instance, resistance to CBZ action is probably mediated by loss of sensitivity of Na^+ channels rather than through multidrug transporters for which CBZ does not seem not to be a substrate (Owen et al. 2001, Remy et al. 2003). Conversely, for PHT, target alterations seem to be less relevant as a mechanism of resistance since its intraparenchymal concentration is strongly regulated by multidrug transporters at the BBB (Potschka and Loscher 2001).

The wide variety of probable mechanisms of PRE reflects a high complexity, which is not completely understood to date. As a result, misdiagnosis of PRE has been reported in patients with non-epileptic seizures as well as incorrect classification of epilepsy type, failure to identify the underlying cause of epilepsy, inaccurate selection or dosage of AED, drug interactions, intolerable adverse effects or poor adherence to therapy have been described (Smith, Defalla, and Chadwick 1999, Alexopoulos 2013). PRE must be promptly recognized so that other non-pharmacological alternatives such as surgery, vagus nerve stimulation or ketogenic diet may be appropriately applied.

Adverse Side Effects

Patients under pharmacological treatment with older AEDs are more susceptible to present chronic toxicity (1). Of all the AEDs, barbiturates are the strongest sedation inductors associated with megaloblastic anemia, metabolic bone disease, changes in connective tissue, peripheral neuropathy, movement disorders, cognitive effects and behavior disorders (Greenwood 2000, Alarcón and Valentín 2012). It is usually observed with PHT that blood levels suddenly increase with small dose changes, producing acute cerebellar syndrome (Brostoff, Birns, and McCrea 2008). Also, PHT causes peripheral neuropathy, hypersensitivity reaction, as well as cosmetic modifications in connective tissue resulting in gum overgrowth, coarsening of face, and hirsutism (Greenwood 2000). Moreover, this drug is a potent liver enzyme inductor, thus reducing the efficacy of other co-administered medicines (Alarcón and Valentín 2012, Greenwood 2000). Hepatoxicity related to AED is a side effect more frequently observed with felbamate, PHT, and CBZ (Björnsson 2008). Also, visual alterations, rash, allergy, and hyponatremia are other common side effects seen with CBZ. Certainly, its rapid absorption and generation of toxic metabolites are promoting factors for the occurrence of side-effects (Neuvonen 1985).

VPA significantly increases the risk of malformations during fetal development (DiLiberti et al. 1984). Also, long-term treatment with VPA is associated with polycystic ovaries, infertility, and weight gain in young women (Alarcón and Valentín 2012). About vigabatrin, an increased risk of visual field constriction has been reported (Kälviäinen et al. 1999). Vigabatrin and topiramate have been associated with psychosis and affective disorders (Nadkarni and Devinsky 2005, Alarcón and Valentín 2012). Behavioral problems have been

commonly observed in patients following AED treatment with phenobarbital, phenytoin, primidone, and ethosuximide (Cavanna et al. 2010).

Antiepileptic Drugs under Development

No significant impact on the prognosis of PRE is observed despite the introduction of fourteen new AEDs during the past thirty years. Hence, the pharmacological treatment of PRE patients relies entirely on the development of new AEDs with novel targets and enhanced safety and pharmacokinetic profiles. Indeed, current aims in AED discovery are focused on finding molecules that will improve the general prognosis by preventing epilepsy development (following a stroke, head injury, neurosurgery, etc.), epileptogenesis and mortality (especially regarding SUDEP). Accordingly, some molecules for the treatment of epilepsy are currently in progress:

Brivaracetam and seletracetam are more potent analogs of levetiracetam with high affinity to the SV2A binding site (Alarcón and Valentín 2012).

2-deoxy-d-glucose (2-DG) has been suggested to be one of the molecules responsible for the antiepileptic activity of the ketogenic diet (Masino and Rho 2012). 2-DG strongly reduces seizure progression in kindling models and upregulates expression of brain-derived neurotrophic factor (BDNF) and its receptor TrkB (Garriga-Canut et al. 2006).

Fluorofelbamate is an analog of felbamate which appears to exhibit the same clinical efficacy without its common side effects (aplastic anemia and hepatotoxicity) (Alarcón and Valentín 2012).

Ganaxolone is a synthetic progesterone-related molecule, belonging to a novel class of neuroactive steroids, known as epalons. Its mechanism of action seems to be exerted via allosteric modulation of $GABA_A$ receptors through a unique recognition site (Reddy and Woodward 2004).

Retigabine is a compound with activity in focal epilepsies. Its mechanisms of action include activation of KCNQ2 and KCNQ2/3 K^+ channels, synthesis inhibition of neuroactive amino acids, enhance of GABA synthesis and stimulation of $GABA_A$ receptors (Alarcón and Valentín 2012, Gunthorpe, Large, and Sankar 2012).

Safinamide is a monoamine oxidase B inhibitor with apparent activity on the modulation of Na^+ and Ca^{2+} currents (Alarcón and Valentín 2012).

Talampanel is a selective antagonist of AMPA receptors displaying a weak affinity for benzodiazepine binding site (Alarcón and Valentín 2012).

Tonabersat is a benzopyran derivative analog of carabersat and AED with efficacy as "add-on" therapy for focal epilepsies. It is implied that tonabersat and carabersat act via a novel mechanism of action by selective modulation of neuronal gap junctions (Bialer and White 2010).

Valrocemide is a glycine derivative of VPA, which is currently in Phase II clinical trials carried out in patients with PRE. Its mechanism of action is not clearly understood to date, but it appears to be mediated by GABA stimulation (Isoherranen et al. 2001).

ANIMAL MODELS FOR EPILEPSY DRUG DISCOVERY

The Zebrafish Model

The zebrafish (*Danio rerio*) has emerged as a promising biological model to study brain disorders since behavioral and neurophysiological responses can be evoked through pharmacological or genetic manipulations (Stewart et al. 2012). Zebrafish constitute a useful and almost inexpensive complementary model to rodents for identification and pharmacological characterization of novel AED candidates. Some advantages of this model are the physiological, pharmacological and genetic similarities to humans, the fast *ex-uterus* development, the body transparency of embryos and larvae, long lifespan, ease of genetic manipulation, simplicity of drug administration (by immersion), as well as lower cost (Stewart et al. 2012, Kokel et al. 2010). Of interest, zebrafish has been suggested as a suitable model for evaluation of neuroactive drugs (Baraban, Dinday, and Hortopan 2013, Berghmans et al. 2007).

Locomotor Activity Evaluation

Exposure of zebrafish to pentylenetetrazol (PTZ) induces a particular sequence of behavioral changes. This includes, first of all, increased swimming activity stage that resembles mostly natural behavior of larvae of that age but is a lot more intense. Second, the convulsive stage comes when swimming is interrupted by uncoordinated involuntary whole-body contortions and trembling. Consequently, this stage resolves into a loss of posture period, when the larva is floating motionless without any control of its posture. These specific features are similar to the sequence of events observed in rodent models (i.e., explore-tremble-fall), and suggests a common nature of these behaviors, especially in terms of the electrographic profile in both cases (Berghmans et al. 2007).

Tectal Field Recordings

Electroencephalographic recordings can be carried out in zebrafish larvae treated and not treated with the AED candidate and in the presence and absence of PTZ using glass electrode filled with artificial cerebrospinal fluid placed into the optic tectum (Figure 1A). Thus, it is possible to quantify the number and average duration of epileptiform discharges (defined as *ictal-like* and *interictal-like* spikes) as well as the total cumulative duration of all forms of epileptiform discharges during the recording period (Baraban et al. 2005).

Based on the fact that larvae are immobilized in agar during the recordings and, that the shape and polarity of the spikes are not affected by sample preparation (whole fish or isolated head), muscle paralysis (tubocurarine pre-treatment, bungarotoxin i.m. injection, or even no pre-treatment), or general sedation (tricaine) (Hortopan, Dinday, and Baraban 2010), it is stated that these changes are specific for electrographic seizures and are clearly distinguishable from movement artifacts. As an example, magnified fragments of recordings are shown below (Orellana-Paucar et al. 2012). Vehicle-treated fish recordings (Figure1B) exhibit relatively slow waves of small amplitude with an upward-pointing maximum. In contrast, PTZ-treated

fish (Figure1C) showed spikes and multi-spike complexes with a very sharp sudden start (10-20 µs from baseline to a minimum) and often high-frequency oscillations of around 40 Hz independent of trembling observed during the recording.

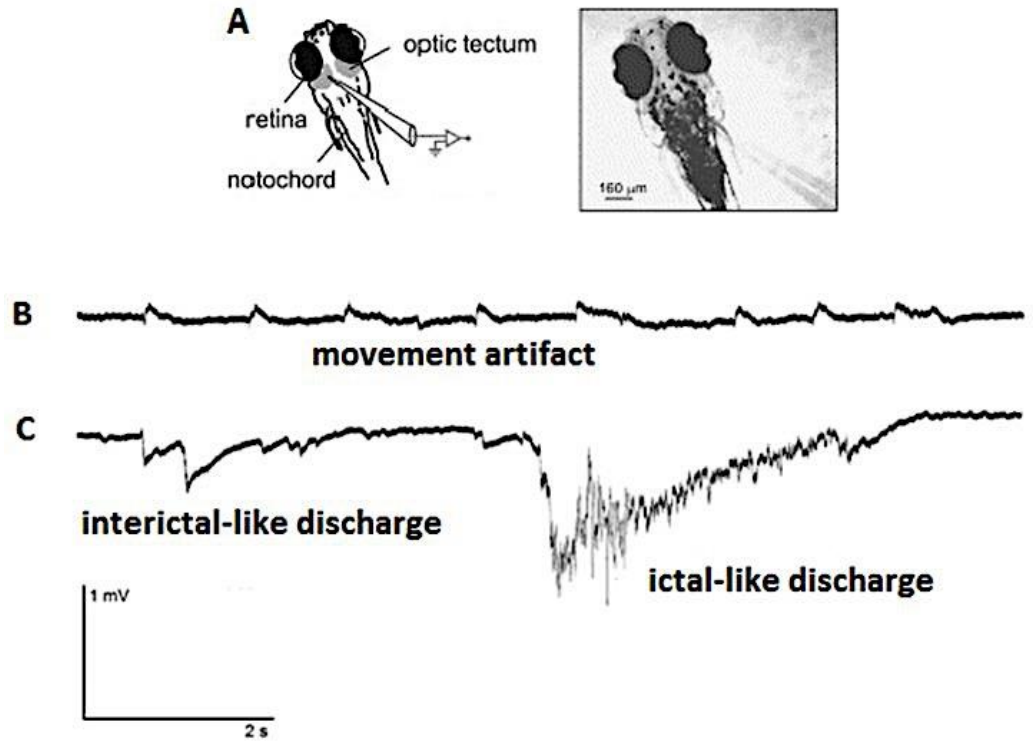

Figure 1. Tectal field recordings in zebrafish larvae. A. Scheme and the picture of tectal field recordings from agar-embedded zebrafish larvae (Baraban et al. 2005). B. Representative field recordings from zebrafish larvae exposed to 20 mM PTZ depicting movement artifact, and, C "interictal-like" and "ictal-like" discharges. Adapted from (Orellana-Paucar et al. 2012).

The Rodent Models

Identification

Despite the introduction of new AEDs, no significant improvement regarding effectiveness in patients with PRE has been achieved during the past two decades. This failure may be explained, at least in part, because of the use of the same conventional tests for AED discovery during the last sixty years. The maximal electroshock (MES) and the subcutaneous pentylenetetrazol (s.c. PTZ) rodent models have been applied for identification of all past AEDs, except for levetiracetam (LEV) and other newer AEDs. Of interest, LEV exerts its anticonvulsant activity mainly, through a novel mechanism action (SV2A binding) (Lynch et al. 2004) and exhibits effectiveness as mono- and combinatorial therapy in the clinical PRE (Gambardella et al. 2008, Li et al. 1993). Thereby, to avoid overlooking interesting compounds (i.e., levetiracetam), the 6-Hz test has been suggested by the National Institutes of Health (NIH) in addition to the conventional rodent models for identification of AED candidates.

The MES test is a model capable of identifying compounds effective against generalized seizures of the tonic-clonic (grand mal) type (Castel-Branco et al. 2009). Following 15-30 min after intraperitoneal (i.p) administration of the test compound, a high-intensity electric current of high frequency and short duration (50-60 Hz; 0.2 s) is delivered to trigger brief tonic flexor convulsion (flexion) and prolonged tonic extensor convulsion (extension) of the hindlimbs (Castel-Branco et al. 2009, Giardina and Gasior 2009). These tonic convulsions may be -or not- followed by clonic convulsions. Blockade of the hind-limb tonic extensor convulsion is considered as an anticonvulsant effect (Giardina and Gasior 2009).

The s.c. PTZ model is used to assess the anticonvulsant or pro-convulsant activity of a compound intraperitoneally administered. After an appropriate period of 15-30 min following the compound administration, the pro-convulsant PTZ (120 mg/kg) is given subcutaneously (Giardina and Gasior 2009). This PTZ dose induces clonic and tonic convulsions as well as death in most mice in approximately 30 minutes after administration. The anticonvulsant effect of the compound in this test is identified by its ability to block clonic and tonic convulsion episodes, mainly drugs controlling generalized absence seizures and/or myoclonic epilepsy (Giardina and Gasior 2009).

In the 6-Hz seizure test, seizures are electrically induced as in the case of the MES test. The difference lies in the characteristics of the electrical stimulus. In the 6-Hz test, convulsions are induced by three-seconds corneal stimulation with a 6-Hz, 0.2-msec rectangular electrical current (Barton et al. 2001, Barton, Peters, and Shannon 2003, Giardina and Gasior 2009). For stimulation, different current intensities can be used (22 and 44 mA). The electrical current of 44 mA in this model, allows further characterization and differentiation of AEDs (Barton et al. 2001, Barton, Peters, and Shannon 2003, Giardina and Gasior 2009). From all available AEDs, only levetiracetam (at high doses), valproate, and newer AEDs such as retigabine, brivacetam, and carisbamate exhibit complete protection (Barton et al. 2001, Löscher 2011). This test induces the so-called 'psychomotor convulsions' characterized by behavioral arrest, forelimb clonus, twitching of the vibrissae and dorsiflexion of the tail (Giardina and Gasior 2009). The ability of a compound to control seizure spread through neuronal tissue evidenced by the absence of the aforementioned behavioral parameters in this test is defined as anticonvulsant activity (Barton et al. 2001, Giardina and Gasior 2009).

Additional rodent models such as the timed intravenous infusion of pentylenetetrazol (i.v. PTZ mouse model) has also been suggested for identification of novel AED candidates (Mandhane, Aavula, and Rajamannar 2007, Giardina and Gasior 2009). In the i.v. PTZ model, the test compound is administered intraperitoneally or intravenously using a dual pump system. Following administration of the test compound, PTZ (7.5 mg/ml; 150 µL/min) is intravenously infused to induce myoclonic twitch, tonic and clonic convulsions, tonic hind-limb extension and finally death (Giardina and Gasior 2009, Mandhane, Aavula, and Rajamannar 2007). Then, the required dose of PTZ (mg/kg) for each behavioral endpoint is calculated. Anticonvulsant activity in this test is identified by a significant increase of PTZ dose needed to trigger a behavioral point.

Quantification

Once a novel AED candidate is identified, the therapeutic window (range between anticonvulsant and neurotoxic doses) should be established (Löscher 2011). Positively, neurotoxic effects such as motor impairment and sedation may influence mice performance in

behavioral tests. For early recognition of motor and balance impairments in rodents, simple models such as rotarod or beam walking tests are used.

The rotarod test allows screening for motor deficits in mice by forcing the animal to run as a response to the natural fear of falling (Shiotsuki et al. 2010). For this purpose, a machine with rotatory drums and automatic timers is used. The mouse is placed on the drum, which is covered with hard chloroethylene, thus avoiding tripping on the surface. After training sessions, mice are habituated to stay on the drum. Performance of treated mice is assessed on their capability to remain in the drum after the acceleration of drum rotation (to 15-20 rpm) (Coughenour, McLean, and Parker 1977). The test ends when the mouse falls. Latency time is then recorded. Usually, computer software connected to the rotarod machine records the number of rpm and the test end (Stanley et al. 2005, Shiotsuki et al. 2010).

On the other hand, the beam walking test can reveal alterations of motor skills in mice caused by an administered compound which, may not be detected by other motor tests (i.e., rotarod) (Stanley et al. 2005, Luong et al. 2011). Fine motor coordination and balance can be assessed by the beam walking assay. Safety of the tested compound is determined by the capability of the treated mouse to stay upright and walk across an elevated narrow beam to a safe platform (Luong et al. 2011). Safety is evaluated on the performance of the mouse on the beam by quantification of the time it takes for the mouse to traverse the beam and the number of foot slips and fallings that occur in the process (Luong et al. 2011).

Differentiation

To further characterize the activity spectrum of the drug candidate against different types of seizures, several rodent models have been proposed. One of them is the kindling model of temporal lobe epilepsy (TLE) in which, seizures are induced by repeated excitatory stimuli via a depth electrode in the limbic system (amygdala or hippocampus) (Sato, Racine, and McIntyre 1990, Löscher 2011). This kindling model effectively mimics seizure susceptibility and brain alterations observed in TLE patients. The corneally kindled mouse model has been suggested as a cost-effective alternative over the amygdala or hippocampal kindling model, for the screening of compounds for the treatment of partial epilepsy due to no need of electrode implants or post-surgery care (Rowley and White 2010). In this model, the brain is transcorneally stimulated through the optic nerve, thus creating a non-invasive route for kindling (Rowley and White 2010). Also, genetic models such as DBA/2 mice with audiogenic seizures or the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) are used for differentiation of the anticonvulsant activity spectrum of AED candidates (Löscher 2011).

Besides, the AED candidate can be tested in specific models of drug-resistant seizures to determine if the drug candidate present advantages about the established AEDs regarding the treatment of refractory seizures (Löscher 2011). For instance, in the lamotrigine-resistant kindled rat model, repetitive electrical stimulation of the amygdala trigger partial and secondarily generalized seizures that increase in severity and length with continue stimulation (Löscher 2011). Exposure to low doses of lamotrigine during the kindling process leads to a reduced response to anticonvulsant and also to the anticonvulsant activity of carbamazepine, phenytoin, and topiramate, in fully kindled rats (Löscher 2011, Postma et al. 2000). The methylazoyxymethanol acetate-exposed rat model is based on the association observed between cortical dysplasia and PRE occurrence (Löscher 2011, Smyth, Barbaro, and Baraban 2002). Thus, in this model, cortical dysplasia in rats is generated *in utero* by exposure to methylazoyxymethanol acetate (MAM) (Löscher 2011, Smyth, Barbaro, and Baraban 2002).

Later, seizures are induced by kainate or AY-9944, a cholesterol synthesis inhibitor. These seizures remain resistant to ethosuximide, valproate, and carbamazepine (Löscher 2011). Another model is the amygdala-kindling rat treated with phenytoin. This test allows the identification of three groups: non-responders (20%), responders (20%) and, variable responders (60%) (Loscher and Rundfeldt 1991). It has been implied that these groups correspond to three clinical scenarios: drug-refractory patients with TLE (non-responders), patients with TLE that achieve complete seizure control with the AED (responders) and the group of patients that achieve reduction of seizure frequency but not a complete seizure control (variable responders) (Löscher 1997, 2011).

An AED candidate can also be assessed in another model o PR, such as the basolateral amygdala (BLA). In this model, SE is achieved in > 90% of rats using prolonged excitatory stimulation of the basolateral amygdala accompanied by simultaneous treatment with phenobarbital (Brandt, Volk, and Loscher 2004). Thus, a reproducible percentage (36-40%) of non-responders is achieved (Löscher 1997, 2011). For this test, it has been reported that 83% of rats from the phenobarbital-resistant group, were also refractory to a subsequent treatment with phenytoin thus fulfilling the minimal requirements for an animal model of PRE (Löscher 2011).

Advanced Studies

Research techniques for pharmacological characterization, identification of potential targets, or determination of mechanisms of action of AED candidates involve proteome analyses, electrophysiology techniques, radiolabel-photoaffinity labeling, among others.

Proteome analyses can be performed using two-dimensional gel electrophoresis (2-DGE) for protein separation coupled with mass spectrometry for identification (Eravci et al. 2007). Using this technique, it is possible to identify altered protein expression in brain tissue after chronic administration of the AED candidate (Tsugita et al. 2000).

Electrophysiological techniques are useful to investigate the potential modulatory effects of the drug candidate on neuronal voltage-gated channels. For instance, by using patch clamping, currents of single ion channels from neuronal membranes (i.e., Na^+, K^+, Cl^-, T-type Ca^{2+} currents) can be measured (Niespodziany, Klitgaard, and Margineanu 2001, Park et al. 2013). An example of this approach is the use of *Xenopus laeves* oocytes from wild type and $GABA_A$ receptor mutants to identify potentiation/inhibition of Cl^- currents in the presence of $GABA_A$ agonists (diazepam) and antagonists (etomidate, loreclezole) (Fernandez et al. 2012).

Final target identification can be achieved by a combination of subcellular distribution studies using a tritiated AED candidate in combination with photoaffinity labeling techniques that photo-induced formation of covalent bonds between the compound of interest and its active site in protein molecules, followed by electrophoresis separation (Ruoho et al. 1973; Pérodin et al. 2001).

MEDICINAL PLANTS AND POTENTIAL DRUG DISCOVERY FOR EPILEPSY TREATMENT

Epilepsy involves an increased risk of comorbidity, anxiety, depression, and morbidity (Moshe et al. 2015). Patients with epilepsy suffer from social stigmatization and stress generated by the suffering of an unpredictable disease that could cause loss of autonomy in their daily activities (Espínola-Nadurille, Crail-Melendez, and Sánchez-Guzmán 2014, Mlinar et al. 2016). Thus, the current aim in AED discovery is the identification of new molecules that will ideally act through novel mechanisms of action to solve the problems of pharmacoresistance and severe side effects affecting around 30-40% of patients with epilepsy.

Although there are currently about twenty-five anticonvulsant drugs available on the market, seizures cannot be satisfactorily controlled in 30% of patients due to the presence of drug resistance or associated adverse effects (Dalic and Cook 2016). Among the frequently described adverse effects are hepatotoxicity, gastrointestinal problems, and cognitive deterioration (Loring and Meador 2004, Moshe et al. 2015). Therefore, to date, there is still an unmet need to identify new active principles capable of effectively controlling seizures and preventing the potential side effects induced by them. Accordingly, improvements regarding animal models for searching these drug candidates have been made. Nevertheless, another important concern is the sources where to look for these molecules.

It has been suggested that some of these potential AED candidates may be present in medicinal plants used in traditional medicine to control seizures (Schachter 2009). Throughout history, natural products have played an important role in medicine and health as they were used as the principal means of treating diseases. Indeed, important information regarding natural compounds and their pharmacological applications has been compiled in relevant ancient bibliographies such as the Egyptian *Ebers Papyrus* (1550 BC), the *Corpus Hippocraticum* by Hippocrates of Cos (*circa* 460–377 BC), the *Materia Medica* of Dioscorides (*circa* 40–90 AD), which describes the use of around 600 plant-derived medicines, and the Chinese medicinal book, *Wu Shi Er Bing Fang* (350 BC) that comprises natural agents, combinatorial drug formulae and, practical advice about properties, efficacy and synergy of natural remedies (Ji, Li, and Zhang 2009).

During the last two decades, natural products have been considered a 'second place' source for drug discovery and development because of the high complexity of the process for isolating active molecules, and also for the introduction of combinatorial chemistry. Nevertheless, the rational design of chemical compounds for specific targets has shown limitations regarding their chemical diversity, which conversely displays a clear advantage for natural products. Thus, there is currently a renewed interest in natural products, mainly due to the important contributions that modern analytical and structural chemistry have made concerning the purification and elucidation of the chemical structures of several natural products. This, in turn, has allowed chemists to synthesize the identified active compounds rather than isolate them from the complex matrix of crude extracts thus making the process less complicated.

Medicinal plants certainly play an important role in drug discovery. It has been estimated that more than 60% of available drugs are directly or indirectly derived from natural products (Newman 2008). The World Health Organization (WHO) has estimated that approximately 4 billion people (80% worldwide population) currently use herbal medicines. Indeed, despite the availability of well-established health care systems, traditional medicinal practices remain as

an important component for primary healthcare. It can be explained, at least in part, because they are part of the culture of peoples and they are also, usually considered to be safer and cheaper alternatives in comparison to pharmacological therapy.

The use of medicinal plants as a source of novel compounds can substantially benefit AED discovery. Not only regarding the supply of molecules with a wide chemical diversity but also the way in which medicinal remedies are prepared for traditional use could serve as a heuristic guide for identification of the chemical nature –lipophilic or hydrophilic- of the active compounds.

TURMERIC

Botanical Description

Curcuma longa L. (syn. *Curcuma domestica*) is commonly known as 'turmeric', a name derived from the medieval Latin term 'terramerita', which means 'meritorious earth' (Gopinath and Karthikeyan 2018). Turmeric is a perennial herb of the Zingiberaceae family, native of Asia, and cultured in tropical/subtropical areas worldwide. Its boiled, dried and powdered rhizomes are traditionally used as a food seasoning, dye, and cosmetic.

Phytochemical Composition of Turmeric

Diphenylalkaloids

The major representatives of this group are diphenylheptanoids and diphenylpentanoids. The group of diphenylheptanoids is mainly constituted (3-5%) by curcumin, demethoxycurcumin and bis-demethoxycurcumin, the yellow pigments responsible for the characteristic color of turmeric. These three compounds altogether are commonly known as "curcuminoids" and possess a mutual precursor, phenylpropanoid CoA (Ravindran, Babu, and Sivaraman 2007). Among the diphenylpentanoids, (1E, 4E)-1,5-bis-(4-hydroxy-3-methoxyphenyl)-1,4 pentadien-3-one and (1E, 4E)-1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadien-3-one, have been identified in *C. longa* (Ravindran, Babu, and Sivaraman 2007).

Phenylpropene Derivatives

Seven monomeric phenylpropene derivatives have been identified, caffeic acid, cinnamic acid, p-coumaric acid, p-methoxycinnamic acid, ethyl-p-methoxycinnamic acid, cinnamal-dehyde, and calebin A.

Terpenoids

A wide variety of mono-, di- and sesquiterpenes have isolated from *C. longa*. In fact, turmeric essential oil is rich in monoterpenes such as cineole derivatives, 2,6-dimethyloctane derivatives, methane-type, and pinane-type. On the other hand, 8(17),12-labdadiene-15,16-dial is the only diterpene identified in *C. longa.* Regarding the sesquiterpenes group, around 140 different sesquiterpenes have been isolated from the genus *Curcuma*. Their most common

constituents are classified into three major types, germacrane, guaiane and bisabolane. Among the Germacrane-type sesquiterpenes, the most abundant one found in turmeric is curdione followed by others such as dehydrocurdione, germacrone-4,5-epoxide, and germacrone-13-al. Approximately 30 guaiane-type sesquiterpenes have been identified in different species of the genus *Curcuma*. The most widely distributed one is isocurcumenol. From the bisabolane-type sesquiterpenes, ar-turmerone is the most common compound in the majority of species of the *Curcuma* genus. Other members of this group identified in *C. longa* are α- and β-turmerone, curlone, and bisacumol.

Traditional Uses of Turmeric

The Hindus attribute turmeric, a sacred character. It is believed that a piece of turmeric used as amulet can protect a person against evil spirits. Another fact regarding the sociocultural importance of turmeric in India is related to its role in the marriage ceremony in which the parents of the bride and of the groom pour turmeric water over their hands, coupled with a thread dyed with turmeric to conclude the ritual (Ravindran, Babu, and Sivaraman 2007).

In traditional medicine, turmeric is used to treat a wide variety of disorders including hepatic and gastrointestinal complaints (i.e., colic, intestinal worms, diarrhea, and constipation), rheumatism, arthritis, body aches, purulent ophthalmia, skin diseases, fever, urinary discharges, inflammation, leukoderma, amenorrhea and sepsis (Villegas, Sánchez-Fidalgo, and Alarcon de la Lastra 2008, Schachter 2009). Recent studies have not only confirmed some of these medicinal properties but have also found new bioactivities such as the stimulation of bile output, its ability to act as a free radical scavenger, as well as antiviral and anticancer activities.

Turmeric Oil

The volatile oil (essential oil) of turmeric presents a light-yellow color, and it is the responsible component for the characteristic aroma of turmeric. It is extracted from the rhizomes providing a total yield of around 3-7%. Among their major components are primarily oxygenated sesquiterpenes.

Extraction of Turmeric Oil

The volatile oil is extracted from powdered turmeric rhizome through steam distillation. By using this method, the volatile oil released from the spice is carried along with the vapors and collected. Then, the mixture of volatile oil and steam is cooled in a condenser. Furthermore, turmeric oil is separated from water and collected to be dried and stored in a cool and dry place, protected from light (Ravindran, Babu, and Sivaraman 2007).

Phytochemical Composition of Turmeric Oil

Turmeric oil is mainly constituted by the turmerones, α-turmerone (30-32%), β-turmerone (15-18%), and ar-turmerone (dehydroturmerone) (17-26%). Turmerones α- and β- change in

the presence of air adopting the configuration of ar-turmerone, which is more stable (Ravindran, Babu, and Sivaraman 2007).

Research on Pharmacological Properties of Turmeric

Safety in Humans

Noteworthy, turmeric is of particular interest for medicinal applications due to its high consumption among Indian population (around of 1.5–4 g/day) without any adverse effect report (Villegas, Sánchez-Fidalgo, and Alarcon de la Lastra 2008, Hutchins-Wolfbrandt and Mistry 2011). Accordingly, the Food and Drug Association of the United States (FDA) granted the Generally Recognized as Safe (GRAS) status for food application to turmeric oleoresin, constituted by 40% curcumin, 20% volatile oil, and 30% fixed oils (Ravindran, Babu, and Sivaraman 2007). Besides, only a few clinical studies report negligible adverse effects associated to oral administration of turmeric extract such as diarrhea for the consumption of up to 3.6 g/day of turmeric (Sharma et al. 2004).

Regarding curcuminoids, Phase I clinical trials have determined the safety of oral intake of up to 12g/day of curcumin for three months. Only minimal adverse reactions like rash, headache, and diarrhea have been reported for curcumin oral administration (Kunwar and Priyadarsini 2016).

Likewise, turmeric oil, the other main constituent of turmeric, was evaluated in a Phase I clinical trial reporting safety for oral consumption of 1 ml/day for two months (Joshi et al. 2003).

Pharmacological Properties of Turmeric

Research on the pharmacological properties of turmeric has been mainly focused on curcumin, the principal curcuminoid present in turmeric, since that it has been considered as the main biologically active phytochemical component of turmeric. Nevertheless, other studies have also been performed in turmeric oil. Research findings have shown interesting pharmacological properties for the volatile oil as well as for its major constituents.

Curcumin Applied in Infectious Diseases

There is evidence supporting the antibacterial, antiviral, and antifungal activity of curcumin. For instance, aqueous curcumin extract is capable of reducing bacterial counts of *Salmonella typhimurium*, *Pseudomona aeruginosa*, and *Escherichia coli*. Also, the antibacterial activity of curcumin and its derivatives has been described for methicillin-resistant *Staphylococcus aureus* strains (MRSA) (Mun et al. 2013)

On the other hand, antiviral activity of curcumin against human immunodeficiency virus (HIV) has been associated to a wide diverse of mechanisms of action such as inhibition of HIV-1-LTR-directed gene expression, Tat-mediated transactivation of HIV-1-LTR, Tat protein acetylation, HIV-1 and HIV-2 proteases, HIV-1 integrase, among others (Li et al. 1993, Sui et al. 1993, Mazumder et al. 1995, Barthelemy et al. 1998).

Regarding anti-viral properties, curcumin acts against influenza viruses H1N1 and H6N1 probably through inhibition of haemagglutination (Chen et al. 2010). Moreover, activity against

human papillomavirus (HPV) by inhibition of oncogenes expression and downregulation of HPV-18 transcription has also been described (Divya and Pillai 2006, Prusty and Das 2005).

In addition, antifungal activity for *Candida albicans* has been reported for curcumin as a synergistic effect with fungicides like voriconazole, itraconazole, ketoconazole, miconazole, fluconazole, amphotericin B, and nystatin. This synergistic action is probably associated with the antioxidant activity of curcumin and therefore, its capability to act as a scavenger of reactive oxygen species (ROS) (Sharma et al. 2010). Likewise, nitrosative stress mediated by curcumin action can inhibit *Trichophyton rubrum* growth (Baltazar et al. 2015).

Inflammatory Diseases and Curcumin

Administration of curcumin improves airway obstruction in patients with bronchial asthma which was verified by the mean forced expiratory volume in 1 (FEV1) values (Abidi et al. 2014).

In line with this, curcumin was also suggested as a potential candidate for gingivitis and chronic periodontitis treatment. This activity is probably associated with its topical anti-inflammatory properties (Gottumukkala et al. 2013, Pulikkotil and Nath 2015).

Regarding chronic kidney disease, it has been reported that curcumin is capable of decreasing inflammatory cytokine IL-6 (Moreillon et al. 2013). Moreover, it seems curcumin can improve endoscopic index as well as the clinical activity index in patients with ulcerative colitis (Hanai et al. 2006). Also, in cases of peptic ulcer, there is evidence from a Phase II clinical trial reporting ulcer resolution by curcumin action (Prucksunand et al. 2001).

Additionally, the efficacy of curcumin in osteoarthritis has also been reported as an adjuvant therapy to reduce pain and improve physical responses by inhibiting Coll2-1, a specific cartilage biomarker for collagen catabolism (Panahi et al. 2014). In this regard, curcumin has displayed antiarthritic effects in patients with rheumatoid arthritis by reducing stiffness and swelling joints (Amalraj et al. 2017).

Curcumin as Treatment of Cardiovascular Diseases

Curcumin has shown pharmacological effects against acute coronary syndrome, acute myocardial infarction, and dyslipidemia. In a clinical trial, this compound was able to reduce low-density lipoprotein cholesterol and total cholesterol (Alwi et al. 2008).

Also, curcuminoids supplementation has been associated with hypolipidaemic effects in humans, evidenced by a decrease in serum concentrations of triglycerides (Mohammadi et al. 2013). Accordingly, a clinical trial carried out in patients with metabolic syndrome revealed that consumption of curcumin for 12 weeks seems to decrease low-density lipoprotein cholesterol concentration and to increase high-density lipoprotein cholesterol concentration (Yang et al. 2014).

In this regard, it has been suggested that the antioxidant and anti-inflammatory properties of curcumin may contribute to its capability to significantly reduce the risk of cardiovascular disease.

Treatment of Metabolic Diseases with Curcumin

In type 2 diabetes clinical trials, curcumin has shown a capability to decrease blood glucose levels and to improve the function of beta cells, to increase HOMA-B levels and reduce C-

peptide levels thus acting as an adjuvant for preventing diabetes onset (Chuengsamarn et al. 2012, Na et al. 2014).

Also, oral administrated curcumin reduced oxidative stress in patients with obesity (Sahebkar et al. 2013).

Cancer and Curcumin

Clinical evidence supports the chemotherapeutic activity of curcumin in colorectal, pancreatic, gastric, and colon cancer as well as in multiple myeloma. The antitumor properties of curcumin have been proven in a Phase I trial by using daily doses up to 2 grams (Jalili-Nik et al. 2018). Although the molecular mechanisms for suppressing tumor growth are not yet completely understood, it has been stated that curcumin is capable of inhibiting cyclooxygenase-2 and of inducing caspase-3-mediated apoptosis by decreasing p53 and pre-mRNA processing factor 4B expression (Goel, Boland, and Chauhan 2001, Shehzad et al. 2013). Also, curcumin has increased the production of T helper 1 cells by stimulating interferon-gamma production (Xu, Yu, and Zhao 2017). Hence, curcumin seems to be capable of regulating immune effector cells function. Additionally, it has been shown that a combinatory therapy of curcumin along with 5-fluorouracil improves the anti-cancer activity of the drug (Anitha et al. 2014).

Curcumin Applied to Neurological Diseases

Several studies have evidenced the fundamental role that turmeric and curcumin play in diverse central nervous system disorders. Despite its precise mechanism of action is not completely understood yet, it has been suggested that its pharmacological activities are mediated through its anti-inflammatory and antioxidant properties.

There is evidence supporting the positive effect of turmeric in emotional fatigue due to its anti-inflammatory properties (Kawasaki, Muroyama, and Murosaki 2018). Accordingly, fatigue symptoms seem to be associated with neuroinflammation originated by an increase of blood inflammatory cytokines and microglial activation (Nakatomi et al. 2014, Sandiego et al. 2015).

Turmeric has exhibited neuroprotective properties through an increase of synaptic plasticity among neuronal dendrites of the limbic system. Consequently, turmeric exerts a capability for preventing neuronal morphology and improving cognitive impairment (Vidal et al. 2017). In addition, turmeric was able to prevent and to alleviate memory impairment in murine models (Eun et al. 2017). These findings are in line with those reporting the protective activity of turmeric against shortages in spatial memory performance as well as in the number of hippocampal pyramidal neurons (Yuliani, Mustofa, and Partadiredja 2018). Hence, considering the neuroprotective and antioxidant properties of turmeric, it could also be useful for dementia prevention since its physiopathology involves oxidative processes mainly.

Moreover, the anti-apoptotic activity of turmeric can be exploited for the treatment of Parkinson's disease since its pathology is associated to an important loss of dopamine-producing cells in the substantia nigra (Ma and Guo 2017).

Also, anticonvulsant properties have been reported for curcumin in pre-clinical studies, including animal models of chemical and electrical stimulation of acute and chronic seizures (Dhir 2018). Noteworthy, curcumin has shown anti-epileptogenic effect in a kainate model of temporal lobe epilepsy (Kiasalari et al. 2013). Nevertheless, its poor brain bioavailability has severely limited further clinical research. Currently, nanoparticles, liposomes, polymeric

micelles, microemulsions, and phospholipid complexes are being tested for curcumin to resolve its neuronal penetration and bioavailability issues (Liu et al. 2016).

Therapeutic Limitations of Curcumin

Curcumin has reduced bioavailability due to deficient gastrointestinal absorption, poor stability, and extensive metabolism (Ratnatilaka Na Bhuket et al. 2017). Consequently, some techniques to overcome this pharmacokinetic problem has been tested to date. For instance, co-administration of curcumin, along with piperine seems to improve curcumin bioavailability and permanence in the body tissues (Baspinar et al. 2018).

Another important limitation is associated with the capacity of curcumin to stimulate p53 degradation and, therefore, increase the number of DNA-damaged cells in healthy people. Findings from preclinical studies indicate that curcumin in low doses exerts an antioxidant effect by reducing lipid peroxidation and reducing cytochrome C release whereas that at higher concentrations, it is capable of inducing hepatotoxicity due to the reduction of glutathione production and activation of caspase-3 (Ghoneim 2009).

Furthermore, it has also been reported that curcumin plays a negative role in iron metabolism by reducing the concentration of hemoglobin, serum iron, spleen, and liver iron (Jiao et al. 2009).

Since that there is also evidence supporting curcumin safety in humans, there is a current need to accurately determine the therapeutic range of curcumin in clinical trials as well as the identification of specific clinical cases in which patients may benefit from the pharmacological properties of this compound.

Pharmacological Properties of Turmeric Oil

Turmeric oil has not been as extensively studied as curcuminoids. The various pharmacological activities of turmeric had been described associated to the properties of curcumin. Thus, some pharmacological applications of turmeric oil and its main constituents are described below.

Infectious Diseases Treated with Turmeric Oil

Turmeric oil has shown antifungal activity against *Trichophyton rubrum*. Furthermore, ar-turmerone, one of the major constituents of turmeric oil, evidenced a more effective anti-dermatophytic activity than ketoconazole (Jankasem, Wuthi-udomlert, and Gritsanapan 2013).

On the other hand, considering that aflatoxins are mycotoxins with high mutagenic, teratogenic and carcinogenic potential and that they could cause serious health problems when present in food, it is noteworthy to mention that turmeric oil is able to inhibit aflatoxin production by down-regulating expression of mycotoxin genes thus interfering with mycelial growth and spore germination (Hu et al. 2017).

Application of Turmeric Oil in Inflammatory Diseases

Turmeric oil has displayed anti-inflammatory and analgesic activity. Also, ar-turmerone stimulates cytokines production and peripheral blood mononuclear cells proliferation (Yue et al. 2010). In the case of inflammatory skin diseases such as psoriasis, ar-turmerone was able to alleviate imiquimod-induced inflammation in preclinical assays (Li et al. 2018). Likewise, it has been reported that ar-turmerone is capable to prevent brain damage mediated by neuroinflammation through inhibition of microglia activation and control of inflammatory

cytokines production (Chen et al. 2018). Additionally, oral administration of turmeric oil was able to reduce gastric ulcers as well as oxidative stress induced by ethanol and ethanol-induced lesions like necrosis and hemorrhage in the stomach wall of murine models (Liju, Jeena, and Kuttan 2015).

Antioxidant Activity of Turmeric Oil

Turmeric oil possesses higher radical-scavenging activity than butylated hydroxyanisole. Thus, it constitutes an important natural antioxidant of great interest in the food industry (Sasaki et al. 2002). Even more, its capability for scavenging superoxides and hydroxyl radicals as well as to control lipid peroxidation advocates turmeric oil as a potent antioxidant to be used for potential health benefits in humans (Liju, Jeena, and Kuttan 2011).

Prevention of Cardiovascular Diseases by Turmeric Oil

Turmeric oil significantly reduced hepatic cholesterol and oxidative stress. Accordingly, preventive action against liver tissue damage induced by high-fat diet has also been reported (Ling et al. 2012).

In addition, there is evidence of its capability to down-regulate plasma levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, and free fatty acids. Furthermore, turmeric oil increases levels of high-density lipoprotein cholesterol (Singh et al. 2013).

Regarding anti-ischemic activity, turmeric oil shown protection against collagen-epinephrine induced thromboembolism in murine models (Prakash et al. 2011). In line with this finding, ar-turmerone in comparison with aspirin displayed more potent activity against platelet aggregation induced by collagen and arachidonic acid (Lee 2006).

Moreover, in a rat embolic stroke model, turmeric oil was able to down-regulate the expression of iNOS, cytochrome C and Bax/Bcl-2. As a result, an increased survival rate of neurons was reported (Dohare et al. 2008).

Turmeric Oil and Metabolic Diseases

Preclinical findings support the capability of turmeric oil to effectively control hyperglycemia and hyperinsulinemia by modulating the expression of genes involved in lipid and glucose metabolism. Noteworthy, through this mechanism of action, turmeric oil could also prevent thrombotic complications related to insulin resistance (Singh et al. 2015).

The Role of Turmeric Oil in Cancer

Cytochrome p450 isoforms (CYP1A1, CYP1A2, CYP2B1/2, CYP2A, CYP2B, and CYP3A) are involved in activation of carcinogens, and it has been reported that turmeric oil is capable of inhibiting their expression (Liju, Jeena, and Kuttan 2014).

Likewise, turmeric oil was able to inhibit hepatoma cell growth and to stimulate Hepa1-6 cell death. Hence, it has been suggested that turmeric oil treatment could act in preventing hepatocellular carcinoma development (Li et al. 2014).

Treatment of Neurological Diseases and Turmeric Oil

An association between the neuroprotective activity of turmeric oil and its antioxidant properties has been suggested. Thus, turmeric oil shown capability of inhibiting nitrosative and oxidative stress as well as delaying neuronal death via a caspase-dependent pathway (Jain et al. 2007, Dohare et al. 2008, Rathore et al. 2008).

In the amyloid beta-induced Alzheimer's model, turmeric oil displayed a more potent activity to enhance cognition when compared with curcumin and donepezil in preclinical assays. It protects rats from spatial working and learning memory impairment (Ittiyavirah and Kuriyakose 2017).

Turmeric oil and its major sesquiterpenoids (a-turmerone, B-turmerone, and ar-turmerone) are capable to prevent dementia in murine models. Moreover, a positive pharmacokinetic behavior has been reported since that these active compounds were detected in serum and brain after oral administration which is a clear advantage if compared to curcumin bioavailability (Matsumura et al. 2016).

In addition, anxiolytic and sedative properties have been attributed to turmeric oil and its major constituent, ar-turmerone (Oyemitan et al. 2017).

TURMERIC OIL, TURMERIC BISABOLENE SESQUITERPENOIDS AND EPILEPSY

The antioxidant and anti-inflammatory properties of turmeric oil have been associated with its neuroprotective activity. Indeed, the bisabolene sesquiterpenoids, especially ar-turmerone, have been suggested to be the responsible compounds for the pharmacological actions of this essential oil.

Since that about 30% of patients with epilepsy present problems related to the currently available pharmacological therapy such as pharmacoresistance or adverse side effects, it is evident the need to look for novel, effective, and safe alternatives for the treatment of epilepsy.

Natural products represent an interesting source of new active ingredients with potential anticonvulsant capacity as the case of turmeric oil. We assessed turmeric oil and its major bisabolene sesquiterpenoids, ar-turmerone, α,β-turmerone, and α-atlantone, through a series of phyto-pharmacologic analysis in the zebrafish and mouse models.

Our findings encourage the development of future research on the subject as well as new studies focused on the pharmacological evaluation of natural products with neurological therapeutic potential (Orellana-Paucar et al. 2012, Orellana-Paucar et al. 2013).

EXPERIMENTAL SECTION

Vegetal Material

The essential oil was extracted through steam distillation from turmeric rhizomes.

Turmeric Oil and Isolation of Active Principles

Further isolation of turmeric oil through column chromatography and high-performance liquid chromatography allowed the identification of the active principles present in the oil (ar-turmerone, α,β-turmerone, and α-atlantone).

Biological Models

The zebrafish and mouse models were used to analyze the safety and the anticonvulsant properties of turmeric oil and its sesquiterpenoid bisabolenes. In the zebrafish model, the locomotor activity, acute toxicity, electrophysiological changes, and the expression of genes related to seizure activity were investigated.

On the other hand, in the mouse model, the ability to control acute convulsive seizures chemically stimulated by intravenous infusion of pentylenetetrazole (PTZ) and electrically induced (6Hz model) was evaluated. Additionally, the ability of the ar-turmerone compound to cross the blood-brain barrier and its time of permanency in brain tissue was determined.

Main Findings

The Zebrafish Model

Turmeric oil showed the ability to decrease the locomotor activity of zebrafish larvae in non-toxic concentrations. Similarly, ar-turmerone was able to reduce locomotor activity in this model (Figure 2).

To identify the factor associated with the decrease in locomotor activity, these analyses were accompanied by an electrophysiological evaluation of zebrafish larvae, demonstrating the capacity of turmeric oil to reduce the number and average duration of epileptic discharges induced chemically by PTZ (Figure 3).

About the expression of genes related to convulsive activity, c-Fos is a marker of neuronal activity that is expressed when neurons are subject to action potentials. Ar-turmerone decreased the expression of c-fos genes in zebrafish larvae (Figure 4).

Mouse Biological Model

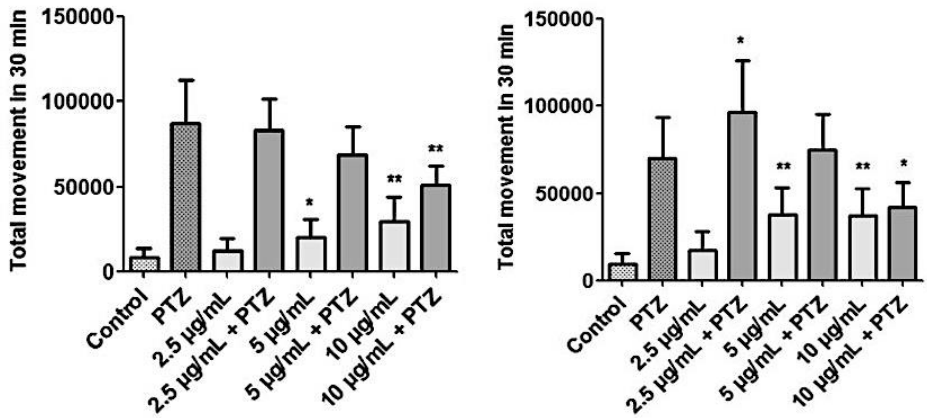

Figure 2. Evaluation of locomotor activity in zebrafish larvae. Left: turmeric oil, right: ar-turmerone. The x-axis represents the tested concentration and the y-axis, the total locomotor activity exhibited by the larvae of zebrafish in 30 minutes. The data are expressed as the mean ± standard deviation (n = 10-12). Statistically significant differences between the control group (light gray) and the group exposed to PTZ (dark gray) are represented with * for $p < 0.05$ and ** for $p < 0.01$. (Orellana-Paucar et al. 2012).

After the chemical induction of seizures by intravenous infusion of PTZ in the mouse, ar-turmerone showed a significant anticonvulsive capacity (Figure 5). Additionally, in this model, psychomotor seizures were induced through corneal electrical impulses (6-Hz) to evaluate the anticonvulsive capacity of ar-turmerone. 100% of mice given ar-turmerone intraperitoneally at concentrations between 0.1 and 50 mg/kg had complete protection against the generation of this type of seizures (Figure 6).

Regarding the determination of ar-turmerone in brain tissue, this evaluation showed its ability to cross the blood-brain barrier after intraperitoneal administration as well as its permanence in brain tissue after 15 minutes and up to 24 hours after administration (Figure 8).

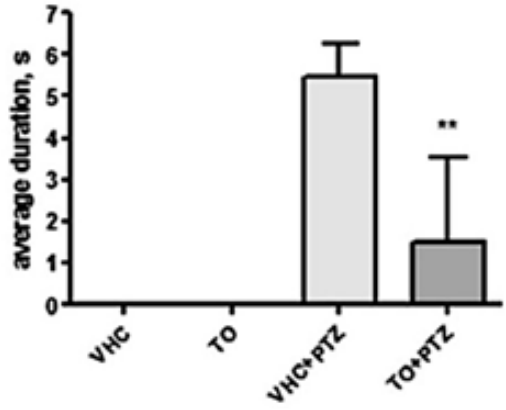

Figure 3. Electrophysiological evaluation of the anticonvulsant activity of turmeric oil in the zebrafish model. The average duration of ictal type discharges is depicted. Statistically significant differences between the control group (light gray) and the group exposed to PTZ (dark gray) are represented with ** for $p < 0.01$. (Orellana-Paucar et al. 2012).

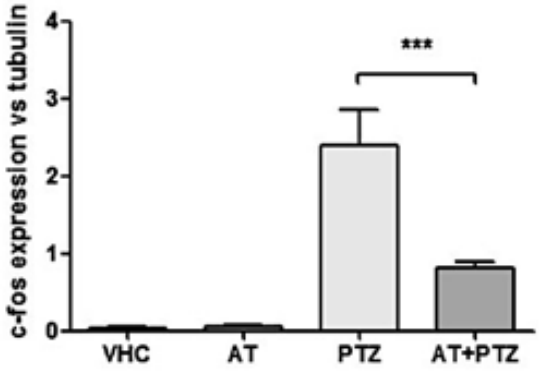

Figure 4. Evaluation of the effect of ar-turmerone on c-fos gene expression. Ar-turmerone (AT) controlled the expression of c-fos gene induced by PTZ. The statistically significant differences between the control groups (VHC), larvae incubated with vehicle and exposed to PTZ (PTZ), larvae treated with ar-turmerone (AT) and larvae treated with ar-turmerone and exposed to PTZ (AT + PTZ) are marked as **** for $p < 0.001$ (one-way ANOVA test). (Orellana-Paucar et al. 2013).

Thus, based on our findings, the combination of traditional remedies in conjunction with recent advances in analytical chemistry for the analysis of plant composition and the use of high-throughput animal models appears as an interesting approach that will likely lead to a significant breakthrough for the discovery and development of new AEDs.

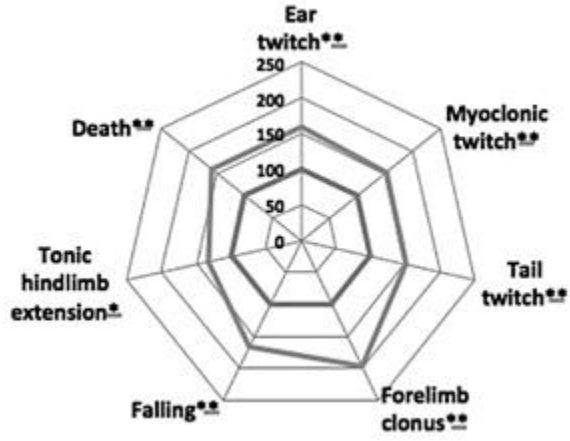

Figure 5. Anticonvulsant activity evaluation of ar-turmerone in the chemically induced seizure model in mice. Graphic representation of the results obtained with ar-turmerone at a dose of 50 mg/kg. Results are expressed as relative values in comparison with the control group (established as 100%). Statistically significant differences between the treated group (second line from the center of the graph) and the control group (first line from the center of the graph) are represented with * for $p < 0.05$ and ** for $p < 0.01$ (Student t-test, unpaired). (Orellana-Paucar et al. 2012).

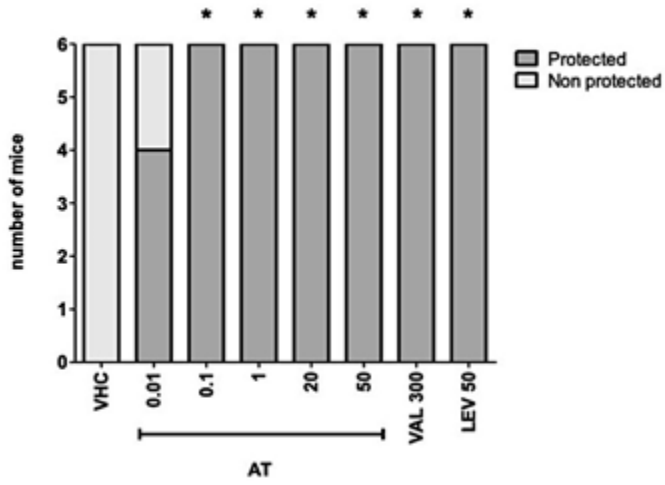

Figure 6. Evaluation of the anticonvulsant activity of ar-turmerone (AT) in the 6 Hz model. Complete protection was observed for ar-turmerone in a dose range of 0.1 to 50 mg/kg following 30 min of i.p. administration. The control group (VHC) was included as a negative control and mice treated with sodium valproate 300 mg/kg (VAL300) and levetiracetam 50 mg/kg (LEV50) as positive controls. Group of mice treated with ar-turmerone (AT) is also depicted. Statistically significant differences between control (VHC) and treated groups are represented with * for $p < 0.05$ (Fisher's exact test). (Orellana-Paucar et al. 2013).

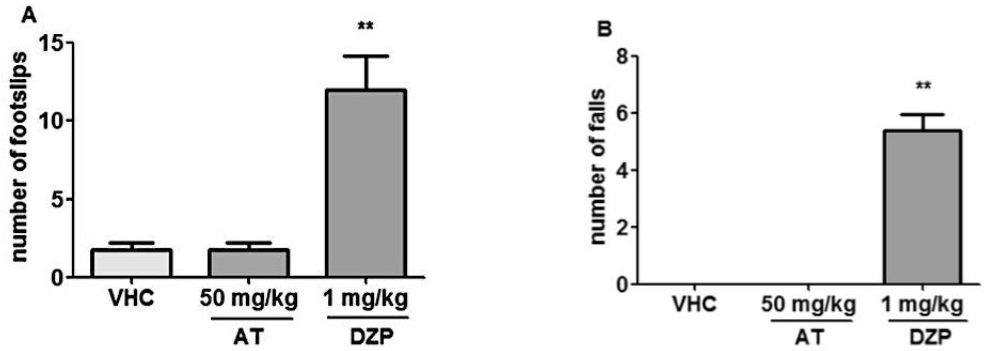

Figure 7. Evaluation of the effect of ar-turmerone on motor function (equilibrium test). An intravenous dose of 50 mg/kg of ar-turmerone (AT) did not generate any alteration of motor skills in mice. The sensitivity of this model was confirmed by the detection of motor and balance deficits induced by diazepam (DPZ) in mice at a dose of 1 mg/kg. Compared to the control group (VHC), the mice treated with DZP showed a significant increase in the number of landslides (left) and fell (right). Statistically significant differences between the control and the treated groups are represented by ** for $p < 0.001$ (one-way ANOVA test). (Orellana-Paucar et al. 2013).

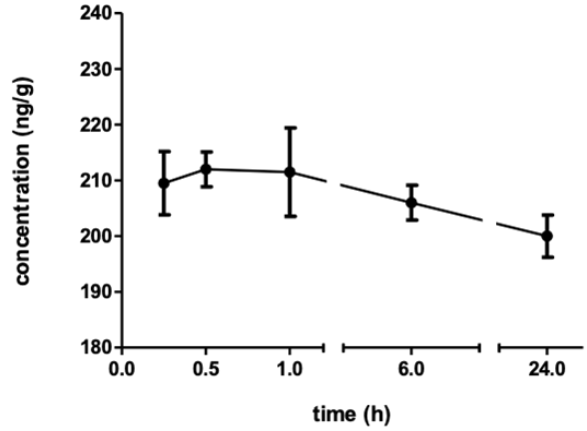

Figure 8. Concentration-time profile of ar-turmerone in mouse brain extract. Chromatographic analysis showed evidence of the presence of ar-turmerone at 15 minutes and up to 24 hours after intraperitoneal administration of 50 mg/kg.

Turmeric Oil, Ar-Turmerone and Metabolic Epilepsy

Neurological symptoms such as mental deterioration, movement disorders, developmental delay, and seizures are common in a wide variety of inherited errors of metabolism (IEM). In the specific case of epilepsy-associated to IEM, main clinical manifestations involve seizures at a very early age, often of refractory type, with the consequent cognitive and sensorial worsening and motor function relapse (Papetti et al. 2013). In metabolic epilepsy, seizures could be generated by metabolism alteration due to deficiency of specific substrates, abnormal

intracellular storage of toxic metabolites or alteration of intracellular osmolality (Messing and Simon 1986).

According to the International League Against Epilepsy (ILAE), eight types of metabolic epilepsies have been identified: biotinidase and holocarboxylase synthase deficiency, cerebral folate deficiency, creatine disorders, folinic acid-responsive seizures, glucose transporter type 1 (GLUT-1) deficiency, mitochondrial disorders, peroxisomal disorders and pyridoxine-dependent epilepsy (PDE) (Berg et al. 2010).

For mitochondrial disorders treatment, it has been suggested the use of antioxidants as adjuvants in the pharmacological treatment with AEDs. As has been described, the main activity of the antioxidants is to reduce excessive oxidative stress generated by an over-production of free radicals (Finsterer and Mahjoub 2013). Thus, considered the free radical scavenger activity described for turmeric oil and its major bisabolene sesquiterpenoid, ar-turmerone, both emerge as interesting potential candidates to be further investigated for metabolic epilepsy treatment. In addition, since that metabolic epilepsy frequently presents the treatment limitation of pharmacoresistance, it is noteworthy the anticonvulsant activity reported for ar-turmerone in tests of pharmacoresistant epilepsy such as the 6-Hz murine model (Orellana-Paucar et al. 2013).

Further evaluation of the potential application of ar-turmerone as a treatment for metabolic epilepsy is needed to prevent possible neurodevelopmental disabilities in newborns that could severely affect their future quality of life.

CONCLUSION

Epilepsy is a common neurological disorder affecting around 70 million people worldwide. Main limitations regarding its pharmacological treatment are associated with the presence of adverse reactions and pharmacoresistance distressing approximately 30% of patients with epilepsy. Hence, there is still an urgent need to identify novel AEDs with improved efficacy and safety. Nature is an interesting source for searching these novel compounds.

Anticonvulsant properties have been described for a wide diversity of medicinal plants around the world. Such is the case of turmeric (*Curcuma longa* L.) whose pharmacological activity for controlling induced seizures has been mainly attributed to curcumin, one of its main constituents. Nevertheless, important problems regarding its bioavailability have limited the scope of further clinical research outcomes. On the other hand, research has also been focused on turmeric oil, although not as extensively as for curcumin. Neuroprotective properties have been described for turmeric oil.

In this context, our research was focused on the evaluation of the anticonvulsant activity of turmeric oil and its major bisabolene sesquiterpenoids. The obtained results from the zebrafish model showed the ability of turmeric oil and ar-turmerone to decrease locomotor activity in larvae. This observation was interpreted in association with the findings of the electrophysiological evaluation and the transcriptional analysis of *c-fos* expression. Thus, these findings altogether support the identification of the anticonvulsant activity and the safety of turmeric oil and ar-turmerone in this model.

Also, the anticonvulsant activity of ar-turmerone was confirmed through chemically induced seizure model (i.v. PTZ test) and electrically stimulated seizure model (6-Hz test) in

mice. About toxicity, the equilibrium test showed that ar-turmerone does not affect the balance of treated mice. Moreover, the determination of ar-turmerone in brain tissue allowed identifying its prolonged brain permanence time from 15 minutes after intraperitoneal administration up to 24 hours. This result added to the identified anticonvulsant capacity (even at concentrations as low as 0.1 mg/kg), would suggest the possibility that in future clinical studies, the administration of minimum daily doses would be necessary to achieve the desired therapeutic effect. Additionally, the use of minimum doses could be associated with a decrease in the probability of presenting adverse side effects.

Hence, ar-turmerone emerges as a promising candidate for the treatment of epilepsy. Of special interest, further research on a potential application of ar-turmerone as a candidate for metabolic epilepsy treatment needs to be developed. This possible application is mainly based on the potent antioxidant properties of ar-turmerone and its relationship with the physiopathology of mitochondrial epilepsy.

Our studies reported for the first time to the scientific community, the anticonvulsant pharmacological activity of turmeric oil and its sesquiterpenoid bisabolenes. This finding allowed the application of patent certification in the European Community, the United States, and, Japan, of which all three patents have been granted to date, European Community (European Patent No. 2729138), United States (United States Patent No 9,782,361) and Japan (Japanese Patent No. 6090867).

Consequently, the results achieved through our studies not only promote the development of future research in the area but also support the execution of new research focused on the bio-discovery of natural products with potential application in the treatment of epileptic syndromes including metabolic epilepsy.

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Chapter 18

CAPSICUM SPP AS A MEDICINAL PLANT FOR THE TREATMENT OF METABOLIC DISORDERS

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ABSTRACT

Capsicum spp. have been consumed regularly all over the world not only for their nutritive properties but also for its physiological and pharmaceutical uses. *Capsicum* spp. is a good source of different phytochemicals including capsaicinoids, capsinoids, flavonoids, phenolic compounds, carotenoids, among others. The present chapter elucidates the mechanism of the different phytochemicals of *Capsicum* spp. face to obesity, cancer, diabetes, hypertension, hypercholesterolemia, and hypertriglyceridemia. *In vitro* and *in vivo* studies showed that various phytochemicals of *Capsicum* spp., especially capsaicin have anti-obesity activity, anti-diabetic activity, anti-hyperlipidemic activity, anti-hypertensive activity, and anti-carcinogenic activity. Therefore, *Capsicum* sp., is a promising medicinal plant to develop natural drugs against metabolic disorders.

Keywords: *Capsicum* spp., metabolic disorders, phytochemicals and capsaicin

INTRODUCTION

From immemorial time, human beings have used plants for curative treatment of diseases. Some of these plants have been grouped as spices as garlic (*Allium sativum*), onion (*Allium cepa*), pepper (*Piper nigrum*), curcuma (*Curcuma longa*), etc. Among the spices, the genus

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Capsicum has been consumed regularly all over the world, not only for their nutritive properties, pungency, aroma, flavor, taste, and color but also for its physiological and pharmaceutical uses.

Archeological artifacts evidenced that *Capsicum* gender peppers have been used since 8600–5600 B.C. by primitive communities in America (Sousa et al. 2006). Nowadays, about 50 *Capsicum* species are distributed throughout the world in subtropical Europe, Southern America, tropical Africa, India, East Africa, and China, ranging from the hottest to sweetest varieties (Saikat Kumar and Amit Krishna 2003). Pepper is an annual herbaceous plant of the *Solenaceae* family (Saikat Kumar and Amit Krishna 2003), the same family of eggplant, potatoes, tomatoes, and tobacco. Table 1 shows the taxonomy of the genus *Capsicum*.

Table 1. Taxonomy of the genus *Capsicum*

Kingdom	***Plantae***
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Solanales*
Family	*Solanaceae*
Genus	*Capsicum*
Species	*C. annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens*

In general, depending on their pungency, *Capsicum* spp. are commonly divided by consumers into two main groups: pungent and non-pungent fruits. The pungency depends on capsaicinoid content, which in turn depends on the variety and maturation stage (Melgar-Lalanne et al. 2017). However, botanically speaking, there are five domesticated species: *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens* (Ornelas-Paz et al. 2010, Peña-Alvarez, Ramírez-Maya, and Alvarado-Suárez 2009). *C. annuum* spp. are the most grown common species, which contains the largest group of pepper varieties (Paran and van der Knaap 2007). Table 2 shows pepper examples of the five domesticated *Capsicum* species.

Table 2. Examples of most consumed Capsicum species

Genus and species	**Examples of pepper type**
C. annuum	Serrano, Jalapeño, Poblano, Guajillo, Caribe, Chilaca, Bell pepper, Cayenne pepper, Cascabel, Piquín among others
C. baccatum	Ají varieties
C. chinense	Habanero and Scotch Bonnet varieties
C. frutescens	Tabasco
C. pubescens	Manzano and Rocoto varieties

PHYTOCHEMICALS IN *CAPSICUM* SPECIES

Capsicum spp. are a good source of different phytochemicals including: capsaicinoids, flavonoids, phenolic compounds, carotenoids, tannins, saponins, cyanogenic glycosides, hydroxycinnamates, vitamins A, C and E, terpenoids, among others (Serrano et al. 2010, Suna

et al. 2006, Wahua, Okoli, and Sam 2013). The concentration of these compounds depends on the cultivar, maturity stage, growing conditions, and postharvest manipulation. *Capsicum* spp. fruits are considered as non-climacteric fruits, then its maturity stage impact directly in the proximate chemical composition, color, and phytochemical content (Melgar-Lalanne et al. 2017). Furthermore, recent researches have shown that regular consumption of *Capsicum* spp. is effective in reducing oxidant stress, rheumatism, arthritis, neuralgia, lumbago, obesity, cancer, diabetes, among other diseases (Kim et al. 2008, Malagarie-Cazenave et al. 2009, Meghvansi et al. 2010). Moreover, *Capsicum* species have also been used as topical analgesic, antiseptic, carminative and counter-irritant (Yaldiz, Ozguven, and Sekeroglu 2010).

Capsaicinoids and Capsinoids

The pungent properties of peppers are attributed to capsaicinoids. The capsaicinoids are derivate from the secondary metabolism of the alkaloid groups, which is formed by acid amides of vanillyl-amide of branched-chain of fatty acids of 8–13 carbons from phenylalanine and valine (Kobata et al. 1999, Peña-Alvarez, Ramírez-Maya, and Alvarado-Suárez 2009). In peppers, fifteen capsaicinoids have been identified and characterized, being capsaicin, dihydrocapsaicin and nordihydrocapsaicin the predominant compounds of fresh peppers extracts (Ornelas-Paz et al. 2010). Among them, capsaicin - (E)-N-[(4-hydroxy-3-methoxy-phenyl) methyl]-8-methylnon-6-enamide) and dihydrocapsaicin - (N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methylnonanamide) – are the two main responsible capsaicinoids of pepper spiciness, about 90% (Perucka and Oleszek 2000). The remaining analogs differ in the length of the hydrocarbonaceous chain, the degree of unsaturation, and the position of the double bond and/or the position of the methyl group (Schweiggert, Carle, and Schieber 2006). Some examples of minor capsaicinoids are Nornorcapsaicin, capsaicin I or II, homocapsaicin I, and II, homodihydrocapsaicin isomers I and II and nornordihydrocapsaicin (Schweiggert, Carle, and Schieber 2006).

Capsaicinoids are found mostly in the placental tissues of Capsicum fruits, where they are biosynthesized by capsaicin synthase through the condensation of vanillylamine, a phenyl propanoid pathway intermediate, and fatty acid moieties (Bennett and Kirby 1968, Iwai et al. 1978). In mild pungent peppers varieties, the concentration of capsaicinoids ranges from 0.5 to 0.3%, and in strong, pungent pepper, the content is higher than 0.3%, reaching about 1% (Perucka and Oleszek 2000). The profile of capsaicinoids in peppers depends on many factors, including ripening stage, geographical origin, type, and cultivar (Ornelas-Paz et al. 2010).

Capsaicinoids produce a burning sensation by activating the central nervous system of noxious stimuli; this property has been used to ease the pain associated with neuropathy, cluster headaches, arthritis, muscle strain, postherpetic neuralgia, and postmastectomy pain syndrome. Moreover, they also exert antioxidant, anti-inflammatory, antidiabetic, antitumor, anti-teratogenic, anti-lithogenic, hypolipidemic and hypocholesterolemic activities (Dömötör, Szolcsányi, and Mózsik 2006, Srinivasan 2005).

On the other hand, capsinoids are capsaicinoid-like substances with non-pungency at an oral tasting. These compounds have been found in non-pungent *Capsicum* spp., and they differ to capsaicinoids because of their difference between their aromatic portions, vanillyl alcohol for capsinoids vs vanillylamine for capsaicinoids (Kobata et al. 1999). The most representative molecules of this group are capsiate (4-hydroxy-3-methoxyphenyl) methyl(E)-8-methylnon-6-

enoate), dihydrocapsiate (4-hydroxy-3-methoxyphenyl)methyl 8-methylnonanoate), and nordihydrocapsiate (4-hydroxy-3-methoxyphenyl)methyl 7-methyloctanoate) (Melgar-Lalanne et al. 2017). Capsiate and dihydrocapsiate are nonpungent ester analogs of capsaicin and dihydrocapsaicin (Kobata et al. 1999). Moreover, it has been reported that these analogs of capsaicin share some of the biological activities of capsaicinoids (Rosa et al. 2002). Figure 1 exposed the chemical structures of capsaicin and capsiate.

Phenolics Compounds

Phenolic compounds are the largest category of phytochemicals and the most widely distributed in the plant kingdom. Plant phenolics include simple phenols, phenolic acids, flavonoids, anthocyanins, stilbenes, tannins, lignans, and lignins (Antonious et al. 2006). The term phenolics involves approximately 8000 naturally occurring compounds, all of which possess one common structural feature, a phenol (an aromatic ring bearing at least one hydroxyl substituent) (Robbins 2003). Based on the number of phenol subunits present, phenolics have been divided into polyphenols and simple phenols (Robbins 2003). Flavonoids structure consists of two aromatic rings linked by three carbons that usually form an oxygenated heterocycle. In plants, they are typically found as glycosides contributing to the color of leaves, flowers, and fruits (blue, scarlet, orange) and as aglycones (Garcia-Salas et al. 2010).

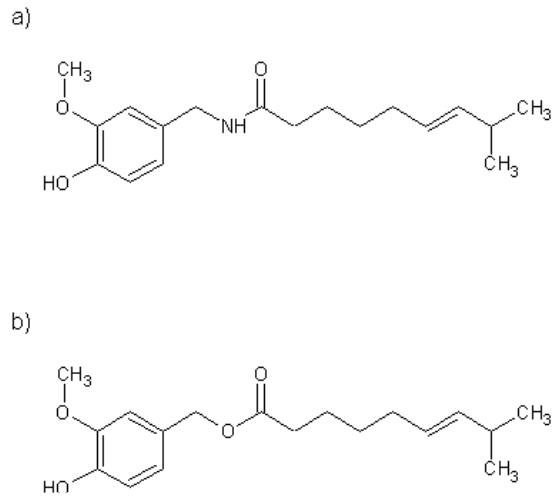

Figure 1. Structure of a) capsaicin and b) capsiate.

In fruits and vegetables, the most abundant phenolic compounds are phenolic acids and flavonoids (Garcia-Salas et al. 2010). In *Capsicum* spp. several studies have been carried out to elucidate their main phenolic compounds. For example, the study of Blanco-Ríos et al. (2013) identified in different cultivars of sweet peppers (*Capsicum annum*), two hydroxycinnamic acids: caffeic acid and chlorogenic acid, and three flavonoids: myricetin, quercetin, and luteolin. Moreover, Marín et al. (2004) also identified five hydroxycinnamic derivatives and twenty-three flavonoids from the pericarp of sweet pepper (*Capsicum annun*). Jeong et al. (2011) also identified by HPLC MS/MS analysis, twenty-eight phenolic compounds

in bell peppers (*Capsicum annum*). Four quercetin, fourteen luteolin, two apigenin, three cinnamic acid derivatives, and five flavonoid components. Table 3 lists some of the most common phenolic compounds found by Marín et al. (2004) and Jeong et al. (2011) in sweet and bell pepper fruits.

Table 3. Identified phenolic compounds in sweet and bell pepper fruits

Polyphenols in sweet pepper fruits (*C. annum* L.)	
Hydroxycinnamic derivatives	
(1) Caffeic acid derivative	
(2) transp-coumaroyl-β-D-glucopyranoside	
(3) caffeic acid derivative	
(4) transferuloyl- β -D-glucopyranoside	
(5) trans-synapoyl- β -D-glucopyranoside	
Flavonoids	
(1) luteolin 6,8-di-C-hexoside	(13) luteolin 6-C-hexoside-8-Crhamnoside
(2) apigenin 6,8-di-C-hexoside	(14) luteolin 6-C-hexoside
(3) quercetin 3-O-rhamnoside-7-O-glucoside	(15) luteolin 6-C-(6-malonyl)-hexoside-8-C-pentoside
(4) luteolin 6-C-hexoside-8-C-pentoside	(16) apigenin 6-C-hexoside-8-C-pentoside
(5) luteolin 6-C-hexoside-8-C-pentoside	(17) luteolin 6-C-rhamnoside-8-C-hexoside
(6) luteolin 6-C-pentoside-8-Chexoside	(18) luteolin 7-O-(2-apiosyl)glucoside
(7) chrysoeriol 6,8-di-C-hexoside	(19) luteolin 7-O-(2-apiosyl-6-acetyl)glucoside
(8) apigenin 6-C-pentoside8-C-hexoside	(20) quercetin 3-Orhamnoside
(9) luteolin 6-C-(6-malonyl)hexoside-8-C-hexoside	(21) luteolin 7-O-(2-apiosyl-6-malonyl)glucoside
(10) luteolin 6-C-pentoside-8-C-hexoside	(22) chrysoeriol 7-O-(2-apiosyl-6-acetyl)glucoside
(11) luteolin 8-C-hexoside	(23) luteolin 7-O-(2-apiosyldiacetyl)glucoside.
(12) chrysoeriol 6-C-hexoside-8-C-pentoside	
Polyphenols in bell pepper fruits (*C. annum* L.)	
1. Feruloyl hexoside	15. Quercetin O-rhamnosyl-O-hexoside
2. Luteolin 8-C-hexoside	16. Quercetin 3-O-hexoside
3. Sinapoyl hexoside	17. Luteolin C-pentosyl-C-hexoside
4. Luteolin 6-C-hexoside	18. Quercetin O-rhamnosyl-O-hexoside
5. Luteolin 6,8-di-C-hexoside	19. Luteolin C-pentosyl-C-hexoside
6. Orientin	20. Luteolin O-(apiosylacetyl)glucoside
7. Feruloyl hexoside	21. Vicenin-2
8. Apigenin C-pentosyl-C-hexoside	22. Luteolin O-malonylpentosyldihexoside
9. Luteolin 6,8-di-C-hexoside	23. Apigenin C-pentosyl-C-hexoside
10. Kaempferol pentosyldihexoside	24. Quercetin-3-O-rhamnoside
11. Quercetin O-rhamnosyl-O-hexoside	25. Luteolin 8-C-hexoside
12. Luteolin O-(apiosyl)hexoside	26. Luteolin 8-C-hexoside
13. Luteolin C-pentosyl-C-hexoside	27. Luteolin C-pentosyl-C-hexoside
14. Isoscoparin	28. Luteolin O-(apiosylmalonyl)glucoside

In all the cases, phenolics compounds varied in function of different factors, being the most important the degree of ripeness, the varieties, the climate, the soil composition, and the geographic location (Haminiuk et al. 2012).

On the other hand, the chemical structure and stereochemistry of phenolic compounds determine their chemical behavior such as metal chelation, ease of oxidation, and free radical scavenging ability (Tucker and Robards 2008). Then, each phenolic compound demonstrates its protective roles against metabolic disorders like hypertension, hypercholesterolemia and some forms of cancer.

Vitamin C

L-ascorbic acid, also known as vitamin C, is commonly found in fruits and vegetables. Within the plant kingdom, fresh pepper is one of the vegetables with the highest content of vitamin C (Serrano et al. 2010). According to some studies, vitamin C of *Capsicum* spp. ranged from 2.54 to 50.44 mg/g on a dry weight basis (Perla et al. 2016). Vitamin C content of yellow and red tomato fruit has been reported between 9 to 44.8 mg/100 g in fresh weight (Georgé et al. 2011).

Moreover, unripe *Capsicum* spp. fruits accumulated more vitamin C than ripened fruits (Perla et al. 2016). Vitamin C, best known for its role in protection against scurvy, has also been used to prevent allergies, reduce the levels of circulating proinflammatory cytokines, modulates gene expression and cell cycle progression, and to prevent some forms of cancer, neurological and cardiovascular diseases (Davey et al. 2000).

Carotenoids

Carotenoids are the pigments responsible for the yellow, orange and red color of many peppers (Ornelas-Paz et al. 2013). They are lipid-soluble compounds comprised of eight isoprenoid units whose order is inverted at the molecule center (Delgado-Vargas, Jiménez, and Paredes-López 2000). They are classified by their chemical structure in two groups: (1) carotenes that are constituted by carbon and hydrogen and (2) oxycarotenoids or xanthophylls that have carbon, hydrogen, and, additionally, oxygen (Delgado-Vargas, Jiménez, and Paredes-López 2000).

In *Capsicum* ssp. fruits, carotenoids are stored in the chromoplasts (Guzman et al. 2010); being identified more than 50 carotenoid structures. β-carotene, α-carotene, zeaxanthin, violaxanthin, β-cryptoxantin, α-cryptoxanthin, antheraxanthin, and lutein are the most responsible carotenoids of yellow and orange color; and capsainthin, capsorubin, and cryptocapsin are the most responsible carotenoids of red color (Hornero-Méndez and Mínguez-Mosquera 2001, Matsufuji et al. 2007). According to literature, the total carotenoids content of *Capsicum* ssp. mainly varied in function of the fruit maturity, and the cultivars (Topuz and Ozdemir 2007). During the ripening process, the content of total carotenoids increases, although many of hydroxylated carotenoids are progressively esterified with fatty acids (Matsufuji et al. 2007). In most of the studies, β-carotene and capsanthin have been identified as the most abundant carotenoids in ripe *Capsicum* ssp. fruits, representing more than 50% of the total carotenoids content (Ornelas-Paz et al. 2013, Topuz and Ozdemir 2007). β-Carotene is a precursor of vitamin A and exerts protective effects against cardiovascular diseases and some forms of cancer (Ornelas-Paz et al. 2013). And capsanthin, uniquely found in *Capsicum* spp., has shown antioxidative and anti-tumor-promotion activities; moreover, in some

epidemiological studies, capsanthin also exerted a potent inhibitory effect on colon carcinogenesis (Kim, Ha, and Hwang 2009).

Carotenoids bearing a k-ring as end group have been shown to have strong reactive oxygen scavenging potential, and this antioxidant effect, have shown health benefits face to many diseases, such as cancer and strokes (Delgado-Vargas, Jiménez, and Paredes-López 2000, Melgar-Lalanne et al. 2017). Therefore, increased intake of peppers carotenoids may be helpful for the improvement of health.

CAPSICUM SPP. AS A MEDICINA PLANT FOR THE TREATMENT OF METABOLIC DISORDERS

Metabolic diseases such as obesity, insulin resistance, type 2 diabetes, and atherosclerosis are on the rise globally. In the United States, about 98 million people have elevated cholesterol levels, 58 million have weight problems, and 16 million have diabetes (Capasso et al. 2003). Therefore, to prevent and treat these diseases, a wide variety of medicinal plants has been studied to elucidate its therapeutic action. Mainly, in this section, scientific evidence is provided about the beneficial effects of *Capsicum* spp. in health. Figure 2 summarized the main protective results of *Capsicum* spp. against metabolic disorders.

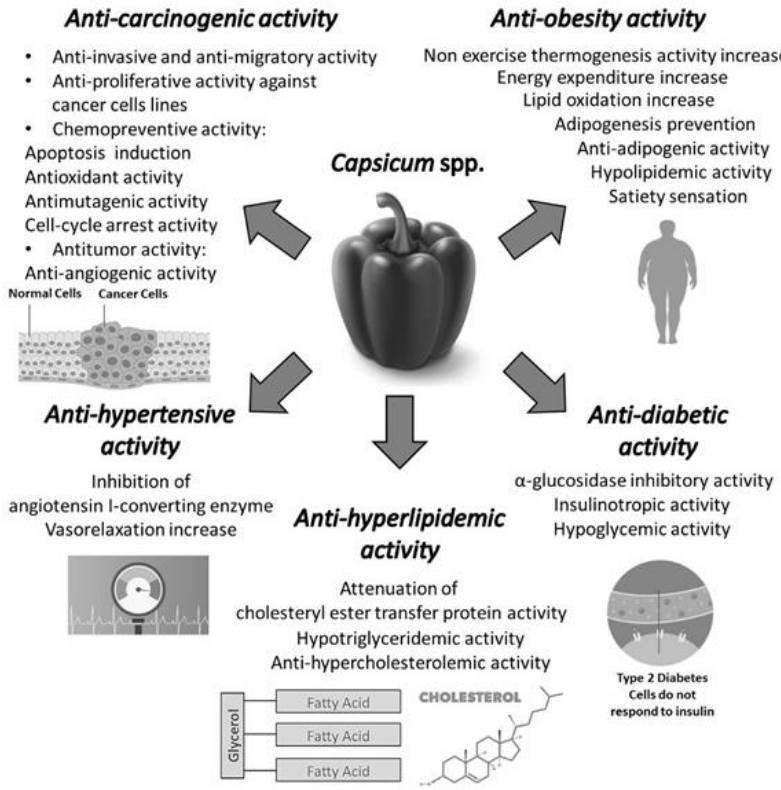

Figure 2. Schematic representation of the beneficial effects of *Capsicum* spp. against metabolic disorders.

Obesity

According to the World Health Organization (WHO), overweight and obesity are the fifth foremost causes of deaths globally. Most of the world's population live in countries where overweight and obesity kills more people than underweight (WHO 2018). The conventional therapy of obesity mainly involves synthetic drugs and surgical procedures, which unfortunately has many harmful side effects and chances of recurrence with severity (Karri et al. 2019). For that reason, in the last years, a lot of studies have been carried out to found natural anti-obesity agents. Among hundreds of medicinal plants, *Capsicum* species have been identified as a promising alternative to improve weight loss and weight maintenance, especially by their capsaicin content.

The study of Kang et al. (2010) showed that dietary capsaicin reduced obesity-induced insulin resistance and hepatic steatosis in obese mice fed with a high-fat diet. For 20 weeks, male C57BL/6 obese mice were fed with a high-fat diet. In the first ten weeks, they only received food, and the second 10 weeks, they were administrated by a supplement of 0.015% capsaicin. Controls were unsupplemented mice. Their results showed that dietary capsaicin lowered fasting glucose, leptin levels, insulin, and reduced the impairment of glucose tolerance in obese mice. Moreover, the levels of tumor necrosis factor-α (TNFα), monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6 mRNAs and proteins in adipose tissue and liver decreased markedly, as did macrophage infiltration, hepatic triglycerides, and TRPV-1 expression in adipose tissue. On the other hand, the mRNA/protein of adiponectin in the adipose tissue and PPARα/PGC-1α mRNA in the liver increased. Additionally, luciferase assays revealed that capsaicin was capable of binding PPARα. This study proposes that dietary capsaicin may reduce obesity-induced glucose intolerance by not only suppressing inflammatory responses but also enhancing fatty acid oxidation in adipose tissue and/or liver, both of which are important peripheral tissues affecting insulin resistance. The effects of capsaicin in adipose tissue and liver were related to its dual action on PPARα and TRPV-1 expression/activation.

Ma et al. (2010) also identified that activation of the TRPV1 channel by dietary capsaicin prevents adipogenesis and obesity in mice. Further, they reported that capsaicin significantly increased the uncoupling proteins (UCP2 and UCP3) expression in mature adipocytes, which indicated that capsaicin promoted fat oxidation. UCP2 and UCP3 play an important role in fat oxidation, and they are also associated with blood pressure regulation. In their study, four months mice on a high-fat diet with added capsaicin markedly prevented the development of obesity and increased UCP2 and UCP3 expression in visceral fat from mice. Moreover, chronic administration of capsaicin also dose-dependently increased the acetylcholine-mediated hypotensive responses in wild-type mice.

Yoshioka et al. (1995); Yoshioka et al. (1999), and Yoshioka et al. (1998) investigated the effects of dietary red pepper on energy metabolism in Caucasian men and Japanese women. In the case of men, their results suggested that the consumption of red pepper (10 g), outcomes in an increase in the energy expenditure, only immediately after the meal ingestion; and, it increases the carbohydrate oxidation without increasing the total energy expenditure for 150 min after the meal. Respect to women, the addition of red pepper (10 g) to a high-fat (HF) and a high-carbohydrate (HC) meals significantly increased diet-induced thermogenesis and lipid oxidation, particularly after the HF meal.

On the other hand, carbohydrate oxidation was significantly decreased by the addition of red pepper to the experimental meals.

Moreover, by adding red pepper (10 g) to an HF and HC breakfast, women decreased their desire to eat and hunger before lunch, and they reduced protein and fat intakes at lunchtime. In the case of men, after ingesting a standardized breakfast, the subjects were given an experimental appetizer at lunch-time: (1) mixed diet and appetizer and (2) mixed diet and red-pepper (6 g), and results showed that the addition of red pepper to the appetizer significantly reduced the cumulative ad libitum energy and carbohydrate intakes during the rest of the lunch and in the snack served several hours later. Moreover, the power spectral analysis of heart rate revealed that this effect of red pepper was associated with an increase in the ratio sympathetic: parasympathetic nervous system activity.

The study of Smeets and Westerterp-Plantenga (2009) also states the anti-obesity effects of a lunch containing capsaicin. Thirty subjects were administrated by two lunch conditions: a) lunch without capsaicin and b) lunch with capsaicin (1,030 mg of red pepper). The capsaicin effects were evaluated on gut-derived hormones (GLP-1, ghrelin, and PYY), energy expenditure, substrate oxidation, and satiety at lunch in the postprandial state. Results showed that capsaicin regulated apatite by acting on ghrelin and glucagon-like peptide 1 (GLP-1). However, respect to satiety, energy expenditure, and peptide YY no difference was found between two lunches.

On the other hand, the study of Josse et al. (2010) determined how ingestion of capsinoids affected energy expenditure, lipid oxidation, and blood metabolites at rest and during moderate-intensity exercise. Twelve healthy young men were administrated with capsules either with 10 mg of purified capsinoids or a placebo, and they were studied on two occasions in a double-blind design at rest, after 90 min of cycling at 55% VO_2 peak, and for 30 min into recovery. Capsules were ingested 30 min before exercise. Their results showed that at rest, the ingestion of 10 mg of capsinoids increased adrenergic activity, energy expenditure, and resulted in a shift in substrate utilization toward lipid. However, under the selected study conditions, at exercise or recovery, capsaicinoids consumption had little effects on energy expenditure and lipid oxidation.

On the contrary, the study of Sahin et al. (2018) showed that capsaicinoids consumption coupled with regular exercise might enhance lipid metabolism. Capsimax, a product that contains 2% of a standardized mix of capsaicinoids: capsaicin (1.2–1.35%), dihydrocapsaicin (0.6-0.8%), and nordihydrocapsaicin (0.1-0.2%), were administered to rats. 28 male Wistar albino rats were divided into four groups: (1) No exercise and no Capsimax, (2) No exercise + Capsimax, (3) Regular exercise, and (4) Regular exercise + Capsimax. Results showed that by combining regular exercise with capsaicinoids consumption, a significant decrease in lactate and malondialdehyde (MDA) levels and an increase in activities of antioxidant enzymes were observed. Moreover, regular exercise + Capsimax treated rats had greater nuclear factor-E2-related factor-2 (Nrf2) and heme oxygenase-1 (HO-1) levels in muscle than regular exercise and no exercise rats. According to this study, the combination of regular exercise with capsaicinoids consumption may enhance lipid metabolism by regulation of gene products involved in lipid and antioxidant metabolism including SREBP-1c, PPAR-γ, and Nrf2 pathways in rats.

In addition to capsaicinoids, capsiate, and dihydrocapsiate of *Capsicum* spp. also stimulate weight loss through thermogenesis mechanism. Ohnuki et al. (2001) investigated the effect of a non-pungent cultivar of sweet red pepper (*Capsicum annum* L.) and capsiate on body

temperature in humans and mice. Their results showed that sweet pepper and capsiate increased thermogenesis and energy consumption in both study subjects. According to the researchers, this effect may be induced by the vanilloid receptors' stimulation of capsiate.

Moreover, contrary to the capsaicinoids, capsiate activates TRPV1 receptors in the gut, not in the oral cavity (Ludy, Moore, and Mattes 2011). Besides, the study of Hachiya et al. (2007) stated that in humans, sweet red pepper activates the sympathetic nervous system and enhances thermogenesis as effectively as hot red pepper, but without its gastrointestinal side effects. Furthermore, they found that the intake of sweet red pepper does not affect systolic blood pressure or heart rate, while hot red pepper transiently elevates them.

On the other hand, the study of Lee et al. (2010) investigated the effects of dihydrocapsiate on adaptive and diet-induced thermogenesis with a high protein very low-calorie diet. After four weeks to follow a very low-calorie diet (800 kcal/day providing 120 g/day protein), 30 healthy men and postmenopausal women were randomly administrated three times per day with capsules with dihydrocapsiate (3 mg or 9 mg) or placebo. After four weeks, results showed 9 mg/day of dihydrocapsiate consumption increased postprandial thermogenesis and fat oxidation.

The study of Joo et al. (2010) elucidated the molecular action of capsaicin on the antiobesity effect in epididymal white adipose tissue of rats (WAT). Five weeks old treated rats received 10 mg/kg capsaicin along with a high-fat diet (HFD). Controls were saline-treated rats. Results showed that compared to the controls, the capsaicin-treated group decreased by 8% its body weight. Moreover, proteomic analysis of WAT revealed significant alterations to different thermogenesis and lipid metabolism-related proteins in HFD fed rats treated with capsaicin: 10 proteins were significantly up-regulated, and ten proteins were remarkably down-regulated. Particular interest was focus on the significant down-regulation of heat shock protein 27 (Hsp27) and Steap3 protein, and the up-regulation of olfactory receptor (Olr1434) in obese WAT. Moreover, most of the identified proteins were associated with lipid metabolism and redox regulation. The authors demonstrated that capsaicin altered the thermogenesis and increased lipid metabolism in WAT through up-regulation of proteins responsible for both mechanisms.

Baboota, Murtaza, et al. (2014) also provided evidence about the anti-obesity mechanism of capsaicin in the high-fat diet (HFD)-fed mice. Swiss albino mice were divided into three groups: a) control, b) HFD fed, and c) HFD fed + capsaicin (2 mg/kg), and they were studied for three months. Results showed that oral administration of capsaicin: modulates hypothalamic satiety associated genotype, alters gut microbial composition, induces “browning” genotype in subcutaneous white adipose tissue and increases expression of thermogenesis and mitochondrial biogenesis genes in brown adipose tissue.

Moreover, to better understand the anti-adipogenic effect of capsaicin and the modulatory role of TRPV1 receptors in adipogenesis Baboota, Singh, et al. (2014) applied *in vitro* and *in vivo* model systems. Their study focused on investigating the effect of capsaicin on adipogenic differentiation with special reference to induction of ‘‘brite’’ phenotype during differentiation of 3T3-L1 preadipocytes. In rodents and neonatal mammals, brown adipose tissue helps to maintain body temperature by converting lipids and glucose into heat, thereby increasing energy expenditure. In adults, in addition to classical brown adipocytes, there exist “brite” (brown-in-white) adipocytes as a physiological response to chronic cold (Rosenwald et al. 2013). *In vivo* studies were carried out in high-fat diet (HFD) fed rats treated with resiniferatoxin (RTX) (a TRPV1 agonist) and in mice administered with capsaicin. And *in vivo*

studies were followed in preadipocytes and matured adipocytes. Results provided evidence that capsaicin has a dual modulatory role in adipogenesis: 1) it inhibited adipogenesis in 3T3-L1 via TRPV1 activation and 2) it induced brown-like phenotype adipocytes.

Besides, Sung, Bang, and Lee (2015) and Sung and Lee (2016) also identified that Capsicoside G suppresses adipogenesis through activation of AMP-activated protein kinase in 3T3-L1 cells. Capsicoside G is a derivative of furostanol saponins isolated from pepper (*Capsicum annuum* L.) seeds, which has demonstrated anti-adipogenic activity. These studies showed that Capsicoside G treatment a) inhibited in a dose-dependent manner (1–20 μg/mL) the accumulation of lipid droplets in adipocytes without cytotoxicity, b) inhibited the expression of the major adipogenic transcription factors and their target genes during differentiation of preadipocytes into adipocytes, and c) significantly increased phosphorylation of 5′-adenosine monophosphate-activated protein kinase (AMPK) α1, thereby modulating fatty acid metabolism.

Moreover, not only *Capsicum* spp. fruits and seeds have shown anti-obesity activity. The study of Marrelli, Menichini, and Conforti (2016) identified the hypolipidemic and antioxidant properties of hot pepper flowers (*Capsicum annuum* L.). In their study, the hypolipidemic effect of hydroalcoholic extract of flowers from *Capsicum annuum* L. was examined through the evaluation of inhibition of pancreatic lipase, and antioxidant activity assays. Results showed that flowers extract inhibited pancreatic lipase (IC_{50}= 3.54 ± 0.18 mg/ml), lipid peroxidation (IC_{50} = 41.69 ± 1.13 μg/ml after 60 min of incubation), and NO production (IC_{50} = 264.3 ± 7.98 μg/ml) without showing any cytotoxic effect. Hot pepper flower extracts could be applied to develop natural drugs for obesity treatment.

On the other hand, even if animal experiments and human studies have shown that *Capsicum* spp. consumption is effective for weight loss; an important world population segment cannot tolerate the pungent flavor. For that reason, Tan et al. (2014) developed capsaicin-chitosan microspheres (CCM). To evaluate the anti-obesity effects of microspheres, an *in vivo* study in obese rats induced by a high-fat diet was carried out. Results showed that by comparing CCM to chitosan microspheres, capsaicin, and Orlistat (an antiobesity drug also named as tetrahydrolipstatin), the CCM showed better ability to control body weight, body mass index, organ index, body fat, proportion of fat to body weight, and serum lipids. The CCM upregulated the expressions of PPARα, PPARγ, UCP2, and adiponectin and downregulated the expression of leptin. The development of this kind of natural product enables more people to enjoy the health benefits of capsaicin consumption.

In the same way, to reduce the spicy taste of hot peppers without sacrificing their anti-obesity activity, Liu et al. (2018) explored the impact of microbial fermentation of peppers. In their study, they compared the anti-obesity effects of extract from multi-strain coupled fermented capsicum (EFC) and extract from fresh capsicum (EC) on obese mice induced by a high-fat diet (HFD). *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Bacillus subtilis* were used for capsicum fermentation. Bodyweight, adipose tissue weight, adipocyte size, energy consumption, intravenous glucose tolerance test, ghrelin, leptin, and lipid concentration in liver, serum, and feces were monitoring. Results showed that obese mice in all experimental groups had similar energy absorption, and both EFC and EC relieved obesity, with better effect in the EFC group than in the EC group. Lower lipid and cholesterol were observed in serum, liver, and feces in HFD-EFC group compared to HFD-EC group.

Moreover, the HFD-EFC group had less visceral fat and smaller adipocyte size. The HFD-EFC group exhibited better sensitivity to hormones, with lower levels of both leptin and insulin

and higher ghrelin level. In summary, fermentation of *Capsicum annuum cv.* reduced its pungent taste and improved its anti-obesity activity.

In addition to capsaicinoids and capsinoids, capsanthin, the main carotenoid in red paprika (*Capsicum annuum* L.) has also performed the anti-obesity activity. The study of Kim et al. (2017) investigated the effects of red paprika and capsanthin on impaired lipid metabolism in diet-induced obese mice. Mice were divided into 4 groups: 1) normal diet (ND), 2) high-fat diet (HD), 3) HD with red paprika, and4) HD with capsanthin. Their results showed that red paprika and capsanthin groups significantly reduced weight gain, ameliorated hypertrophy of the liver, and adipose tissues. Moreover, these groups improved serum lipid profile, adipokine secretion, and ameliorated hepatic steatosis. Also, in epididymal adipose tissue, red paprika and capsanthin inhibited adipogenesis and decreased lipid droplet size.

Evidence indicates that regular consumption of *Capsicum* spp. could help in good weight management through several mechanisms as reducing the accumulation of WAT, the increased expression of PPARα and TRPV-1 etc., the inhibition of ghrelin, the regulating gene expression, the decreasing lipid accumulation, among others. Therefore, based on the results of cited studies, the phytochemicals of *Capsicum* spp. could be used to develop promissory natural drugs with anti-obesity activity.

Diabetes

Diabetes mellitus (DM) is a group of metabolic, endocrine disorder characterized by elevated blood glucose level resulting from the defects in insulin secretion, insulin action, or both, the balance of glucose homeostasis (Loizzo et al. 2013). Among the types of diabetes, Type 1 and type 2 diabetes are the most predominant. Type 1 is mostly associated with inadequate insulin secretion, which results in persistent hyperglycemia, and Type 2 is characterized by a rapid increase in blood glucose levels due to hydrolysis of starch by pancreatic α-amylase and absorption of glucose in the small intestine by α-glucosidase (Chukwuma et al. 2019, Ranilla et al. 2010). To treat diabetes, the currently available antidiabetic agents include sulfonylureas, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors; however they have limited use because of high rates of secondary failure and undesirable pathological conditions (Surya et al. 2014). Then, many studies have been focused on looking for alternative antidiabetic agents with fewer side effects. At his respect, many plants as *Curcuma longa* L, *Momordica charantia* L, *Psidium guajava* L, and *Capsicum frutescence* L., have been found to be important reservoirs of antidiabetic compounds, some of which are already being formulated into medicine for the treatment of Type 2 diabetes (Njume, Donkor, and McAinch 2019). Correctly, in the case of *Capsicum* spp., several studies have reported important hypoglycemic activities of secondary metabolites from different parts of the plant (e.g., seeds, flowers, and fruits).

Chaiyasit, Khovidhunkit, and Wittayalertpanya (2009) studied the effect of fresh *Capsicum frutescens* fruit consumption on the plasma glucose level. They performed a crossover study in 12 healthy volunteers, who consumed 5 grams of capsicum (26.6 mg capsaicin) vs. a placebo. First, a glucose tolerance test was applied to the volunteers, they were given 75 grams of glucose diluted in 150 ml of water, and secondly, they were administered by capsicum and placebo. Blood samples were collected to measure the glucose, insulin and capsaicin levels in plasma. Results showed that plasma glucose levels in volunteers who received capsicum were

significantly lower than those in the placebo group. Furthermore, plasma insulin levels were significantly higher at 60, 75, 105, and 120 minutes ($p < 0.05$). When comparing before and after capsicum intake, their results showed that the insulin levels were maintained. This study correlated the plasma glucose level decrease to the presence of capsaicin.

Dömötör, Szolcsányi, and Mózsik (2006) also studied the role of capsaicin consumption in carbohydrate metabolism of humans. Fourteen healthy subjects were subjected to a glucose loading test without and with capsaicin (400 μg orally given). And the glucose, insulin, C-peptide and glucagon levels in plasma were measured every 15 min during 4h. Results showed that the plasma level of glucose increased significantly from 30 to 150 min, and the plasma glucagon level increased from 90 to 180 min after the glucose loading when capsaicin administered. The plasma levels of insulin and C-peptide risen from 90 to 165 min after glucose loading, but there was no significant difference between the results obtained without and with capsaicin administration. The study concluded that capsaicin increases the glucose absorption from the gastrointestinal tract and increases the glucagon release loading during glucose loading tests.

According to Wang et al. (2012), dietary capsaicin can regulate glucose homeostasis through the activation of transient receptor potential vanilloid 1 (TRPV1). Wild type and TRPV1 knockout mice were feed dietary capsaicin for 24 weeks, and their results showed that TRPV1 enhanced gut glucagon-like peptide-1 (GLP-1) secretion in the intestinal cells and tissues. GLP-1 is a potent incretin hormone produced in L-cells of the distal ileum and colon. Moreover, chronic dietary capsaicin not only improved glucose tolerance and increased insulin levels but also lowered daily blood glucose profiles and increased plasma GLP-1 levels in wild type mice.

Islam and Choi (2008) also showed the insulinotropic activity of dietary red chili (*Capsicum frutescens* L.) in a rat model of Type 2 diabetes. Five-week-old male Sprague-Dawley rats were fed an HF-diet for a duration of 2 weeks, and they were randomly divided into 4 groups: 1) healthy control (NC), 2) diabetic control (DBC), 3) red chili low (RCL, 0.5% of total dietary intake), and 4) red chili high (RCH, 2.0% of total dietary intake) groups. Diabetes was induced in groups 2, 3 and 4, by an intraperitoneal injection of STZ (40 mg/kg BW). After four weeks free access to the allocated diets, animals were sacrificed, and various diabetes parameters measured. Results showed that fasting blood glucose concentrations in both red chili fed groups were not significantly different. Serum insulin concentration was significantly ($p < 0.05$) increased in the RCH group compared to the DBC and RCL groups, and better glucose tolerances were observed in the red chili fed groups compared to the DBC group during the intraperitoneal glucose tolerance test. Blood HbA1c level, liver weight, liver glycogen, and serum lipids were not influenced by feeding of red chili containing diets.

On the other hand, Yuan et al. (2016) also determine the positive effect of capsaicin supplementation in women with gestational diabetes mellitus (GDM). Forty-four pregnant women with GDM at 22 - 33 gestational weeks were randomly assigned to the capsaicin group (5 mg/d of capsaicin) or the placebo group (0 mg/d of capsaicin) for four weeks in a randomized, double-blind, placebo-controlled trial. The concentrations of fasting plasma glucose and serum insulin, 2-h postprandial plasma glucose (2-h PG) and serum insulin (2-h INS), and fasting serum lipids, liver and kidney function parameters, and calcitonin gene-related peptide (CGRP) were measured at 0 and four weeks. Results showed that in women with GDM, chili supplementation improved postprandial hyperglycemia and hyperinsulinemia, as well as fasting lipid metabolic disorders.

Additionally, the study of Kwon et al. (2013) also highlights capsiate as a secondary metabolite able to improves glucose metabolism. 90% pancreatectomized (Px) diabetic rats were divided into three treatment groups: 1) capsaicin (Px-CPA), 2) capsiate (Px-CPI) and 3) dextrose (Px-CON); and they were provided with high-fat diets (40 energy % fat) containing assigned components (0.025% capsaicin, capsiate, or dextrose) for 8 weeks. Overnight-fasted serum glucose levels and body weights were measured every week. Moreover, an oral glucose tolerance test was performed every three weeks in overnight fasted animals by orally administering 2 g glucose/kg body weight. Serum glucose levels were measured by tail bleeding every 10 min up to 90 min and at 120 min after glucose loading while serum insulin levels were determined at 0, 30, 60, 90, and 120 min. Results showed that both capsaicin and capsiate reduced body weight gain, visceral fat accumulation, serum leptin levels, and improved glucose tolerance without modulating energy intake in diabetic rats.

In comparison to the control, both capsaicin and capsiate potentiated first and second and phase insulin secretion during hyperglycemic clamp. Both also increased β-cell mass by increasing proliferation and decreasing apoptosis of β-cells by potentiating insulin/IGF-1 signaling. However, only capsiate enhanced hepatic insulin sensitivity during the euglycemic hyperinsulinemic clamp. Capsiate reduced hepatic glucose output and increased triglyceride accumulation in the hyperinsulinemic state and capsiate alone significantly increased glycogen storage. Then, the study concluded that even if capsaicin and capsiate improve glucose homeostasis, capsiate has better antidiabetic actions than capsaicin.

In-vitro hypoglycaemic activity of four *Capsicum annuum* L. cultivars (Fiesta, Acuminatum, Orange Thai, and Cayenne Golden) was also evaluated by Tundis et al. (2013). Hypoglycaemic effects of two stages of fruits ripening (immature and mature) were examined via the inhibition of α-amylase and α-glucosidase enzymes. Results showed that Fiesta, Orange Thai, and Cayenne Golden cultivars showed the highest inhibitory activity on α-amylase in the immature stage. The lipophilic fraction exhibited a selective inhibitory activity against α-amylase with IC50 values ranging from 9.1 μg/ml to 28.6 μg/ml in the unripe stage. The study proposes that the hypoglycaemic activity of *Capsicum annumm* could be linked to the presence of phytol and different fatty acids, such as myristic acid, methyl stearate, and methyl linoleate, as well as to capsaicin, dihydrocapsaicin, total soluble phenolics compounds, and total flavonoids. Moreover, Kwon, Apostolidis, and Shetty (2007) also confirmed the anti-diabetic potential of nine varieties of *Capsicum annuum* phytochemicals by using *in vitro* enzyme assays for α-glucosidase and α-amylase. Results showed that several pepper extracts presented a high α-glucosidase inhibitory activity and, a less or no inhibitory effect on the α-amylase activity, which indicates the potential for reduced side effects.

On the other hand, Biro et al. (2018) studied the effect of allithiamine (N-[(4-amino-2-methylpyrimidin-5-yl)methyl]-N-[(2E)-5-hydroxy-3-(prop-2-en-1-yldisulfanyl)pent-2-en-2-yl]formamide), a natural fat-soluble thiamine derivative obtained from seeds of Hungarian red sweet pepper (*Capsicum annuum*), on streptozotocin-induced diabetic mice with neuropathy. Their results showed that after 5 weeks of allithiamine treatment, the tail-flick time latency was increased, indicating improvement in neuropathic pain sensation in diabetic mice. Allitamine presented the same results as benfothiamine, which is a synthetic thiamine monophosphate analog commonly used on diabetic neuropathy. Moreover, according to Leiherer, Mündlein, and Drexel (2013) another important active component used for neurological disorders such as diabetic neuropathy is capsaicin.

Even if, there are several commercial drugs available for the treatment of diabetes, there exists scientific evidence about the potential of medicinal plants to better manage this disease. Respect to *Capsicum* spp. *in vitro* and *in vivo* studies have shown that fruits and other parts of the plant presented α-glucosidase inhibitory activity, insulinotropic activity, and hypoglycemic activity. Therefore, these findings support that regular dietary consumption of capsicum could be applied as a strategy for treating diabetes.

Hypertension

Hypertension, also known as high or raised blood pressure, is one of the significant threats to public health and the socioeconomic status of most parts of the world. According to the World Health Organization (WHO), it is estimated that hypertension has caused 7.5 million worldwide deaths, which represents about 12.8% of the total of all deaths (WHO 2019b). The clinical presentation of hypertension has been defined by WHO as systolic (SBP)/diastolic (DBP) blood pressures of ≥ 140/90 mmHg; however, as there are several metabolic processes that can influence the development, progression and complications of this disease, the pathophysiology of this disease is not limited to abnormal blood pressure (Chukwuma et al. 2019). The relationship between blood pressure and the increased risk of cardiovascular diseases (CVD) is graded and continuous, starting at blood pressures as low as 115/75 mmHg, well within what is considered to be the normotensive range (Oparil et al. 2018).

Over several decades synthetic antihypertensive drugs have been used to treat hypertension. However, these drugs had triggered side effects like dry mouth, dizziness, emotional distress, gastrointestinal disturbance, visual disorders, among others (Sultana and Asif 2017). For that reason, some plants, as *Capsicum* spp. has been studied to search for cheaper and non-toxic compounds against hypertension.

The study of Kwon, Apostolidis, and Shetty (2007) evaluated the anti-hypertension activity of nine types of *Capsicum annuum* fruits through an angiotensin-converting enzyme (ACE) inhibition assay. Their results showed that water extracts of four peppers presented high ACE inhibitory activity: Yellow pepper (84.1%), Cubanelle (79.9%), Red (76.5%), and Red Sweet (73.0%). The angiotensin-converting-enzyme plays a significant role in converting enzyme angiotensin I into angiotensin II which is responsible for increasing the blood pressure (Sultana and Asif 2017). Therefore, the inhibition of ACE has been considered a useful therapeutic approach in the treatment of high blood pressure (Crook and Penumalee 2004). Moreover, the study of Ranilla et al. (2010) also evaluated the potential of some types of *Capsicum* spp. against hypertension. Their results showed that ACE inhibitory activities of pepper samples ranged from 41% to 92% (at 2.5 mg of dried sample) and showed an excellent dose-dependent response. Paprika and red pepper (*Capsicum annuum*) presented the highest ACE inhibition (92% and 84%, respectively), followed by Rocoto and yellow pepper (*Capsicum baccatum*) with (71% and 41%, respectively). These studies suggested that consumption of some pepper types may control hypertension via modulation of angiotensin I-converting enzyme (ACE).

On the other hand, the study of Yang et al. (2010) investigated the role of the transient receptor potential vanilloid type 1 (TRPV1) activation in blood pressure regulation by dietary capsaicin. And their results showed that long-term stimulation of TRPV1 by capsaicin improved vasorelaxation, and lowered blood pressure in genetically hypertensive rats. Chronic TRPV1 activation increased the phosphorylation of protein kinase A and endothelial nitric

oxide synthase and thus the production of nitric oxide (NO) in endothelial cells. According to this study, TRPV1 activation by dietary capsaicin improves endothelial function, and the TRPV1-mediated increase in NO production may represent a promising target for therapeutic intervention of hypertension.

Among the phytochemicals of *Capsicum* spp., capsaicin is the most studied anti-hypertensive compound; however, even if, this molecule has shown the capacity of inhibiting the angiotensin I-converting enzyme, while increasing vasorelaxation, there is a need to expand research of other potential hypotensive and antihypertensive agents of peppers as well as to elucidate its mechanism of action.

Hypercholesterolemia and Hypertriglyceridemia

Even though cholesterol is one of the most valuable substances in the human body, raised cholesterol increases the risks of heart disease and stroke. Overall, is estimated that raised cholesterol cause 2.6 million deaths (4.5% of total) and a third of ischemic heart (WHO 2019c). On the other hand, hypertriglyceridemia, defined as high amounts of triglyceride in the blood, also has been associated with cardiovascular disease risk (CVD), and an increased risk of acute pancreatitis when hypertriglyceridemia is very severe (Yuan, Al-Shali, and Hegele 2007). Therefore, to maintain the cholesterol and triglycerides at normal levels, several studies have focused on the anti-hyperlipidemic activity of medicinal plants, as *Phyllanthus Niruri*, *Curcuma Longa*, *Allium Sativum*, *Capsicum spp.* (Mukherjee 2003). Specifically, this section focuses on the performance of *Capsicum spp.* in lowering cholesterol and triglycerides levels.

The studies of Srinivasan M.R. and Satyanarayana M.N. (Srinivasan and Satyanarayana 1988, Srinivasan and Satyanarayana 1987, Srinivasan and Satyanarayana 1989) showed that in high sucrose diet-fed rats, capsaicin lower or tend to lower liver weight, liver triglycerides, free fatty acids, phospholipids, serum total, VLDL+LDL and HDL triglycerides, VLDL+LDL cholesterol, free fatty acids and also elevate serum total and HDL cholesterol. Moreover, they indicated that common dietary capsaicin triggered lipid-lowering action in Wistar rats fed high-fat diets. In female rats, capsaicin lowered the rate of weight gain, adipose tissue, liver and serum triglycerides. In male rats, it lowered only the liver and serum triglycerides. Furthermore, according to their results capsaicin enhanced serum triglyceride uptake by muscle tissue and in turn lowered triglyceride levels.

Moreover, the studies of Negulesco, Young, and Ki (1985) determined the anti-hyperlipidemic activity of capsaicin and dihydrocapsaicin on turkeys and rabbits. Rabbits maintained on a 0.5% cholesterol diet, were daily administered over a five-week experimental period with a dose of 8 mg of capsaicin. And results showed that capsaicin decreased total plasma cholesterol, triglycerides, and total cholesterol to HDL-C ratio as compared to cholesterol level of controls. Turkeys maintained on a 0.2% cholesterol diet, were daily administered with a dose of 4 mg of capsaicin or dihydrocapsaicin. Results showed that triglycerides, total cholesterol, and LDL-cholesterol decreased significantly with dihydrocapsaicin treatment. Furthermore, both compounds reduced VLDL-cholesterol and increased HDL-cholesterol. In cholesterol-fed animals, both capsaicin and dihydrocapsaicin presented anti-hyperlipidemic activity.

Sambaiah and Satyanarayana (1982) also highlighted that the inclusion of capsaicin on Wistar rats led to a lowering of total lipids, particularly triglycerides in the liver. According to

the authors, the effect of capsaicin could be linked to a reduction of hepatic lipogenesis, an enhanced transport of lipids to serum, and increased oxidation of fats in the tissues.

Kawada, Hagihara, and Iwai (1986) also study the effects of capsaicin on lipid metabolism in rats fed a high-fat diet (30% lard). In their work, the capsaicin supplementation lowered the level of serum triglyceride and the perirenal adipose tissue weight in high-fat rats. Moreover, the hepatic enzyme activities of glucose-6-phosphate dehydrogenase and adipose lipoprotein lipase were higher when capsaicin was added to the diet. Levels of serum cholesterol and pre-β-lipoprotein were not affected by the supplementation of capsaicin.

Gupta, P Dixit, and Dobhal (2002) also evaluated the hypocholesterolemic effect of *Capsicum spp.* on animals, specifically gerbils. Gerbils were divided into three groups: a) Control group fed with a regular diet, b) a group fed with an atherogenic diet, and c) a group fed with an atherogenic diet supplemented with capsicum oleoresin (75 mg/kg body wt/day). Results showed that in comparison with atherogenic fed controls, oleoresin supplementation reduced serum cholesterol and triglycerides by 70% and 66%, and liver cholesterol and triglycerides by 70.9% and 68.7% respectively. Moreover, the fecal excretion of cholesterol and triglycerides were significantly increased in oleoresin fed gerbils. This work concludes that capsicum oleoresins could prevent the prevented the accumulation of cholesterol and triglycerides in the liver and aorta.

On the other hand, according to Kwon et al. (2003) another hypolipidemic activity of peppers is linked to the attenuation of cholesteryl ester transfer protein (CETP) activity. CETP plays a major role in regulating plasma HDL by mediating the random transfer of neutral lipids between lipoprotein cores, such as cholesteryl ester and triglycerides. In this study, red pepper supplementation to cholesterol-fed rabbits (1%), inhibited plasma CETP, lowered the concentration of total cholesterol, TG, LDL-C, VLDL-C, and VLDL-TG, increased the concentration of HDL-C, and delayed the progression of atherosclerosis in the aorta and aortic arch. Red pepper played an important role in regulating the hypolipidemic and/or antiatherogenic effect.

Additionally, to the hypolipidemic potential of capsaicin, this compound offers protective erythrocyte integrity in high-fat-fed rats and inhibits the grow of cholesterol gallstone. Kempaiah and Srinivasan (2006) fed rats with capsaicin, and their results showed the inclusion of this compound in the diet produced a significant hypotriglyceridemic effect, corrected the osmotic fragility of erythrocytes due to high-fat diet, and significantly reduced the activity of Ca^{2+}, Mg^{2+}-ATPase in erythrocyte membrane. The decreased activity of membrane-bound Ca^{2+}, Mg^{2+}-ATPase could have probably contributed to the accumulation of intracellular calcium leading to the diminished deformability of the erythrocytes in high-fat-fed rats. Moreover, capsaicin caused regression of pre-established cholesterol gallstones (CGS) in mice. Cholesterol gallstones, composed predominantly of cholesterol crystals, are a result of abnormalities in cholesterol metabolism (Acalovschi 2001). The study of Hussain and Chandrasekhara (1994) showed that after eight weeks, feeding of capsaicin diet triggered 80% of CGS regression in mice. With increase in the duration of capsicum feeding, the biliary cholesterol decreased, and phospholipids and bile acids increased. Then, capsaicin had the ability to influence both the biliary lipid metabolism and pathogenesis of CGS.

In addition to capsaicinoids, the study of Tani et al. (2004) showed that capsinoids, the non-pungent components of peppers, also could improve serum and liver lipid metabolism. Male Wistar rats, received a high-fat diet containing 1% cholesterol, were divided into four groups: 1) control group, 2) synthetic capsaicin group (0.1 mmol/kg), 3) capsinoid-I group (0.1

mmol/kg) and 4) capsinoid-II group (1 mmol/kg). Capsinoid was a mixture of capsiate and dihydrocapsiate (63:37). Results showed that compared with the control group, serum lipid levels in both capsinoid groups and liver lipid contents in the capsinoid-II group showed the same reduction as that of the capsaicin group. In the capsaicin and capsinoid-I groups, fatty acid synthase activities were lower, and hepatic triacylglycerol lipase activities tended to be higher than those of control group. Lipoprotein lipase activity in adipose tissue was higher in the capsaicin and capsinoid-II groups than in the control group.

On the other hand, the study of Aizawa and Inakuma (2009) showed that also carotenoids, as capsanthin, have a positive effect on lipids metabolism. Wistar rats fed with purified capsanthin, increased its plasma high-density lipoprotein cholesterol (HDL). Quantitative analyses of hepatic mRNA levels revealed that capsanthin administration resulted in up-regulation of mRNA for apoA5 and lecithin cholesterol acyltransferase, without significant differences in other mRNA levels related to HDL-cholesterol metabolism. As it has been suggested that low levels of HDL-cholesterol constitute an independent risk factor for CVD, this study suggests that dietary intake of peppers rich in capsanthin can improve plasma lipid profiles.

Furthermore, the study of Kempaiah, Manjunatha, and Srinivasan (2005) suggested that dietary capsaicin-induced protective effect to LDL oxidation both *in vivo* and *in vitro* under normal situation, while in hypercholesterolemic situation where the extent of LDL oxidation is already lowered, capsaicin does not offer any further reduction.

Moreover, according to Kenig et al. (2018) it is very important to regulate the dose intake of capsaicinoids of peppers, this because their study revealed that low, moderate consumption of capsaicinoids (4.4 mg per day) has beneficial health effects, such as decrease in glucose level, LDL cholesterol, and C-reactive protein; however, in higher daily dose of capsaicinoids (16.7 mg/day) those effects were lost. Therefore, this work suggests that a regular moderate intake of *Capsicum* spp. could be most profitable than a high dosage.

The proper management of hypercholesterolemia and hypertriglyceridemia could prevent cardiovascular diseases as well as other metabolic disorders, such as diabetes mellitus. Based on animal studies, secondary metabolites of *Capsium spp.*, as capsaicinoids, capsinoids, and carotenoids, have shown a relevant anti-hyperlipidemic potential. However, to better understand the mechanism and clinical usefulness of this phytochemicals it is needed to carry out extensive human studies.

Cancer

Cancer is a generic term for a large group of diseases characterized by the growth of abnormal cells beyond their usual boundaries. It is considered the second leading cause of death globally and is estimated to account for 9.6 million death in 2018 (WHO 2019a). Chemotherapy and radiation therapy have been the most applied cancer treatments; however, they often include side effects as hair loss, fatigue, anemia, among others. Therefore, prevention, suppression, and/or delaying the onset of the disease are important (Chung, Lim, and Lee 2013). In this respect, various phytochemicals from *Capsicum spp.* have shown chemopreventive, chemotherapeutic, antimutagenic and antitumor activity against different types of cancer like prostate, breast, gastric, colorectal, stomach and cervix cancer. Among all the phytochemicals of *Capsicum spp*., capsaicin is one of the most studied. It has been highlighted that its main

anticancer mechanisms are cell-cycle arrest activity, apoptosis induction, anti-invasive and anti-migratory activity, and anti-angiogenic activity (Clark and Lee 2016).

The study of Moon et al. (2012) showed the mechanism through capsaicin enhanced apoptosis induced by TRAIL. TNF-related apoptosis-inducing ligand (TRIAL) triggers the induction of apoptosis in a variety of tumor cells; however when giving repeated applications, cancer cells develop resistance to TRAIL through multiple mechanisms. Thus, by combining TRIAL treatment with capsaicin, it was possible to induced cell death in a variety of tumor cells. Capsaicin sensitizes TRAIL-induced apoptosis through activation of the calcium-CaMKII-Sp1 pathway. This feature prompts capsaicin as an excellent molecule for improving TRAIL-based cancer therapy.

Specifically, in the case of skin cancer, Hail and Lotan (2002), showed that the majority of the cutaneous squamous cell carcinoma underwent apoptosis after 12-hour exposure to 100 μM capsaicin. The induction of apoptosis was associated with inhibition of mitochondrial respiration. Moreover, capsaicin treatment inhibited cell proliferation in the respiration-deficient clones by promoting G1 arrest. Both results suggested that capsaicin could be useful for preventing or treating skin cancers or other hyperproliferative skin disorders.

On the other hand, *in vitro*, and *in vivo* capsaicin treatments also inhibited the growth of human leukemia cells. According to Zhang et al. (2003), capsaicin could be considered as chemopreventive for adult T-cell leukemia (ATL) cell lines, thanks to its induction of cell cycle arrest and apoptosis. Capsaicin also induced the degradation of Tax and up-regulation of Iκ-Bα, resulting in the decrease of nuclear factor (NF)-κB/p65 DNA binding activity. Tsou et al. (2006) also indicated that capsaicin decreased the percentage of viable human leukemia HL-60 cells via the induction of G0/G1-phase cell cycle arrest and apoptosis. This study suggested that capsaicin-induced apoptosis could be a result of the activation of caspase-3 and the intracellular Ca^{2+} release pathway. Moreover, the *in vitro* and *in vivo* study of Ito et al. (2004), capsaicin suppressed the growth of leukemic cells, inhibited tumor growth and induced apoptosis in NOD/SCID mice with no toxic effects. According to the authors, capsaicin-induced apoptosis was in association with the elevation of intracellular reactive oxygen species production and with the induction of G0-G1 phase cell cycle arrest.

Capsaicin has also shown protective effect against lung cancer. Anandakumar et al. (2012) indicated the chemo modulatory effect of capsaicin against benzo(a)pyrene-induced lung carcinogenesis in mice. And Brown et al. (2010) demonstrated that capsaicin has anti-proliferative activity against human small cell lung cancer (SCLC) in cell culture and nude mice models. Capsaicin potently suppressed the growth of H69 human SCLC tumors *in vivo*. The anti-proliferative activity of capsaicin was correlated with a decrease in the expression of E2F-responsive proliferative genes like cyclin E, thymidylate synthase, cdc25A and cdc6, both at mRNA and protein levels. Capsaicin inhibited cell proliferation through the recruitment of E2F4 and p130 on E2F-responsive proliferative promoters.

In the management of prostate cancer, capsaicin has also shown a positive role. The studies of Sánchez et al. (2006) and Sánchez et al. (2007) showed that in the androgen-independent prostate cancer PC-3 cells, capsaicin inhibits cell growth and induces apoptosis through reactive oxygen species (ROS) generation, c-Jun N-terminal kinase (JNK) activation, ceramide accumulation, and extracellular signal-regulated protein kinase (ERK) activation. Moreover, the study of Ziglioli et al. (2009) displayed that capsaicin mediated apoptosis in prostate cancer cells follows two pathways: 1) through a TRPV-1 independent mechanism (direct path) and 2) a TRPV-1-dependent mechanism (indirect path). The direct path was linked to two

mechanisms, the delivery of reactive oxygen species (ROS) generated by NADH-oxidoreductase inhibition and the interaction with caspases, particularly caspase 1 and 3. And the indirect pathway was linked to the intracellular calcium growth, provoked by the TRPV-1 receptor. Besides, according to the *in vitro* and *in vivo* study of Mori et al. (2006), capsaicin induces apoptosis in prostate cancer cell lines (PC-3, DU-145, and LNCaP) associated with an increase of p53, p21, and Bax. Furthermore, when given orally to treated mice, capsaicin slowed the growth of PC-3 prostate cancer by size and weight.

Respect for human breast cancer, capsaicin has also evidenced promising results. *In vitro* and *in vivo* tests carried out by Thoennissen et al. (2010) showed that capsaicin inhibited the growth of five breast cancer cell lines (MCF-7, T47D, BT-474, SKBR-3, and MDAMB231) and also blocked the cell migration. In mice, oral consumption of capsaicin decreased by 50% the size of MDAMB 231 breast cancer tumors and inhibited the development of pre-neoplastic breast lesions by up to 80% without evidence of toxicity. Moreover, the study of Chang et al. (2011) determined that capsaicin could inhibit cancer cell growth in caspase-3-deficient human breast cancer cells (MCF-7 and BT-20) through inducing cell apoptosis and arresting the cell cycle in the S phase. This fact is relevant because most breast cancer patients are resistant to chemotherapy or radiotherapy due to the down-regulation or lack of caspase-3 expression. Capsaicin induced cell apoptosis through the mitochondrial pathway, and PARP-1 subsequently cleaved by activation of caspase-7.

Furthermore, Lu et al. (2010) reported that capsaicin also inhibited colon cancer cell and tumor growth. In human colon cancer, "Colo 205" cells, capsaicin-induced apoptosis and cytotoxic effects, and increased reactive oxygen species (ROS) and Ca^{2+}. And in mice, capsaicin effectively inhibited tumor growth. In this study, capsaicin-induced apoptosis was associated with the activations of caspase-8, -9, and -3. Similarly, Kim et al. (2004) also reported that capsaicin-induced apoptosis in HT-29 human colon cancer cells. And they suggest that apoptosis could be associated with the activation of the peroxisome proliferator-activated receptor γ (PPARγ).

In vitro and *in vivo* studies have shown that capsaicin-induced apoptosis in pancreatic cancer cells lines (AsPC-1 and BxPC-3) and suppressed the growth of AsPC-1 pancreatic tumor of athymic nude mice (Zhang et al. 2008). Capsaicin induced apoptosis has been associated with the generation of ROS and the persistent disruption of mitochondrial membrane potential (Zhang et al. 2008, Pramanik, Boreddy, and Srivastava 2011). Moreover, according to Zhang et al. (2013), in the pancreatic cancer cell line PANC-1, capsaicin may function as an inhibitor of cell growth due to the downregulation of the phosphoinositide 3-kinase/Akt pathway.

Regarding the effect of capsaicin in Korean human gastric cancer cells (SNU-1 and cisplatin-resistant SNU-668), the studies of Kim et al. (1997) and Huh et al. (2011) showed that capsaicin-induced apoptotic cell death in both human gastric cancer cells. Kim et al. (1997) suggested that capsaicin-induced apoptosis of SNU-1 cells may be possibly mediated by the overexpression of *p*53 and/or *c-myc* genes. And Huh et al. (2011) indicated that combined treatment with capsaicin and cisplatin, induced apoptosis through induced G1/S arrest. Moreover, the combined treatment of capsaicin and cisplatin performed higher apoptotic cell death than only capsaicin or only cisplatin treatment.

By examining the study of Wang et al. (2011), capsaicin also induced apoptosis in SNU-1 gastric and tumor-associated NADH oxidase knockdown sensitized TMC-1 cancer cell lines. In SNU-1 cells, capsaicin-induced significant cytotoxicity with increases in oxidative stress, PARP cleavage, and apoptosis. And in tNOX knockdown TMC-1, capsaicin led to decreased

cell growth and induced apoptosis and G1 phase accumulation. Induced apoptosis of capsaicin was associated with the down-regulation of tumor-associated NADH oxidase (tNOX) mRNA and protein.

Moreover, capsaicin has also exhibited anticancer activity on human cervical epithelial carcinoma (HeLa) cells (Šaponjac et al. 2014), human esophagus epidermoid carcinoma CE 81T/VGH cells (Wu et al. 2006) and murine MBT-2 bladder tumor cells (Lee et al. 2004).

Capsaicin is a potent bioactive molecule that possesses anticarcinogenic effects. Several *in vitro* and *in vivo* studies have reported that the effect of capsaicin depends on cell type, doses used as well as the length of the treatment. Moreover, several mechanisms have been elucidated to understand the ability of capsaicin to act against cancer initiation, promotion, progression, and metastasis. However, to be able to propose a therapeutic strategy, it is necessary to understand better the molecular mechanisms triggered by capsaicin against cancer.

CONCLUSION

Capsicum spp. is a promising medicinal plant to develop natural drugs against metabolic disorders. The present chapter aimed to elucidate the potential of the different phytochemicals present on fruits and parts of the plant of *Capsicum* spp. face to obesity, diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia, and cancer. *In vitro* and *in vivo* studies allowed us to understand the mechanism through *Capsicum* spp. phytochemicals improve health. Face to obesity, *Capsicum* spp. showed lipid oxidation increase, adipogenesis prevention, anti-adipogenic activity, hypolipidemic activity, satiety sensation, energy expenditure increases, and non-exercise thermogenesis activity increase. Respect for diabetes, bioactive components of peppers presented insulinotropic, hypoglycemic and α-glucosidase inhibitory activities. In the case of hyperlipidemia, capsaicinoids, capsinoids, and carotenoids showed hypotriglyceridemic, anti-hypercholesterolemic and attenuation of cholesteryl ester transfer protein activities. For hypertension, it was highlighted the capacity of capsaicin to inhibit the angiotensin I-converting enzyme and to increase vasorelaxation. Finally, respect to cancer, capsaicin showed chemopreventive, chemotherapeutic, antimutagenic and antitumor activity against leukemia and prostate, breast, gastric, colorectal, skin, lung, pancreatic and cervix cancer. *Capsicum* ssp. is not only an exotic pungent spice, it could be also a key tool to prevent or treat metabolic disorders.

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Chapter 19

PREVALENCE OF METABOLIC SYNDROME IN ADOLESCENTS WITH OBESITY FROM A MEXICAN PUBLIC JUNIOR HIGH SCHOOL

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ABSTRACT

Metabolic Syndrome (MetS) is defined as a set of risk factors (obesity, hyperglycemia, high triglyceride levels, low HDL cholesterol levels, and hypertension), which are considered as precursors of cardiovascular and metabolic diseases. The objective of this work was to determine the prevalence of Metabolic Syndrome in adolescents with obesity in a public junior high school in San Juan del Río. The anthropometric measurements as indicators of obesity (Waist Circumference, WC); and measurements of blood pressure, fasting glucose, HDL cholesterol, and triglycerides were made in 75 adolescents of both genders, with an average age of 13.07 years. The prevalence of MS was 21.33% (7 men and nine women). The age group with the highest prevalence was 14 years, with 6 cases. High blood pressure was detected in 26.67% of participants, while altered levels of triglycerides, HDL cholesterol, and glucose were found in 21.33%, 17.33%, 53.33%, respectively. Obesity in children and adolescents has become a growing risk factor for the development of multiple complications for health, including the metabolic syndrome that has had a significant increment in recent years. So, it is important to implement appropriate activities in health and specific action plans aimed at guiding adolescents and teaching the administrative school staff to know the impact of this and other metabolic diseases.

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Keywords: metabolic syndrome, obesity, adolescents

INTRODUCTION

The Metabolic Syndrome

The Metabolic Syndrome (MetS) is defined as a set of risk factors (abdominal obesity, hyperglycemia, hyperinsulinemia and insulin resistance, hyperlipidemia and hypertension) which initially appeared in adulthood; however, recently that set has been associated with children and adolescents (Araujo-Herrera 2015, Burguete-García, Valdés-Villalpando, and Cruz 2014, Burrows et al. 2007). Clinicians have traditionally evaluated each of the significant risk factors contributing to metabolic syndrome on an individual basis. There is evidence, however, that the risk factors are more than additive (Sherling, Perumareddi, and Hennekens 2017).

The concept of metabolic syndrome has existed for at least 80 years. It was first described by a Swedish physician, as the clustering of hypertension, hyperglycemia, and gout; and latter related to abdominal obesity (Eckel, Grundy, and Zimmet 2005).

Although the metabolic syndrome is not a disease per se, it highlights traits that may have an increased risk of disease, approximately 2-fold for cardiovascular disease and 5-fold or more for type 2 diabetes mellitus (Samson and Garber 2014).

Obesity Association to MetS

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. Obesity is defined as an excess of body fat. For practical reasons, body weight has been used as an indirect measure of the degree of adiposity, which is not easy to measure with usual tests. In the 1980s, the concept of body mass index (BMI) was introduced, and cut-off points were defined to define overweight and obesity in adult women and men (WHO 2016b, Alegría, Castellano, and Alegría 2008).

BMI, which is the weight in kilograms divided by height in squared meters, is used to identify obesity. For adults, a BMI of 25.0 to 29.9 kg/m^2 is defined as overweight and a BMI of 30 kg/m^2 or higher is defined as obese. BMI is not used for children and adolescents age 2 to 18 years; instead, it is recommended that a percentile scale based on the child's sex and age be used. In this population, overweight is defined as a BMI in the 85th to 94th percentile, and obesity is a BMI at or above the 95th percentile (Apovian 2016, WHO 2016b).

The rising number of obese children and adults has solicited in recent years the creation of indexes able to accurately define weight excess, body composition, and in particular visceral obesity because the latter is a marker for higher risk of cardiometabolic diseases (Radetti et al. 2019). Body Mass Index (BMI = kg/cm^2) is by far the oldest and most common index used. However, BMI does not always give accurate information about health status. Other parameters have been used to define not only obesity but also MetS such as Body Mass Fat Index (BMFI) (Radetti et al. 2019), levels of C-Reactive protein (Williams et al. 2017), and Intra-abdominal fat measurement (Novais et al. 2019).

To assess the presence of obesity, the anthropometric variable that is used to estimate abdominal fat is the waist circumference (WC), a simple measurement that has a low error and correlates adequately with the amount of intra-abdominal fat (Vargas et al. 2011, Gaston, Tulve, and Ferguson 2019).

According to the World Health Organization (WHO 2016a), the term adolescence is defined as the period of growth and human development between 10 and 19 years, after childhood and before adulthood, considering two phases: early adolescence that covers from 12 to 14 years, and late adolescence considered from 15 to 19 years.

Childhood obesity is one of the most serious public health challenges of the 21st century. The problem is global and is steadily affecting many low- and middle-income countries, particularly in urban settings. The prevalence has increased at an alarming rate. Globally, in 2016, the number of overweight children under the age of five is estimated at over 41 million. The prevalence of overweight and obesity in adolescents is defined according to the WHO growth reference for school-aged children and adolescents. The prevalence of obesity has increased globally, and the most change, from11% to15%, occurred among 6 to 19 years of age group (WHO 2016b, Mahbuba et al. 2018).

Prevalence of MetS in Adolescents

The criteria contemplated by the National Cholesterol Education Program (NCEP) and the Adult Treatment Panel III (ATP-III) established that in developing countries, the MetS reached a prevalence between 4.2 to 15.4% for adolescents from 10 to 19 years old. However, the WHO reports a broader range, between 4.5 and 38.7% for the same population (Álvarez et al. 2014). In Mexico, the prevalence of MetS reaches up to 50% in obese young people, while in moderately obese patients, it is 38.9% (Álvarez et al. 2014).

According to Romero-Velarde et al. (2016) and Radetti et al. (2019), the global epidemic of overweight and obesity in recent decades is responsible for the occurrence of conditions in children and adolescents that were previously described only in adults, for instance, MetS. According to data obtained in the Medical Information and Clinical File Area (ARIMAC, 2016) of the Clinic No. 7 of the Mexican Institute of Social Security (IMSS by its initials in Spanish) located in the municipality of San Juan del Río, every 100 children who attend to the first level consultation, 40 present obesity, and of these 5 have high levels of blood glucose. They are classified as patients with type 1 diabetes mellitus (DM1) in the absence of additional information such as anthropometric and biochemical measurements that yield the particular diagnosis of MetS.

Prevention of MetS by Using Medicinal Plants

Metabolic syndrome is of substantial concern, as the incidences of both type 2 diabetes and cardiovascular disorders have reached epidemic proportions worldwide. Unlike acute diseases, such as those caused by pathogens, metabolic syndrome is a complex, progressive disorder that can develop over many years and can vary between individuals both in terms of its extent and characteristics. Botanicals may serve as effective agents for the treatment or prevention of metabolic syndrome because they often contain diverse collections of biologically active

compounds with multiple mechanisms of action that may potentiate each other's activity or have a synergistic effect, providing more significant benefit than a single chemical entity (Graf et al. 2010).

An approach to treat MetS is to use decoctions of plants attributed to glucose and lipid levels modulation activity. Sanguayin is a plant traditionally used in ancient Chinese medicine. Insulin resistance represents one of the major components of MetS, and the main impairment includes gluconeogenesis, dysregulated lipogenesis, and defective glycogen synthesis. Zheng et al. (2020) demonstrated that sanguayin decoctions significantly suppressed the glucose metabolism disorder in rats by downregulation of HOMA-IR (Homeostatic Model Assesment for Insulin Resistance) score, and inhibition of glucose intolerance and gluconeogenesis-related gene expression, suggesting that sanguayin improved glucose metabolism and alleviated the insulin resistance.

C. glandulosum exerts a protective effect against experimentally induced MetS by bringing about corrective pharmacological changes in plasma lipid profile, lowering plasma glucose and insulin, and improving insulin resistance (Jadeja et al. 2010).

Cinnamon has demonstrated positive effects on other markers of metabolic syndrome in addition to blood glucose. The antihypertensive action of cinnamon extract was shown successfully in both spontaneously hypertensive rats and patients with metabolic syndrome (22 men and women treated with a 500-mg extract daily for 12 weeks) (Leach and Kumar 2012, Mang et al. 2006, Altschuler et al. 2007). Moreover, triglyceride-lowering effects with cinnamon were observed in several animal models (Maierean et al. 2017, Khan et al. 2003).

Obesity is the major risk factor for increasing incidences of diabetes. Therefore, search towards a molecule that can mitigate both hyperglycemia and obesity is of higher priority for the management of the metabolic syndrome.

Natural plant products are widely used in healthcare or as dietary supplements. The majority of plant extracts are not single compounds but rather a mixture of different molecules, therefore their mechanism of action usually targets several organ and cellular systems. The phytochemicals would act through their inhibitory activities for pancreatic lipases, adipocyte differentiation or by increasing thermogenesis and anorexia. The important phytochemicals include flavonoids, terpenoids, saponin, phenols, and alkaloids (Sharma and Kanwar 2018, Karri et al. 2019). A class of compounds found in herbs and spices that are generally bioactive and have generated a lot of interest are the polyphenols. Polyphenols are natural substances with variable phenolic structures and are enriched in vegetables, fruits, grains, bark, spices, roots, tea, and wine (Graf et al. 2010, Panickar 2013).

Another approach to treat metabolic disorders is to reduce blood lipids. In this regard, a hydroethanolic extract of *Curatella americana* L. leaves reduce oxidative stress by free radical scavenging and protects against lipid peroxidation and is also able to manage hyperlipidemia by decreasing serum level of cholesterol and triglycerides, similarly to standard drugs (Karri et al. 2019).

Sideritis hyssopifolia is a little woody plant endemic to western and southwestern Europe. Aqueous extracts of *S. hyssopifolia* showed anti-hypercholesterolemic effects and modify the atherogenic index (Coto et al. 2019).

Based on the examples mentioned above of medicinal plants, it is possible to prevent and even to treat metabolic syndrome. However, previously to treat MetS, it is necessary to detect it.

Then, based on the above, this work aimed to measure blood glucose, cholesterol, and triglyceride (TG) levels, as well as taking anthropometric measurements (WC and height), and taking the blood pressure (BP) in schoolchildren of San Juan del Río, in order to establish the prevalence of MetS in those who are obese. Therefore, the subsequent investigation had a quantitative, observational, transversal and descriptive approach.

METHODS

Sample Selection

The population under study was established on the basis of WC measures of the total number of junior high school students, resulting in 106 adolescents of both genders who presented abdominal obesity. Subsequently, the sample size was calculated through the formula for finite population:

$$n = Nz2pq/(d2(N-1) + z2pq \quad \text{Eq. (1)}$$

where: "n" is the sample size; "N" represents the size of the population; "Z" corresponds to 95% (confidence interval = 1.96); "P" represents the estimated proportion of the population under study, which is expected to have a certain characteristic (if it is not known, 50% is used, which is 0.5); "q" is equal to 1-p (1-0.5 = 0.5); and "d" is the desired degree of precision (0.05 is usually used) (Pineda & Alvarado, 2008).

As a result, the representative sample size was made up of 83 students. However, according to the inclusion, exclusion and elimination criteria, a total of 8 participants were discarded because they were not fasting for 8 hours or because they had missed the day of capillary and BP sampling; finally, a representative sample of 75 students of both genders was selected.

Data Collection

The present investigation was reviewed and approved by the Undergraduate Research Subcommittee of the Faculty of Nursing of the Universidad Autónoma de Querétaro and by the Ethics Subcommittee of the Faculty of Nursing. After approval, authorization was requested from the Principal of the Escuela Secundaria General Antonio Caso in the municipality of San Juan del Río, Querétaro, presenting the authorized documentation. Once the school permission was granted, the date of work and the place were established, selecting the school chemistry laboratory to take blood samples.

In addition, permission was obtained from the parents or advisors of the students through an informed consent in which they expressed their kinship with the participating individual and the phrase "I accept" if they authorized the inclusion of the student in the said investigation, or otherwise the phrase "I do not accept." The same was granted to the students with informed consent, being necessary since the participants are juveniles. The participation in the study was completely anonymous, respecting the integrity of the teen-agers.

The identification of everyone's sample was done with a folio conformed with successive Arabic numbers beginning with the number 001. Everyone had a unique identification code made up of gender and age (letter F (female) for women and M (male) for men); the age was recorded in years and was asked directly to the participant.

Anthropometric Measurement

To measure WC, the methodology recommended by Casanueva et al. (2015) was used. After eight hours-fast, participants were indicated to remove their coat to avoid wrong measurements. The subject must be standing, its abdomen relaxed, the arm at the sides, the feet together, and the weight distributed equally between both. The lowest part of the ribs and the iliac crests were identified at the level of the axillary midline. The perimeter of the waist waste taken between those lines, at the height of the umbilical scar (Figure 1).

All collected data were registered in the format presented in Table 1.

Table 1. The format used to register collected data

Code	**Sociodemographic data**		**Risk factors for metabolic syndrome**				
	Gender	Age	WC (cm)	BP (mmHg)	Glucose (mg/dl)	TG (mg/dl)	C-HDL (mg/dl)

WC = waist circumference as an index of abdominal obesity. Glucose = level of glucose after an 8 h fast. TG = Triacylglycerides. C-HDL = Cholesterol-High Density Lipoproteins level.

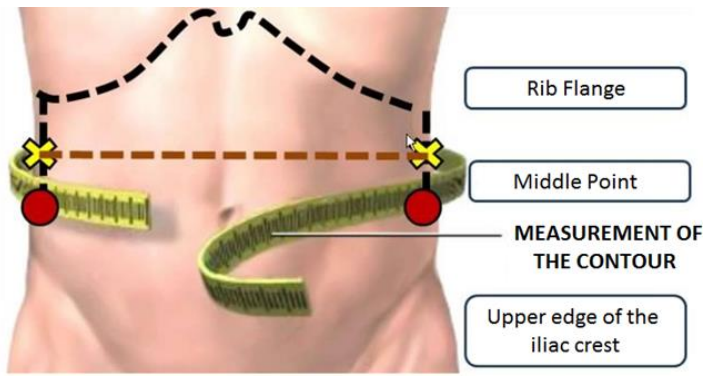

Figure 1. Measurement of waist circumference (https://agita.cl/centimetros-en-tu-cintura-vs-riesgo-cardiovascular/. Date of consultation: September 18th, 2017).

Blood Pressure Measurement

For the measurement of BP, the technique described by the NOM-030-SSA2-2009 (2009) was followed. A stethoscope and a sphygmomanometer were used, making sure that the bracelet was of a suitable size to the arm of each subject. The patient should have been at rest for at least 5 minutes in a sitting position. The observer positioned himself so that his sight was at the meniscus level of the mercury column. The cuff was placed over the humeral artery and placing the lower edge 2 cm above the fold of the elbow. After locating the pulse of the humeral artery, the cuff was inflated rapidly until the pulse disappeared in order to determine the level

of systolic pressure, then deflated at a rate of approximately 2 mmHg/sec. The appearance of the first Korotkoff noise marked the level of the systolic pressure and, the fifth, the diastolic pressure.

Blood Glucose Level

The capillary glycemia is a test in which the glucose level of the moment is evaluated using a small drop of blood and an apparatus for reading the blood glucose concentration. It was carried out following the procedure proposed by Morales (2010) using a glucometer. Previously to taking a blood drop, the procedure as explained to the teenager.

Blood Lipids Level

The measurement of HDL cholesterol can be done without fasting, while the TG needs a fast of 8 to 12 hours (DOF, 2012c). Blood lipids were measured by the procedure described by Quevedo and Seringe (1999) using test strips.

Data Analysis

The collected data were analyzed by SPSS Statistics 23®.

RESULTS AND DISCUSSION

Sociodemographic Data and Abdominal Obesity

Figure 2 shows the prevalence of obesity, obtained in the "Antonio Caso" junior high school in the students of the morning shift. From a total of 828 students registered, 106 showed abdominal obesity (12.8%) according to the waist circumference measure.

From the 75 participants in the study, 47 were women, and 28 were men, reflecting a higher prevalence of obesity in females, unlike the survey conducted by Martínez et al. (2017) where they found a prevalence of obesity of 34%, resulting in a majority in men with 76%. Also, Yoon (2014) reported a higher obesity rate in men than in female Korean adolescents. On the other hand, Cárdenas-Villarreal et al. (2010), reported a total obesity prevalence of 20.1%, with 20.2% for men and 80% for women while Romero-Velarde et al. (2016) found a prevalence of obesity in women of 53% and men of 26.3%.

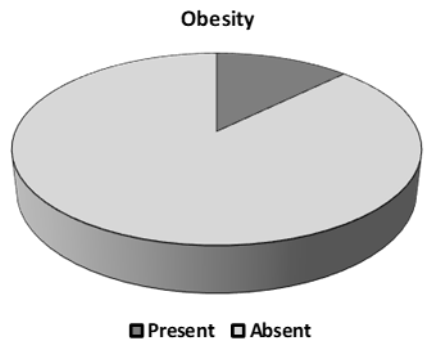

Figure 2. Presence of abdominal obesity according to waist circumference measure.

Adolescents of the sample were from 11 to 14 years old, with an average age of 13.08 years. All of these teenagers presented obesity. This result is like that of Romero-Velarde et al. (2016), who reported that the average age of the adolescents with obesity was 12.6 years, while Martínez, Perea-Martínez, and López-Navarrete (2016) obtained an average age of 13.1 years in Colombian teenagers whereas Cárdenas-Villarreal et al. (2010) reported obese adolescents with an average age of 14.3 years.

Childhood obesity tends to track to adolescence and latter to adulthood, and many of the metabolic and cardiovascular complications of obesity have their origin during childhood.

Blood Pressure

High blood pressure in children and adolescents is a growing health problem that is often overlooked. In children younger than 13 years, elevated blood pressure is defined as blood pressure in the 90th percentile or higher for age, height, and sex, and hypertension is defined as blood pressure in the 95th percentile or higher. In adolescents 13 years and older, elevated blood pressure is defined as blood pressure of 120 to 129 mm Hg systolic and less than 80 mm Hg diastolic, and hypertension is defined as blood pressure of 130/80 mm Hg or higher (Riley, Hernandez, and Kuznia 2018).

Figure 3 shows that 26.67% of the students in the sample presented high blood pressure.

McNiece et al. (2007) reported that almost 20% of the adolescent population is already at risk for future cardiovascular disease due to high blood pressure values. Tony et al. (2016) reported that the prevalence of pre-hypertension among study subjects (children and adolescents) was 21.3% (19.65-22.95%), while the prevalence of systolic pre-hypertension was found to be 21.4% (95% CI 19.74%- 23.06%) and diastolic prehypertension 5.3% (95% CI 4.4%-6.2%). In Hispanic adolescents, (Cárdenas-Villarreal et al. 2010) found that elevated pressure arterial systolic and diastolic blood pressure were 5.9% and 9.1%, respectively.

Normal and elevated blood pressure values for children one to 12 years of age are based on the normative distribution of blood pressure in healthy children of normal weight and should be interpreted based on age, height, and sex.

Table 2 shows the relationship between age, gender, and blood pressure. Twenty adolescents had high blood pressure, of which 9 (45%) were men, and the remaining 11 (45%) corresponded to women. Similar results (21%) of hypertension have been reported for adolescents from 13 to 17 years (Tony et al. 2016).

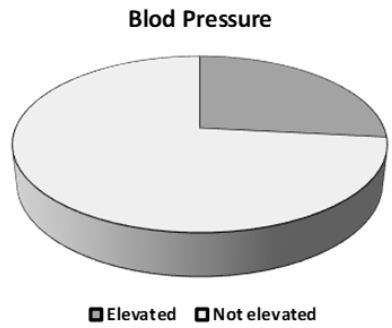

Figure 3. Presence of high blood pressure in students.

Table 2. High blood pressure concerning the gender of the students in the sample

	Blood Pressure		
Gender	Elevated	Not elevated	Total
Male	9	16	25
Female	11	39	50
Total	20	55	75

Blood Glucose

The prevalence of Type 2 diabetes mellitus among children and adolescents is increasing worldwide, mostly due to the epidemic of overweight and obesity in these age groups. Since more young individuals develop Type 2 diabetes, their propensity to develop diabetes' related complications is more significant. Hence, identifying adolescents at higher risk of developing the disease must be a priority (Brandão, Lopes, and Ramos 2013).

In our study, students that presented high glucose levels were 53.33%.

The study of Brandão, Lopes, and Ramos (2013) reported that from 1276 participants, 332 had fasting plasma glucose above the 75th percentile (91 mg/dl), representing 26%. The difference in the prevalence of high blood glucose levels could be due to the kind of population studied. While Portuguese students included all teenagers, either normal weight or obese, in our study, only obese teenagers were included. It is well known that obesity increases the risk of impaired glucose levels.

In this study, women with high glucose levels almost triplicate the number of men with this condition (Table 3). By the contrary, Alemzadeh and Kichler (2014) reported that the number of male adolescents that have high glucose levels was more elevated than female.

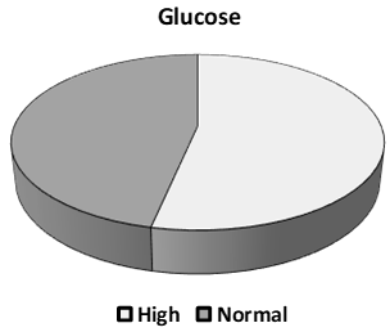

Figure 4. Percentage of students with high glucose levels.

Table 3. Cases with high glucose level according to gender

	Fasting glucose level		
Gender	High	Normal	Total
Male	11	14	25
Female	29	21	50
Total	40	35	75

Blood Lipids

Dyslipidemia is associated with significant comorbidities and complications, and with cardiovascular risk factors (obesity, diabetes mellitus, hypertension, and smoking) (Yoon 2014).

In this study, 16 adolescents showed high triacylglycerides levels, representing 21% of the sample (Figure 5). A similar percentage of hypertriglyceridemia has been reported for Chilean teenagers with 19,2% in risk and 11,5% at high risk of cardiovascular disease (Barja Yáñez et al. 2015). Another study reported a higher incidence of dyslipidemia in obese children, up to 30% (Montero 2010).

For hypertriglyceridemia, there was not a difference between male and female cases (Table 4). On the other hand, Barja Yáñez et al. (2015), reported a higher incidence of hypertriglyceridemia in men than in women, 36.2% vs. 27.4%, respectively.

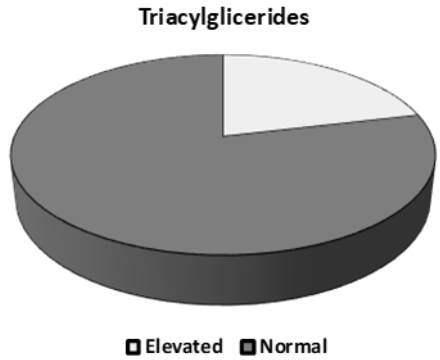

Figure 5. Percent of students and Triacylglicerides level.

Table 4. Triacylgliceride blood levels in adolescents

	Triglyceride concentration		
Gender	Elevated	Normal	Total
Male	8	17	25
Female	8	42	50
Total	16	59	75

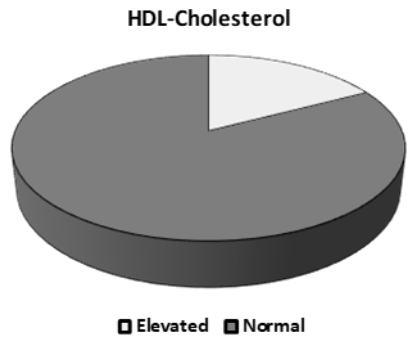

Figure 6. Percentage of students and Cholesterol-HDL levels.

HDL-cholesterol is known as "good" cholesterol. It helps remove LDL from the blood. It also helps prevent plaque in the blood vessels. The higher the HDL-C levels the better. In this study, all HDL-C values could be considered good because they are in or above the standard (Figure 6).

Presence of Metabolic Syndrome in Adolescents

The utmost importance of metabolic syndrome is that it helps identify individuals at high risk of both type 2 diabetes and cardiovascular disease (CVD). Several expert groups have therefore attempted to produce diagnostic criteria. The criteria had insulin resistance or its surrogates, impaired glucose tolerance or diabetes, as essential components, together with at least two of: raised blood pressure, hypertriglyceridemia and/or low HDL-cholesterol, obesity (as measured by waist/hip ratio or body-mass index), and microalbuminuria (Alberti, Zimmet, and Shaw 2005, Alegría, Castellano, and Alegría 2008, Álvarez et al. 2014, Apovian 2016, Baron and Márquez 2010, Bolado et al. 2015).

The presence of MetS was established after the subjects presented at least three impaired parameters. After correlating all the parameters: abdominal obesity, glucose level, blood pressure, triacylglycerides, and HDL-Cholesterol, seven males and nine females presented MetS (Table 5). The sum of cases represented a MetS prevalence of 21% independently of gender.

Burrows et al. (2007) reported that the prevalence of MetS was significantly different among subjects with or without risk of obesity, no-insulin resistant, and insulin-resistant, however, these authors did not observe differences attributed to gender (28.4% vs. 26.0% for male and female, respectively). In the study conducted by Romero-Velarde et al. (2016) the prevalence of MetS was higher in women (60%) than in men (48%). While in the Cárdenas-Villarreal et al. (2010) study, a prevalence of 9.4% of metabolic syndrome was obtained.

Several studies have shown that the amount of visceral adipose tissue correlates directly, both in men and women, with a severely altered metabolic risk profile, which precedes the development of Type 2 diabetes mellitus and cardiovascular disease. Therefore, although obesity indeed increases the risk of chronic diseases, it seems clear that it is patients with visceral obesity who form the subgroup of individuals with the most severe metabolic disorders. Thus, it has been shown that regional accumulation of fat in visceral deposits is a more reliable predictor of cardiovascular risk than the total amount of body fat (Alegría, Castellano, and Alegría 2008, Castillo-Durán, Le Roy, and Osorio 2012).

Table 5. Prevalence of MetS by gender

Gender	**MetS**			
	Present		**Absent**	
	Cases	**%**	**Cases**	**%**
Male	7	43.8%	18	30.5%
Female	9	56.3%	41	69.5%

The pathogenesis of the metabolic syndrome is complex and genetic and environmental factors are involved. The findings suggest that obesity, and subsequently insulin resistance,

participate in this process and are both closely linked. Obesity has an important role, since adipose tissue, especially visceral or abdominal, releases different substances that can favor the appearance of a proinflammatory state, insulin resistance, and endothelial damage. The non-esterified free fatty acids that are generated, increase in plasma and promote the increase of gluconeogenesis, of the production of triglycerides, of substances with prothrombotic activity and decrease of high-density lipoproteins (HDL-cholesterol) (Araujo-Herrera 2015, Castillo-Durán, Le Roy, and Osorio 2012).

The assessment of risk factors for metabolic syndrome is required for all obese children and adolescents, and a well trained professional team must manage it.

CONCLUSION

There was a strong relationship between abdominal obesity, hypertension, and high levels of glucose and TG and low HDL cholesterol in adolescents of the junior high school "Antonio Caso."

A high prevalence of MetS was observed in obese adolescents. Obesity was the more significant criterion that helped to define the presence of the metabolic syndrome.

It is essential and necessary to implement timely activities on health promotion, disease prevention, and specific action plans aimed at orienting adolescents, their families, their communities, and the teaching and administrative staff of educational institutions so that they know the impact of this and other metabolic and chronic diseases. These plans would be focused on combat, in particular, the problems of obesity detected, in addition to lifestyles that include changes in diet and the performance of physical activity within an individualized plan.

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Chapter 20

USE OF MEDICINAL PLANTS FOR THE TREATMENT OF PARKINSON'S DISEASE: A BRIEF REVIEW

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ABSTRACT

Parkinson's disease (PD) is a progressive neurological disorder that presents several motor and non-motor features in a variable degree, which directly affects the patient's health-related quality of life, affecting mainly adults and the elderly. Parkinson's disease patients lose 60 to 80 percent of the dopamine-producing cells in the substantia nigra, an important area of the brain that regulates muscle movement. On the other hand, the non-motor alterations are more significant, among which neuropsychiatric, sensory or gastric-intestinal, cognitive (like depression and anxiety) symptoms stand out. Modest personality changes can also be present over time in this kind of patient. PD is considered a multifactorial disease resulting from both environmental factors and genetic susceptibility. Several studies have demonstrated that age is an unequivocal risk factor for this disease as the incidence increases with age increasing. The etiology of the PD is not clear; for that reason, the disease remains incurable. Levodopa is the most used medication for the treatment of PD, used to simulate the dopamine effect. Different studies have identified compounds with antiparkinson activity in *Mucuna pruriens*, *Nardostachys jatamansi*, *Withania somnifera*, *Gingko biloba*, and *Bacopa monnieri*, species that represent a potential alternative for the treatment of this disease.

Keywords: PD, neurological, dopamine, medicinal plants

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INTRODUCTION

Dr. James Parkinson discovered Parkinson's disease (PD) in 1817, who called the condition the "shaking palsy." PD is a chronic neurodegenerative process that occurs in adulthood and is the second most common degenerative disease after Alzheimer's disease (DeMaagd and Philip 2015).

PD is the most common progressive neurodegenerative movement disorder affecting more than 10 million people worldwide, and the incidence of PD increases with age. According to the World Health Organization (WHO), Parkinson's disease affects 1 in 100 people over 60 years. Currently, there are about 7 million people with this disease in the world, and WHO predicts that by 2030, they will reach more than 12 million. The individuals suffering from PD tend to be between 55 and 65 years old, although in some cases, the disease can appear before 40 years old (Srivastav, Fatima, and Mondal 2017). The risk of suffering from the illness as age advances, as well as the global aging of the population, makes the number of people suffering from this pathology also increase. In recent years, PD has become the main focus of scientific research since it is one of the most common neurodegenerative disorders in the United States (Hermanns and Engebretson 2010). According to the Parkinson's Disease Foundation, there are 1 million Americans that suffer from PD. The burden associated with PD is also increasing (Rocca 2018). Many people affected by neurological disorders, or those who care for them or their families, have difficulty accessing appropriate care; that is the reason why the WHO advocates that neurological care is integrated into primary health care. The exact cause of Parkinson's disease is unknown, but it is known that genetic and environmental factors influence its development (Hermanns and Engebretson 2010, Rocca 2018).

PATHOPHYSIOLOGY OF THE PARKINSON DISEASE

Studies show that most people with Parkinson's disease have lost 60 to 80 percent of the dopamine-producing cells in the substantia nigra once symptoms appear. Also, this can affect nerve endings that produce norepinephrine and affect the nerve endings that produce norepinephrine, which is the principal chemical messenger of the sympathetic nervous system, which is part of the nervous system responsible for regulating many of the body's automatic functions such as blood pressure and pulse. The loss of norepinephrine may help explain many of the non-motor symptoms present in Parkinson's disease, such as fatigue and abnormalities related to the regulation of artery pressure (NIH 2016).

PD is characterized by the loss of neurons in certain parts of the brain, specifically in the region known as the substantia nigra, neurons located in this area of the brain produce a chemical substance called dopamine. Dopamine is the neurotransmitter responsible for transmitting signals between the substantia nigra and the striatum to provide uniform and deliberate movements (Aldred, Buck, and Vall 2009).

PD is a disease of the extrapyramidal system which links the higher motor centers and effector motor cells of the spinal cord. Two types of dopamine receptors belong to the extrapyramidal system: D1, excitatory type, and D2, inhibitory type. Other elements of the extrapyramidal system include the internal globus pallidal segment (GPi) of the ventral striatum and the pars reticulata allocation of the substantia nigra (SNpr). When dopamine activity starts

to diminish, it causes a boost in the activity of the GPi/SNpr circuits, which then leads to a flaw in the gamma-aminobutyric acid (GABA) and consequently generates an inhibition of the thalamus. Eventually, the thalamus fails to activate the frontal cortex and without the activation of the frontal cortex, there is a loss of motor activity, an important characteristic in PD. The loss of dopamine activity can also result in the development of cholinergic activity (DeMaagd and Philip 2015).

Another important histopathological feature of PD includes the existence of Lewy bodies (LBs). LBs are atypical clumps of main protein, but can also contain lipids and other materials, that form inside of the brain and have been associated with several neurodegenerative diseases, including PD and dementia. In PD, LBs have been in the substantia nigra, where the dopaminergic neurons are based, in the form of round fibril bodies. Research has shown that the formation of LBs may be caused by a genetic mutation in the synthesis of alpha-synuclein (αSyn) protein that causes the presence of insoluble fibrils (DeMaagd and Philip 2015, Wakabayashi et al. 2007).

LBs are formed when there is an overproduction of ubiquitin proteins (Ub). Ub-proteins are small proteins that are involved in the intracellular degradation of other proteins. An accumulation of Ub-proteins causes an impairment in the ubiquitin-proteasome system (UPS) (DeMaagd and Philip 2015). The presence of ubiquitin in LBs suggests that they can eliminate damaged cellular elements. LBs also contains fatty acids, sphingomyelin, and polysaccharides (Wakabayashi et al. 2007).

The development of LBs can be divided into four stages, where the first stage consists of the diffuse cytoplasmatic anti-α-synuclein staining, the second stage consists of the occurrence of irregularly shaped staining of moderate-intensity, the third stage is described as the discrete staining corresponding to pale bodies and the last step is the ring-like staining of a typical LB with a central core and a surround halo (Halbach, Schober, and Krieglstein 2004).

Parkin is a 465-amino acid protein that is responsible for acting as an ubiquitin ligase. Parkin is metabolized by the UPS. The mutation in the parkin gene (PRKN) causes autosomal-recessive juvenile Parkinsonism (ARJP) since the E3 ubiquitin-ligase function is damaged, inactivating the Parkin protein (Miller and Muqit 2019, Snyder and Wolozin 2004).

Ubiquitin C-terminal hydrolase isozyme L1 (UCH-L1) is responsible for catalyzing the hydrolysis of C-terminal ubiquityl esters and amides. This allows the release of ubiquitin from substrates. When there is an overexpression of UCH-L1, this causes an accumulation of αSyn. The accumulation of αSyn is found in LBs, and several studies suggest that finding the factors that promote synuclein accumulation is a key factor in understanding the pathophysiology of PD (Snyder and Wolozin 2004).

Pale bodies are found in the pigmented neurons of the substantia nigra and locus coeruleus, those pale staining and poorly circumscribed lesions are referred to as pre-inclusions or cortical type Lewy bodies (Dickson 2018). They are immunohistochemically like LBs, which suggests that they may be precursors to LBs. Dale et al. (1992) proposed that pale bodies are either formed parallel to LBs in the substantia nigra in patients with PD or pale bodies are a stage in the formation of LBs.

Another protein that plays a significant role in the development of Parkinson's is Synphilin-1. The protein interacts with αSyn under pathological conditions, and they are present in LBs. In healthy humans, the role of both proteins has not been determined, but in patients that have PD Synphilin-1 binds to αSyn, and this causes the formation of cytosolic inclusions. LBs contain an unusual amount of αSyn, which is why LBs also contain a high amount of Synphilin-

1 since it is their interaction partner. Research shows that neuronal expression patterns of αSyn and parkin mRNAs closely related, which proposes that both proteins are involved in the development of PD. In patients with PD αSyn is ubiquitinated by parkin, while in healthy patients αSyn does not interact with parkin (Halbach, Schober, and Krieglstein 2004).

EPIDEMIOLOGY

As mentioned, PD is a neurodegenerative disease that, as it progresses, the motor and cognitive symptoms decline, neuropsychological problems and treatment-related complications appear, which directly affects the patient's health-related quality of life (HR-QoL). According to studies conducted by the Global Parkinson's Disease Surveys, other factors besides the severity of the disease and its treatment, such as the patient's satisfaction with the explanation of their diagnosis, their emotional state and the optimism about the condition are also related with HR-QoL (Chen and Tsai 2010).

Two key factors must be considered when an epidemiological study of PD is carried out: the case definitions used and the population studied. Another consideration is to classify Parkinsonism cases into different variants. PD is a term used by neurologists for Lewy body disease; however, its diagnosis can be made accurate only by autopsy. The distinction of different Parkinsonism variants from PD is difficult, even by evaluating the response to levodopa. Shy-Drager syndrome (SDS), striatonigral degeneration (SND), progressive supranuclear palsy (PSP), and multiple system atrophy (MSA) are some Parkinsonism variants. Classification into these different variants also requires autopsy studies to confirm the diagnosis, which is not possible in epidemiological surveys; therefore, an epidemiological study is considered only descriptive (Rajput et al. 1984, Tanner and Goldman 1996).

Prevalence and Incidence

Tanner and Goldman (1996) define the term of incidence as the number of new cases of a disorder first diagnosed at a specific time interval within a specified population and the prevalence as the total number of persons with the disease at a fixed point in time. Chen and Tsai (2010) reported a range of crude incidence from 65.6 per 100,000 to 12,500 per 100,000 in Europe countries, and the incidence from 5 per 100,000 to 346 per 100,000. Fleury et al. (2018) identified 1235 living patients with Parkinsonism in Switzerland, from which 80% presented with a degenerative form and 20% with a non-degenerative form of Parkinsonism. Among the former, PD was the most frequent diagnosis (81%) followed by dementia with Lewy bodies (9%), progressive supranuclear palsy (3.9%), multisystem atrophy (1.9%) and corticobasal syndrome (1.4%). It is inferred that the full ranges obtained are due to the methodologies used by the different research groups, which include case findings protocols, diagnostic criteria, and the age of the study population.

Risk Factors

PD is considered a multifactorial disease resulting from both environmental factors and genetic susceptibility. The etiology of the PD is not clear; for that reason, the disease remains incurable (Chen and Tsai 2010). However, some risk factors have been identified and related to PD. Based on the research *Epidemiology of Parkinson's disease* (Tanner and Goldman 1996), they are the following:

- *Age*. Several studies have demonstrated that age is an unequivocal risk factor for PD as the incidence increases with increasing age; their exact relationship is unknown, but it is generally attributed to age-related neuronal vulnerability (Halbach, Schober, and Krieglstein 2004).
- *Gender*. Women usually have greater longevity than men; therefore, they constitute an increasing percentage of the population as age increases, then it would be expected a higher prevalence of PD in women. However, some studies found that men have a modestly increased age-adjusted PD prevalence. This relationship is also unknown. According to Lyons et al. (1998), in a 330 patients study (half men, half women), there were no gender differences for the age of diagnosis or the mentation and activities of daily living. However, as PD progresses, gender differences emerge, with men exhibiting more severe parkinsonian motor features and women experiencing more levodopa-induced dyskinesia.
- *Race ethnicity*. The PD prevalence is higher in Europe and North America; Japan, China, and Africa registered a lower number of cases. Additionally, based on studies in the United States and Africa, PD prevalence is lower in people with dark skin. However, door-to-door studies performed in Copiah County, Mississippi found a similar prevalence within black and white people; and a Parsi colony in Bombay, India, the prevalence was similar to that found in Europe and North America. This relationship can be attributed to socioeconomic and environmental factors more than genetic characteristics according to the results (Halbach, Schober, and Krieglstein 2004, Dahodwala et al. 2009).
- *Genetic Predisposition*. An autosomal-dominant pattern was suggested for PD by some authors. However, neither of these studies used population-based methods; therefore, these results are not generalizable to the entire population. On the other hand, twin studies support a multifactorial inheritance with symptoms dependent on environmental factors because similar concordance rates between monozygotic and dizygotic twins the obtained (monozygotic twins show a higher concordance in autosomal-dominant disorders). Referring to multifactorial theories of PD etiology, some variant alleles for cytochrome P450 isoenzyme CYP2D6, and specific monoamine oxidase haplotypes are more frequent in patients with PD; therefore they could be involved in an increased risk. All these possibilities require further research as the role of genetic factors in PD etiology is not established yet (Piccini et al. 1999, Balck et al. 2019).
- *Toxicant Exposure*. It is known that PD is caused by numerous chemicals — similar anatomic and clinical features, and in individuals who administered intravenously the compound 1-methyl-1,2,4,6-tetrahydropyridine (MPTP). Therefore, there is the

hypothesis that exposure to an exogenous agent might cause PD. Related to this, some studies based on antiparkinsonian drug sale to estimate prevalence, found a higher disease prevalence in areas where vegetable farming, wood pulp mills, and steel alloy industries were established. Other factors such as rural residence, well water drinking, and exposure to herbicides/pesticides are also related to an increased risk for developing PD. However, all of the studies were performed on a small scale and using different methods, for that reason, the results cannot be generalized (Calne and William Langston 1983, Ascherio et al. 2006). Many studies show that rotenone and paraquat are linked to increased PD risk and PD-like neuropathology. Organochlorines have also been linked to PD in human and laboratory studies. Organophosphates and pyrethroids have limited but suggestive human and animal data linked to PD. Metals such as iron are elevated in PD brain tissue, but the pathophysiological link is unclear. PD due to manganese has not been demonstrated, but a parkinsonian syndrome associated with manganese is well-documented (Nandipati and Litvan 2016).

- *Infection*. The Parkinsonism was a typical late sequela of encephalitis lethargica; consequently, all cases of PD were related to the exposure to that infectious agent. However, currently, few cases of Parkinsonism are identified as postencephalitic. On the other hand, some authors found increased *Nocardia* antibody titers in PD patients; this bacterium presents a specific affinity for substantia nigra neurons, causing a levodopa-responsive movement disorder in mice. The exposure to infectious agents also represents an important factor associated with rural residence, which involves the increased risk of developing PD (Jang et al. 2009).
- *Trauma*. Generally, individuals with a chronic illness relate their disease with prior experiences; for example, patients with Alzheimer's disease show an association between head trauma and the disease. PD patients who sustain head trauma have not demonstrated a change in the course of their condition. Only if the prospectively collected information indicates an association, trauma is considered to increase the risk of PD (Sundman, Hall, and Chen 2014).
- *Emotional Stress*. Stress-produced changes in central dopamine systems have been reported by laboratory studies, which could contribute to the development of PD. Likewise, the PD patients experience transient worsening of their symptoms during stressful periods. However, evaluation of the emotional or physical stress factors that could contribute to the onset of PD has to be studied more deeply to confirm their role in the disease (Dallé and Mabandla 2018).

Symptoms

The main symptoms of Parkinson's disease are motor alterations, although it also presents non-motor disorders. Within the first group of changes are mainly the typical cardinal signs such as the resting tremor that occurs in 70% of cases and have a frequency of 4 to 6 Hz or the akinesia of both spontaneous and voluntary movements. On the other hand, the non-motor alterations are more significant, among which neuropsychiatric, sensory, or gastric-intestinal symptoms stand out (Table 1).

Table 1. Non-motor symptoms in Parkinson disease (Martínez-Fernández et al. 2016)

Neuropsychiatric	Depression, anxiety, apathy, hallucinations (commonly visual), and delusions. Soupminergic dysregulation syndrome (related to dopaminergic agents and panic attacks.
Sleep disorders	REM sleep behavior disorders, excessive daytime hypersomnia, sleep attacks, insomnia, restless pie syndrome, and fatigue.
Sensitive	Pain, hyposmia, and visual disturbances (blurred vision, diplopia, and alteration of colors).
Autonomic dysfunction	Urgency and urinary frequency, nocturia, and sexual dysfunction.

According to the NHS, there are many different symptoms associated with Parkinson's disease. These symptoms are separated into three categories: Main, physical, and Cognitive and psychiatric. There is no rule of the order in which the symptoms could develop or the severity of each, but it´s uncommon that a person develops all the signs. The three main symptoms of Parkinson's disease affect physical movement.

Tremor or shaking is an involuntary, rhythmic, and movement with an oscillatory behavior of body parts. PD's tremors occur in rest when the affected body part, most commonly hands and arms, is supported against gravity (Smaga 2003). This symptom is present in 70% of patients with Parkinson's disease (Gironell et al. 2018). The slowness of movement (bradykinesia) causes movements much slower than normal, which can make everyday tasks difficult and can result in a distinctive slow, shuffling walk with tiny steps (NHS 2019). Muscle stiffness (rigidity) is a change of a mechanical property, associated with its structural and neural drive (Marusiak et al. 2012). It is present in more than 90% of patients (Sama et al. 2017).

Non-motor symptoms include balance problems that can make someone with the condition more likely to have a fall and injure themselves (NHS 2019). The loss of sense of smell is a first feature characteristic of PD, present before clinical motor symptoms for years, so they can be a factor that allows you to assess the risk of developing PD. This loss has a bilateral and general sense in the PD, in which each olfactory domain is involved, being independent of the duration and severity of the disease (considering that progressive deterioration has been observed in the early motor stages) (Haehner, Hummel, and Reichmann 2014).

Nerve pain can cause unpleasant sensations, such as burning, coldness, or numbness. Pain is regulated within the nervous system by dopamine. Unexplained pain is a common non-motor symptom of PD. In a study carried out by the DOPAMIP in southwest France, 450 patients were surveyed, and 62% of the patients reported to have some chronic pain. Different types of pain can be symptoms of PD: musculoskeletal pain, PD-related chronic pain, fluctuation-related pain, nocturnal pain, coat hanger pain, oro-facial pain, and peripheral limb or abdominal pain. For example, fluctuation-related pain includes dyskinetic pain, "off" period dystonia-related pain, and "off" period generalized pain (Chaudhuri and Schapira 2009).

The loss of dopamine in the brain also affects the urinary tract. Problems with urination include having to get up frequently during the night to urinate or unintentionally passing urine (urinary incontinence). The detrusor muscle is affected by dopamine receptors, which cause the urge to urinate frequently. Patients with PD lose bladder contractility or have an atypical sphincter action, which causes voiding. It has been shown that apomorphine injections can solve this problem. In some cases, levodopa can either improve or worsen the symptoms of

urination urgency. Nocturia, the need to urinate in the middle of the night, is one of the most common urination problems in patients with PD (NHS 2019).

Constipation is a non-motor symptom of PD that occurs in two-thirds of all patients, resulting in psychological and social problems, affecting their quality of life. It is characterized by colonic and anorectal symptoms and has a wide range of causes (lifestyle, physical weakness, low fluid intake, among others). About PD, the delay in colonic traffic is a consequence of an alteration of the regulation of the disordered central and peripheral parasympathetic system (Pedrosa Carrasco, Timmermann, and Pedrosa 2018).

Sexual inability to obtain or sustain an erection (erectile dysfunction) in men and difficulty becoming sexually aroused and achieving an orgasm (sexual dysfunction) in women is related to neuronal degeneration affecting central sympathetic neurons and postganglionic fibers. Hypersexuality is a side effect of long term use of dopamine agonists, as well as other dopaminergic therapies (Chaudhuri and Schapira 2009).

A sudden drop in blood pressure can cause dizziness, blurred vision, or fainting when moving from a sitting or lying position to a standing one. Visual impairment is considered a premotor marker for PD since the lack of dopamine causes a decrease in retinal dopamine. Few studies suggest that blurred vision can be treated with dopaminergic therapies (Chaudhuri and Schapira 2009).

Swallowing difficulties (dysphagia) in PD can lead to malnutrition and dehydration. Another no-motor symptom is excessive production of saliva (drooling); while problems sleeping (insomnia) can result in excessive sleepiness during the day. Dopamine plays an important role in the sleeping cycle, which is why many patients with PD present sleeping problems. Insomnia is one of the most common sleeping dysfunctions in PD. Sleep-onset insomnia is a type of insomnia where the person has trouble falling asleep while sleep-maintenance insomnia is where a person has difficulty staying asleep for certain periods. Levodopa and carbidopa have shown to improve the sleep patterns of patients with PD and also improved nocturnal akinesia. Levodopa and benserazide can also help enhance nocturnal akinesia and disruptive nocturnal symptoms (Chaudhuri and Schapira 2009).

On the other hand, cognitive and psychiatric symptoms are depression and anxiety that can range in severity and may improve with Parkinson's disease treatment, medications, and "talking therapy" or psychotherapy, such as cognitive-behavioral therapy (APDA 2019). Depression is a severe matter to anyone. For people with Parkinson's, it can affect critical elements of disease management, such as staying socially connected, exercising, and proactively seeking needed care (Richard and Kurlan 2006). It could also be mild cognitive impairment, slight memory problems, and problems with activities that require planning and organization.

Dementia is a consecutive PD syndrome, which has an incidence of 40% of patients. It is characterized by a cognitive slowdown, attention deficit and visuospatial, executive, and memory deficits. It is not determined how this pathology correlates with dementia associated with PD, but Lewy bodies outside the black substance have been evidenced, with the presence of amyloid plaques and neurofibrillary clews (Emre et al. 2004).

Modest personality changes can present over time in PD patients. Also, Parkinson's medication can, in some instances, cause impulse control disorders (ICD). An ICD can manifest as hypersexuality, pathological gambling, and other impulsive behaviors (APDA 2019).

Finally, psychosis associated with Parkinson's disease causes patients to experience hallucinations and/or delusions. More than half of all patients with Parkinson's disease eventually develop psychosis symptoms throughout their illness (APDA 2019).

In the normal midbrain, the substantia nigra is darkly pigmented. In idiopathic Parkinson's disease, marked pallor is due to degeneration and loss of dopaminergic neurons. Some of the surviving neurons contain characteristic eosinophilic Lewy body inclusions. Through a microscope, intracellular Lewy bodies can be seen. The neurons of the substantia nigra pars compacta are particularly affected, which results in depletion of dopamine to its primary projection area, the striatum. The overall outcome of this depletion is an overactive subthalamic nucleus, which increases the activity of the major inhibitory output nuclei (globus pallidus and substantia nigra pars reticulata), which in turn results in increased thalamic inhibition and problems with motor output.

Diagnostic

In general, the PD diagnosis focuses on the patient's history and a physical examination. The presence of PD is estimated if a person presents one or more of the fundamental characteristics of the disease (rigidity, bradykinesia, tremor at rest, asymmetric start and postural instability), also known as cardinal signs (except for postural instability, since it only occurs in 37% of advanced patients, considering it from 5 years of the disease; whereas, in general, it is a more common symptom of the onset of atypical parkinsonisms). Among its various characteristics, the typical resting tremor is an important factor that increases the possibility of presenting PD. However, about 20% of the patients did not show such characteristics (Savitt, Dawson, and Dawson 2006).

Since clinical manifestations are insufficient in terms of sensitivity and specificity by themselves, combinations of clinical parameters have been established to identify different levels of diagnosis (Table 2) (Tapia-Núñez and Chaná 2004).

Table 2. Parkinson's disease diagnosis criteria (Tapia-Núñez and Chaná 2004)

Diagnosis	Criteria
Possible	If there are at least 2 of the 4 cardinal signs (one must be tremor or bradykinesia).
	If there is an absence of atypical symptoms.
	Response to the treatment of levodopa or dopaminergic, as well as the absence thereof.
Probable	If there are at least 3 of the 4 cardinal signs.
	If there is an absence of atypical symptoms, for at least 3 years.
	Response to the treatment of levodopa or dopaminergic.
Confirmed	If all the possible criteria for diagnosis are presented.
	Autopsy.

According to Savitt, Dawson, and Dawson (2006), regarding the patient's history, associated symptoms, and evidence suggesting another possible diagnosis are sought. Examples of related symptoms are anxiety, constipation, depression, sleep disorders, and

fatigue. Similarly, at an early stage of the disease, the patient may present symptoms such as tremor, slowness, imbalance, and rigidity, even having obtained a normal neurological examination. Another point to consider for PD diagnosis is an important and prolonged clinical response to dopaminergic therapy (explained ahead), as well as a lack of it, as a considerable point to explore the option of an alternative diagnosis. The rate of misdiagnosis for PD is approximately 10 to 25% and its complexity lies in its clinical heterogeneity. Patients may present or lack a tremor, and patients who exhibit stiffness and/or postural instability tend to the more rapid development of the disease than those who experience an early tremor. This inaccuracy and the objective to diagnose pre-symptomatic patients opens the door to the identification of biomarkers that allow the application of optimal clinical trials and techniques.

On the other hand, the approach of gene analysis has allowed important advances in the understanding of the causes and mechanisms of PD, as well as other neurodegenerative diseases.

Some Mendelian forms of PD are: Defective gene with autosomal dominant transmission: sinuclein / PARK1; Defective gene with autosomal recessive transmission: parkin / PARK2; Mutation in the ubiquitin-hydroxylase-C-terminal-L1 gene: UCH-L1 / PARK5 (no clear pathogenic role has been shown); Autosomal recessive forms: PARK6 and PARK7; Dominant forms of PD: PARK3, PARK4, and PARK8 (localized genetic defect, but unknown genes responsible).

Non-Mendelian forms usually occur sporadically; their causes are unknown and monogenic models tend to be inefficient for their explanation. They present a complex interaction of many genetic and non-genetic factors. However, the molecular study of Mendelian forms has allowed exposing important mechanisms to the pathogenesis of common non-Mendelian types. Besides, the disposition of data acquired by genomic studies in common non-Mendelian types opens the door to the development of a rapid way to the identification of genomic regions with genetic risk factors, or that alter the risk, or phenotype of classic PD (Tapia-Núñez and Chaná 2004).

PHARMACOLOGICAL TREATMENT OF PD

According to *European Parkinson's Disease Association* (EPDA) in its official web site, there is no specific treatment for PD. Treatment is based on every patient since every case of PD is a little different from person to person. There are certain types of medications that can be used to control the symptoms of PD.

Levodopa

Levodopa is one of the main drugs used in the treatment of PD. Levodopa is used to replace the dopamine that is not formed in patients with PD. Since symptoms of PD start to appear when dopamine levels are low, due to the death of the cells that produce dopamine. When taking levodopa, a chemical that naturally occurs in the body and is a precursor for dopamine, nerve cells can produce dopamine. The peripheral enzyme dopa-decarboxylase (DDC) is responsible for breaking down levodopa. By blocking the action of DDC, using a DDC

inhibitor, the amount of levodopa needed in the medication is less, which lowers the likelihood of the patient suffering from side-effects, such as nausea and vomiting. Levodopa is usually always prescribed with a DDC inhibitor, either carbidopa or benserazide. Levodopa can be used at all stages of PD. Apart from nausea and vomiting, which is controlled by DDC inhibitors, levodopa can cause severe stiffness in the body where the patient sometimes is not even able to move, known as "off" periods. Another significant side-effect of long-term use of levodopa is involuntary movements, known as dyskinesia (Goldenberg 2008).

COMT (Catechol-O-Methyltransferase) Inhibitors

As mentioned previously, the symptoms involved in PD is due to the lower levels of dopamine in the brain, caused by the death of the cell that produces this chemical. COMT is responsible for breaking down dopamine. COMT inhibitors block COMT from breaking down the dopamine.

There are two types of COMT inhibitors used in PD patients: entacapone and tolcapone. It is important to take into account that these medications are used to help levodopa work more efficiently since COMT inhibitors are not effective on their own (EPDA, 2018).

Dopamine Agonists

Dopamine agonists are used in the treatment of PD because of their ability to provide antiparkinsonian benefits, thanks to the direct stimulation of dopamine receptors. There are several mechanisms by which dopamine agonists can provide neuroprotection to a patient with PD: reduced need for levodopa, inhibition of STN-induced excitotoxicity, stimulation of dopamine autoreceptor, direct antioxidant effects (Olanow, Jenner, and Brooks 1998).

MAO-B (Monoamine Oxidase B) Inhibitors

Dopamine agonists need to be taken in conjunction with other medication since it is not considered to be an effective treatment for PD. MAO-B inhibitors allow the brain to make use of the dopamine that it can produce. MAO-B breaks down dopamine in the brain but by inhibiting the action of MAO-B there is more dopamine available in the brain, which helps relieve the symptoms of PD. Rasagiline, selegiline, and safinamide are all types of MAO-B inhibitors.

Amantadine

Amantadine allows the control of tremors and stiff muscles in patients with PD. Although patients usually show symptomatic improvement when included in their treatment, their mechanism is still uncertain. Some proposed mechanisms are increased synthesis, release or

decrease of dopamine reuptake, anticholinergic effects (causing striatal D2 receptors to reach high-affinity states or affect other neurotransmitter systems (Uitti et al. 1996).

Anticholinergic Medication

Anticholinergics block the action of acetylcholine since patients with PD there is an overproduction of this chemical. This medication is not commonly prescribed today, but it can be prescribed in the early stages of PD before using levodopa to reduce the symptoms of tremors and muscle stiffness. This medication only works when symptoms are mild and can be taken on its own (EPDA, 2018).

MEDICINAL PLANTS USED IN THE TREATMENT OF PD

An herb is a part of a plant or a plant used for its scent, flavor, or therapeutic properties. Herbal medicines are one type of dietary supplement. They are sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. People use herbal medicines to try to maintain or improve their health. They have side effects, and regulatory procedures differ from country to country, so it is not always clear what the remedy contains, in what concentration, or whether it was manufactured properly. As a result, the safety of herbal medicines is often questioned. Herbal products for PD have been used worldwide in traditional medicine (Kartika, Palayyan, and Rahman 2010).

Mucuna pruriens

Mucuna pruriens is a legume native of India, Central, and South America; which presents trifoliate leaves, purple flowers, hairy pods, and seeds to which benefits have been attributed as effective therapeutic agents for the treatment of various diseases of the human nervous and reproductive system. Although the content of levodopa in its seeds was known, the amounts that used to be used were not sufficient for an optimal explanation of its role in the treatment of PD. The seeds have different components; however, only the antiparkinson activity of levodopa has been demonstrated (Manyam, Dhanasekaran, and Hare 2004).

In India, seed preparations were used for the treatment of Parkinson's disease. In 1937 the levodopa of seeds of *Mucuna pruriens* was isolated for the first time, finding a factor of value for the treatment of PD and reviving the interest in the study of abundant plants in L-dopa (Katzenschlager et al. 2004). Because there were no randomized controlled studies that demonstrated the effectiveness of *Mucuna pruriens* extracts in the treatment of PD, (Katzenschlager et al. 2004) developed a survey in which a comparison was made between a formulation of *Mucuna* seed powder and synthetic L-dopa, about its activity in said treatment. With what they obtained that with a *Mucuna* preparation of 30 g, the onset of the effect is obtained with greater rapidity (average time of 37 min); and lower latency to obtain maximum plasma concentrations of L-DOPA (110%) — concluding that the formulation of *Mucuna* seed

powder represents a natural source of L-dopa that could offer advantages over the conventional synthesis of L-dopa, for the treatment of PD.

Nardostachys jatamansi

Nardostachys jatamansi (Jatamansi) is a small, perennial herb commonly known as jatamansi, Indian nard, balchar, or spikenard. It is a hairy, rhizomatous medicinal herb growing in steep, moist, rocky, undisturbed grassy slopes from 2,200 to 4,800 m asl in random forms (Ved, Saha, Ravikumar, & Haridasan, 2015).

Nardostachys is a supposedly calming herb from Ayurveda that has been used for anticonvulsant and antiepileptic applications. It may enhance learning in youth and act as a neuroprotector, also is protective against pancreatitis (Patel, K., 2013). The functions of this herb are redox stabilization, improves mitochondrial function, attenuate a-synuclein aggregation, attenuate apoptosis and improve cognition. The important components for Parkinson's treatment are bacopaside and bacoside.

Ahmad et al. (2006) studied the neuroprotective effects of ethanolic extract of *Nardostachys Jatamansiin* a 6-OHDA model of Parkinson's disease. They evaluated ethanolic extract which is an antioxidant and enhancer of biogenic amines and can slow the neuronal injury in a 6-OHDA-rat model of Parkinson's. In this study, rats were treated with 200, 400, and 600 mg/kg body weight of ENj for three weeks. Then they infused 2 µL of 6-OHDA (12 µg in 0.01% in ascorbic acid-saline) into the right striatum, while the sham-operated group received 2 µL of vehicle. Three weeks later of the 6-OHDA injection, these rats were tested for neurobehavioural activity and sacrificed after 6 weeks for the estimation of lipid peroxidation, reduced glutathione content, the activities of glutathione-S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase, quantification of catecholamines, dopaminergic D2 receptor binding and tyrosine hydroxylase expression. They found that this extract significantly and dose-dependently inhibits marked increase in drug-induced rotations and deficits in locomotor activity and muscular coordination, which is a reliable marker for nigrostriatal dopamine depletion. Increased D2 receptor population in striatum, increased activities of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) significantly restored by pretreatment with *Nardostachys* by GSH enhancing or antioxidant effect in 6-OHDA lesioned rats and increased TH-IR fiber density by pretreatment signifies the dose-dependent increase in the number of surviving neurons and confirming the anti-Parkinson effects of Ethanolic extract of *Nardostachys Jatamansi*.

Withania somnifera (Indian ginseng or Ashwagandha)

Withania somnifera (WS) is an important herb from the Ayurvedic medical system used for the treatment of debility, emaciation, impotence, and premature aging. Not surprisingly, it has been dubbed the 'Indian ginseng.' Its Indian name, ashwagandha, is said to refer to the 'smell and strength of a horse' and possibly alludes to its reputed aphrodisiac properties, although it could also relate to the smell of the root. Pharmacological research on *Withania* has stressed its antitumor and adaptogenic actions, reinforcing its comparison with Panax ginseng. However, it lacks the potential, stimulating effects of the latter. It has a mild sedative action, as

indicated by its specific name 'somnifera.' It is, therefore, ideally suited to the treatment of overactive but debilitated patients, in whom Panax might tend to aggravate the overstimulation. Many parts of the plant have been used in traditional medicine, including the leaves, bark, and root. It's important to mention that *'Withania'* refers to the use of the root (Bharti, Malik, and Gupta 2016).

Srivastav, Fatima, and Mondal (2017) found out in many studies that *WS* root extract tends to normalize oxidative stress in MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine) induce mouse model of PD by increasing glutathione (GSH) and glutathione peroxidase (GPx) levels. It also tends to increase DA levels in striatum along with improved motor functions in Ws treated a mouse model of PD. A study on 6-Hydroxydopamine (6-OHDA) induce rat model of PD showed that *WS* extract depreciates oxidative stress by normalizing the antioxidant level and also improves TH expression. Another study of Maneb-PQ on a mouse model of PD has reported that the ethanolic extract of *Withania* causes a reduction in iNOS expression and improves locomotor function in the mouse. Therefore, studies on mouse and rat models of PD depict the potential of *Withania* against PD. However, unlike the rat or mouse model, studies done in *Drosophila* model of PD are contradictory, as a couple of studies suggest that *Withania* extract supplementation is ineffective in rescuing PD phenotype. However, the other groups have shown that *Withania* administration to *Drosophila* model rescues PD phenotype. Henceforth, more studies are required that will clarify the potential of *Withania* in *Drosophila* model of PD.

Further, the mitochondrial function also improves upon *Withania* treatment thereby strengthening its potential against PD. PD is characterized by the loss of dopaminergic neurons, and thus the rate of apoptosis should be normalized. In this milieu, *Withania* administration to the maneb-PQ mouse model of PD causes increase in Bcl-2 expression while suppressing Bax thereby regulating apoptosis. Therefore, *Withania* seems to offer a good platform in drug designing against PD; however, more studies are required to validate its potential further.

Gingko biloba (Maidenhair tree)

Ginkgo biloba is a popular tree in the traditional medicine of many countries, mainly China, Japan, and India. Several studies have described that Maidenhair tree leaves have properties antioxidant, anti-inflammatory, anti-aging, and neuroprotective (Srivastav, Fatima, and Mondal 2017). According to (Tanaka et al. 2013), EGb 76, extract obtained from the leaves of the Gb tree, contains flavonoids and terpenoids in a proportion of 24% and 6% respectively, chemical components that provide neuroprotection in animal models induced with EP, for this reason, EGb 76 extract is considered of great help against neurodegenerative diseases, including Parkinson's, Alzheimer's and dementia.

Srivastav, Fatima, and Mondal (2017) proposed a combination of levodopa with EGb 76 extract in an optimized dose that can provide better therapeutic efficacy than either drug alone. Gb 761 offers several advantages since it not only tends to stabilize the redox state in the EP but also helps to rejuvenate mitochondrial functioning and locomotor activity.

Bacopa monnieri (Brahmi or water hyssop)

Brahmi is an herb that is commonly used in the treatment of anxiety, intellect, and poor memory. It is currently marketed as a memory enhancer and contains different active ingredients, including alkaloids and saponins (Roodenrys et al. 2002). The presence of these chemicals gives the herb antioxidative, anti-inflammatory, antimicrobial, neuroprotective, and memory-enhancing properties (Srivastav, Fatima, and Mondal 2017). The Brahmi plant has also been used in holistic medicine as a sedative and anti-epileptic. Experiments performed on rats showed improved motor-learning, acquisition and retention of memory. The memory-enhancing effects of *B. monnieri* are due to the presence of saponins.

In a study done in 2001, *B. monnieri* was given to a group of randomized, double-blind, placebo-controlled unimpaired people, and it was shown that the herb improved how the group processes visual information, and it showed a reduction in anxiety levels. In 2008 a new study showed that Brahmi improved memory accuracy. It is important to take into account that these studies aren't conclusive and don't prove that the herb has cognitive-enhancing properties (Pitt and Leung 2016). In animal models, studies have reported that *B. monnieri* is neuroprotective since it protects lipid peroxidation, cell death and has antioxidant properties. Even though the herb is neuroprotective this still does not prove that it may be a cognitive-enhancing plant. It is believed that *B. monnieri* can enhance memory and could be neuroprotective because bacosides, one of the main saponins found in Brahmi, can alter Ca2+, which improves blood flow to the brain. By augmenting blood flow to the brain, this increases neuronal activity and allows for the brain to work better. This does not prove that *B. monnieri* acts on the central nervous system (CNS), which is important if the herb is to be used as a treatment for PD. An important factor to consider with Brahmi is that in rat models it showed to enhance the concentration of certain neurotransmitters, including serotonin, acetylcholine, GABA, and glutamate (Pitt and Leung 2016). The fact that Brahmi can enhance serotonin concentration is an important factor in considering the herb to treat common PD symptoms. Serotonergic dysfunction is a common symptom in patients with PD, and it just occurs at a much slower rate than dopaminergic dysfunction (Politis and Niccolini 2015).

CONCLUSION

PD is one of the most common neurodegenerative diseases that can be caused by different factors, mainly age and genetic components. However, PD etiology is not completely understood, the evidence previously collected shows that there is a high amount of studies, but treatment is mainly limited to the use of levodopa. Conventional treatments (pharmacological treatments), can have an aggressive effect on the organism, which emphasizes the importance of inversion and investigation of alternative treatments, such as the use of medicinal plants, which has turned to be an effective option to control PD symptoms.

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