We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Inhibitory Properties of Phenolic Compounds Against Enzymes Linked with Human Diseases

Sandra Gonçalves and Anabela Romano

Additional information is available at the end of the chapter

Abstract

Some drugs currently used are inhibitors of enzymes involved in mediating many disease processes. Concerns over the toxicity and side effects of synthetic enzyme inhibitors have led to a search for new safe and effective inhibitors particularly from natural sources. Owing to their wide range of biological effects, plant phenolic compounds are one of the most studied families of natural products. This chapter aims to provide an overview of the potential of phenolic compounds as enzyme inhibitors. Extensive research has been conducted to study the enzyme inhibitory capacity of many phenolic compounds against several enzymes linked with important human conditions. Investigations conducted are mainly focused on the inhibition of angiotensin I-converting enzyme, α-amylase and α-glucosidase, lipase, cholinesterases, proinflammatory enzymes (cyclooxygenases and 5-lipoxygenase) and tyrosinase, which are related with hypertension, type II diabetes, obesity, Alzheimer's diseases, inflammation and skin hyperpigmentation, respectively. Overall, among phenolics, flavonoids are probably those with great capacity to inhibit the activity of the enzymes revised. Several studies demonstrated the potent antioxidant and anti-inflammatory properties of flavonoids, which highlight the therapeutic potential of these compounds. Although our literature survey showed that a huge number of phenolic compounds have been studied and there are some promising compounds depending on the enzyme, more *in vivo* tests and subsequent steps to be a drug candidate are required before therapeutic application.

Keywords: Alzheimer's disease, diabetes, flavonoids, hyperpigmentation, hypertension, inflammation, obesity

1. Introduction

Due to their essential catalytic role in several physiological processes, enzymes are considered to be one of the most attractive targets for drug intervention in human diseases [1]. Indeed,

the therapy of some important human aliments, namely hypertension, metabolic disorders, inflammatory diseases and neurodegenerative diseases, includes the use of enzyme inhibitors. Nevertheless, some of the inhibitors currently in use (**Table 1**) are reported to have side effects, including hepatotoxicity, gastrointestinal disturbances and diarrhea [2–4]. Consequently, there is a great interest in finding new effective natural inhibitors without undesirable effects.

Diseases	Enzyme	Main standard inibitor(s)
Hypertension	Angiotensin-converting enzyme	Aptopril, benazepril, enlapril
Diabetes	α-Amylase and α-glucosidase	Acarbose
Obesity	Pancreatic lipase	Orlistat
Alzheimer's diseases	Cholinesterases	Tacrine, donepezil, rivastigmine, galantamine
Inflammation	Cyclooxygenases and 5-lipoxygenase	Indomethacin
Skin hyperpigmentation	Tyrosinase	Kojic acid

Table 1. Some standard enzyme inhibitors commonly used.

The discovery of enzyme inhibitors to be used in human therapeutics is an active and actual area of research. Several studies provided evidence about the beneficial effects of phenolic compounds in human health due to their wide range of biological properties, namely antioxidant, anticancer, and antimicrobial [5]. The biological actions of phenolic compounds involve different mechanisms including the interaction with enzymes [6]. In the last years, a great number of reports were published describing the inhibitory potential of phenolic compounds against human enzymes. The present chapter aims to systematize the information about the enzyme inhibitory properties of phenolic compounds against key enzymes associated to several human diseases, namely angiotensin I-converting enzyme, α-amylase, α-glucosidase, lipase, cholinesterases, cyclooxygenases (COXs), 5-lipoxygenase (5-LOX) and tyrosinase.

2. Human diseases and phenolic compounds

Chronic diseases or non-communicable diseases are adverse health conditions of long duration and, generally, also of slow progression, causing 63% of all deaths worldwide (36 million out 57 million global deaths) [7, 8]. These diseases can be classified into five main groups: cardiovascular diseases; cancer; chronic respiratory diseases, such as chronic obstructed pulmonary disease and asthma; diabetes; and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. Hypertension is one of the major risk factors for cardiovascular diseases and is estimated to affect one-third of the Western population [9]. Obesity is also considered a global epidemic problem and is associated with multiple chronic diseases [10]. Overproduction of oxidants and chronic inflammation is responsible for the pathogenesis of many chronic diseases, and experimental and epidemiological studies demonstrated that plant antioxidants play an important role in the prevention and treatment of these diseases.

Plant-derived antioxidants, particularly phenolic compounds, can reduce the oxidative stress in the body maintaining, a balance between oxidants and antioxidants due to their reducing, free radical scavenging or metal chelating properties [8]. Phenolic compounds can easily donate hydrogen from hydroxyl groups positioned along the aromatic ring to terminate free radical oxidation of lipids or other biomolecules, and the aromatic phenolic ring can stabilize and delocalize the unpaired electron within its aromatic ring [11].

More than 8000 different structures of phenolics have been identified up to now [12]. Phenolics may be classified into different groups depending upon the number of phenol rings and the structural elements that bind these rings to one another [13]. The main groups of phenolic compounds are flavonoids, phenolic acids, tannins, stilbenes and lignans [13]. Flavonoids are probably the most important group of phenolic compounds. These are molecules of low molecular weight that have a common C6–C3–C6 structure consisting of two aromatic rings (A and B) linked through a three carbon chain, usually organized as an oxygenated heterocycle (ring C) (**Figure 1**) [6]. Flavonoids can be characterized as flavanones, flavones, flavonols, isoflavones, flavanols (essentially, flavan-3-ols) and anthocyanidins. This classification depends on the degree of unsaturation and oxidation of the oxygenated heterocycle [6]. Many authors demonstrated that the position and number of substituents in the flavonoid basic structure significantly affect the biological function [6, 14].
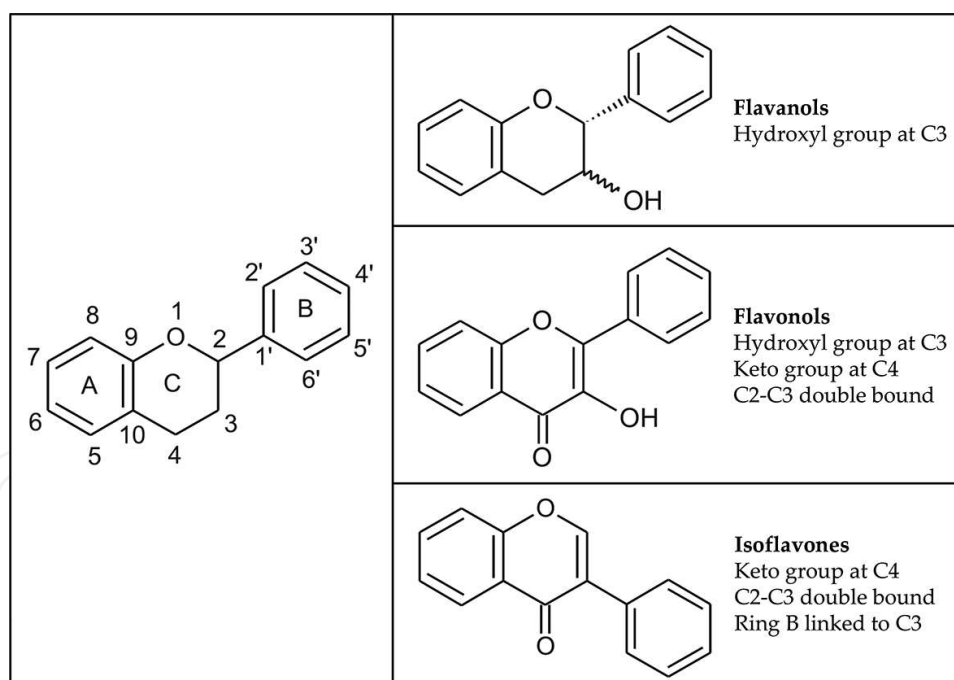

Figure 1. Chemical structure of selected flavonoids. Adapted from Fraga et al. [6].

The biological action of phenolic compounds involves different mechanisms including nonspecific and specific mechanisms [6]. Nonspecific mechanisms are for instance the free radical scavenging and metal sequestration capacity of phenolic compounds and their interactions with membranes. On the other hand, specific mechanisms include

the interaction of phenolics with enzymes, with transcription factors or with receptors. The complexity of some diseases leads to the recent search for alternative therapeutic strategies based on combined therapy protocols and multifunctional compounds. Thus, phenolic compounds, showing a wide range of biological effects through different mechanisms, have a great potential to be used in the prevention and treatment of several human diseases (**Figure 2**).
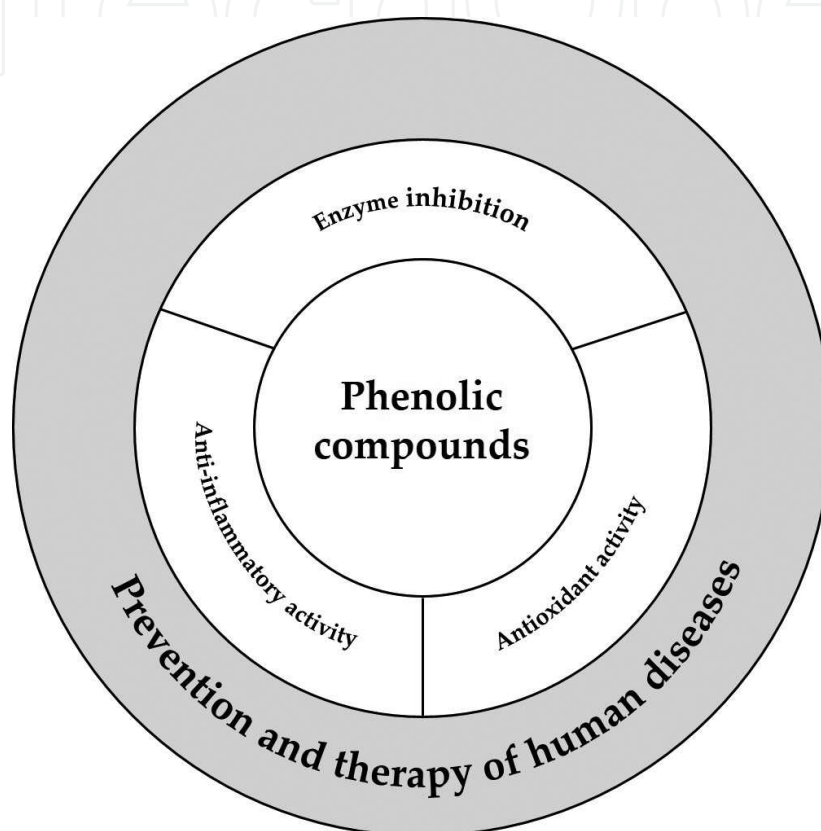

Figure 2. Snapshot of some of the health benefits of phenolic compounds.

3. Enzyme inhibitors

Enzyme inhibitors can have many applications in pharmaceutical, environment and biochemical industries, having a great impact on healthcare and medical sector. The control of some important human diseases includes the use of enzyme inhibitors which represent a great part of the drugs in clinical use. Specific enzyme inhibition will remain a major focus of pharmaceutical research for the foreseeable future [1]. Enzymes are protein molecules acting as catalyst in biological systems. Enzymes specificity assures high coordination and harmonious interplay among different metabolic activities essential to sustain life. The enzyme activity depends on numerous factors, for example, the most important the enzyme concentration,

the amount of specific enzyme substrate, the electrochemical reaction of medium for enzyme activity (pH) and the presence of activators or inhibitors.

Enzyme inhibitors prevent enzymes from their catalytic function by interfering with any step in the catalytic cycle. They are low molecular weight compounds that in small quantity can reduce or completely inhibit the enzyme activity [15]. Some human enzyme inhibitors, such as antithrombin and antitrypsin, control the enzyme activity in the body, and under physiological conditions, they guarantee their action. Among natural enzyme inhibitors, there are intermediary products produced during some metabolic pathways. The inhibition of products is a restricted way of control or modulation of substrate flux through the pathway. If enzymes are sensitive to product inhibition, the output of end product of the pathway will be suppressed [16].

An inhibitor can modify one amino acid or several side chain(s) required in enzyme catalytic activity. To protect enzyme catalytic site from any change, ligand binds with critical side chain in enzyme. Enzyme inhibitors are conceptually classified as specific and nonspecific. Inhibitors can reduce or completely inhibit the enzyme catalytic activity reversibly or irreversibly. Irreversible inhibitors usually change the enzyme chemically. Reversible inhibitors bind non-covalently to produce different types of inhibition, depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both. Most drugs that function through enzyme inhibition interact with their target enzyme through simple, reversible binding mechanisms [1]. Reversible inhibitors can be classified into competitive, noncompetitive or uncompetitive (**Figure 3**). In competitive inhibition, the substrate and inhibitor cannot bind to the enzyme at the same time; therefore, the competitive inhibitor competes with the substrate for the active site. A noncompetitive inhibitor binds equally well to both free enzyme and the enzyme-substrate complex. An uncompetitive inhibitor binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate-inhibitor complex.

4. Phenolic compounds as inhibitors of enzymes linked with human diseases

The use of plant-based enzyme inhibitors is encouraged nowadays because there is concern about the critical side effects of synthetic pharmaceutical agents. In the following sections, the enzyme inhibitory properties of phenolic compounds are reviewed. Investigations have been mainly focused in angiotensin I-converting enzyme, α-amylase and α-glucosidase, lipase, cholinesterases, proinflammatory enzymes (COXs and 5-LOX) and tyrosinase, which are linked with hypertension, type II diabetes, obesity, Alzheimer's diseases, inflammation and skin hyperpigmentation, respectively (**Table 2**). These were selected to be included in this chapter although there are evidences of the inhibitory properties of phenolic compounds against other enzymes like monoamine oxidase and catechol-O-methyl transferase.
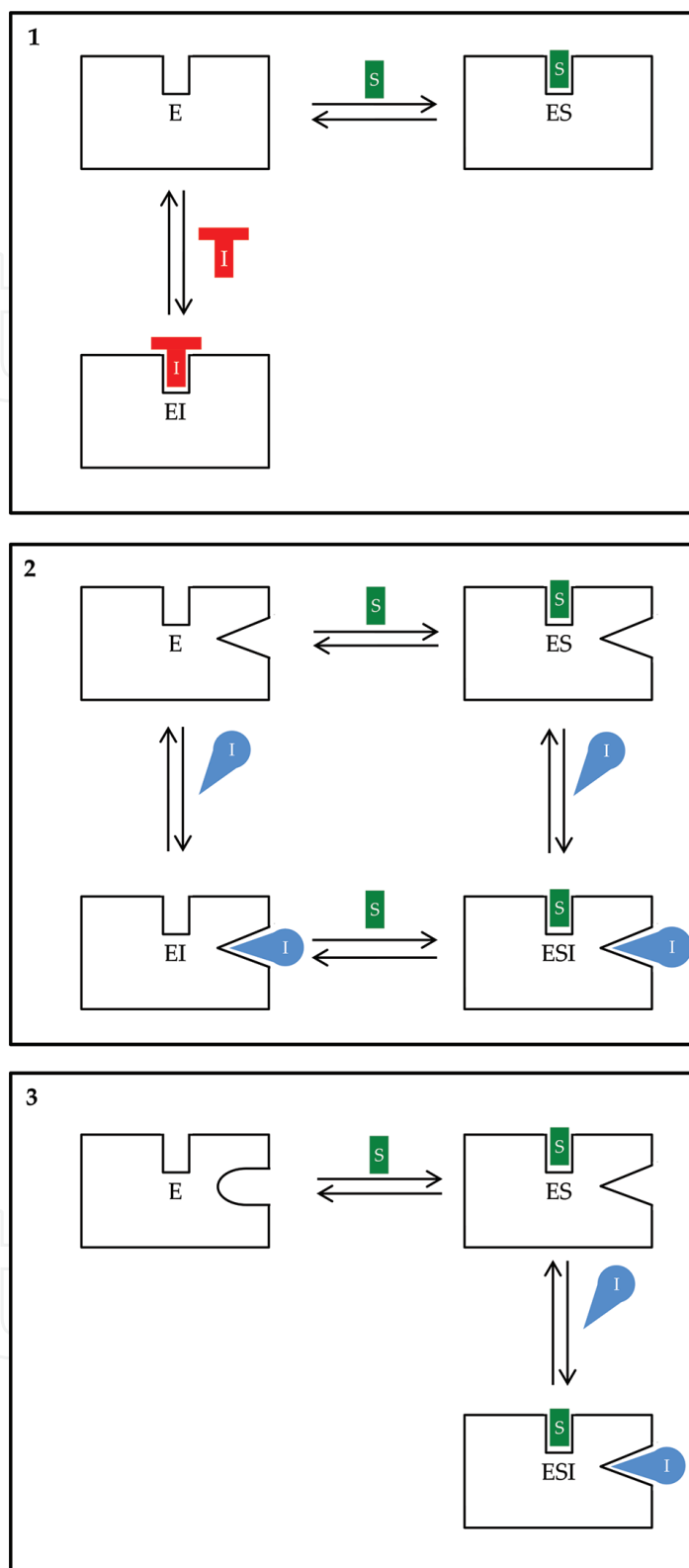
Figure 3. Scheme representative of the three major forms of reversible inhibitor interactions with enzymes: (1) competitive inhibition; (2) noncompetitive inhibition; (3) uncompetitive inhibition. S: Substrate; E: free enzyme (E); ES: enzyme-subtract complex; I: inhibitor; ESI: enzyme-subtract-inhibitor complex. Adapted from Copeland [17].

Diseases	Enzyme	Compound(s)	Important references
Hypertension	Angiotensin-converting enzyme	Flavonoids	[14]
		Tannic acid	[19]
		Anthocyanins	[27]
		Proanthocyanidins	[28]
Diabetes	α-Amylase and α-glucosidase	Flavonoids	[37, 39, 40]
Obesity	Pancreatic lipase	Flavonoids, phenolic acids	[47]
Alzheimer's diseases	Cholinesterases	Flavonoids	[53, 54]
		Coumarins	[59]
Inflammation	Cyclooxygenases and 5-lipoxygenase	Flavonoids	[62, 64–67]
		Stilbenes	[61, 62]
Skin hyperpigmentation	Tyrosinas	Flavonoids	[71, 73]
		Stilbenes	[72, 74, 75]
		Chalcones	[72]

Table 2. Main phenolic compounds described as inhibitors of enzymes linked with human ailments.

4.1. Hypertension: inhibition of angiotensin-converting enzyme (ACE)

Hypertension is a common and often progressive disorder that poses a major risk for cardiovascular diseases and related complications [18]. Hypertension is an important and increasing public health problem worldwide, and some data indicate that about one-quarter of the adult population suffers from this condition [19]. Inhibition of angiotensin-converting enzyme (ACE) is considered to be a target for discovery of lead antihypertensive agents and an important therapeutic approach in the treatment of high blood pressure [20]. ACE catalyzes the conversion of the precursor angiotensin I into angiotensin II, a peptide responsible for triggering vasoconstrictive effects, and it degrades bradykinin, a potent vasodilator [21, 22]. Some of the widely used ACE inhibitors such as aptopril, benazepril, enlapril and other [23] have revealed certain limitations like susceptibility to proteolytic degradation leading to side effects. Therefore, research has been conducted to find new ACE inhibitors from natural sources particularly from plant origin. Many investigations indicate that polyphenol-rich food is effective in the protection and treatment of hypertension namely via ACE inhibition [24]. In a recent review, Patten et al. [25] described 74 families of plants that exhibited significant ACE inhibitory activity. Also, Field and Newton [26] have shown that cocoa polyphenols are bioavailable molecules that induce an antihypertensive response including through ACE inhibition.

Guerrero et al. [14] evaluated the ability of 17 flavonoids belonging to five structural subtypes to inhibit ACE and showed that the highest activity was obtained for luteolin. Results from these authors allow concluding that the combination of substructures on the flavonoid skeleton that increase ACE activity is made up of the catechol group in the B-ring, the double

bond between C2 and C3 at the C-ring and the cetone group in C4 at the C-ring. Al Shukor et al. [19] investigated the ACE-inhibitory capacity of 22 phenolic compounds belonging to different classes and subclasses. Among analyzed compounds, tannic acid exhibited the highest ACE-inhibitory activity. Results indicated that the number of hydroxyl groups on the benzene ring plays an important role for activity of phenolic compounds and that substitution of hydroxyl groups by methoxy groups decreased activity. Furthermore, phenolic acids and flavonoids inhibit ACE via interaction with the zinc ion, and this interaction is stabilized by other interactions with amino acids in the active site. Resveratrol and pyrogallol may inhibit ACE via interactions with amino acids at the active site blocking the catalytic activity of ACE [19].

Several studies suggest anthocyanins as important ACE inhibitors. Ojeda et al. [27] demonstrated the ACE-inhibitory capacity of the anthocyanins delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside isolated from *Hibiscus sabdariffa*, a plant used in folk medicine, as antihypertensive. Moreover, kinetic determinations suggested that those compounds inhibit the enzyme activity by competing with the substrate for the active site. Studies from Eriz et al. [28] with *Vitis vinifera* extracts demonstrated that the inhibitory activity of proanthocyanidins (condensed tannins) against ACE is associated to a higher availability of OH groups, larger mean degree of polymerization and presence of epicatechin gallate.

4.2. Type II diabetes mellitus (DM): inhibition of carbohydrates-hydrolyzing enzymes

Disorders of carbohydrate uptake may cause severe health problems such as diabetes, obesity and oral diseases [29]. Diabetes mellitus (DM) is one of the most serious and chronic diseases and can be attributed to hyperglycaemia, a condition characterized by an excessive concentration of glucose circulating in the blood. Two types of DM are known, type I that is characterized by insufficient insulin production and type II that results from ineffectiveness of insulin [30]. Type II DM accounts for approximately 90% of diabetes cases worldwide and is attributed to greater prevalence of sedentary lifestyle, unhealthy diet and rise of obesity within modern society as well as an increasing number of elderly populations [31, 32]. Type II DM has become a serious medical concern worldwide and is frequently correlated with many complications such as cardiovascular diseases, hypertension, kidney failure, blindness, and neurological complications [33].

One therapeutic approach for treating DM type II is to decrease the postprandial glucose levels, which could be done by retarding the absorption of glucose through the inhibition of the carbohydrates-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. These enzymes are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. Inhibitors of these enzymes delay the rate of glucose absorption by preventing carbohydrate digestion and consequently blunting the postprandial plasma glucose rise [34]. The use of synthetic agents as acarbose is an important clinical strategy for controlling postprandial glycemia [35]. Acarbose reduces blood glucose levels and is approved by Food and Drug Administration (FDA); however, it causes critical side effects, such as liver disorders [4], and its use is now restricted [36]. To avoid or decrease the adverse effects of currently used synthetic inhibitors, it is necessary to employ naturally

occurring alternatives. In the last years, several reviews have been published focusing on the antidiabetic potential of natural products including on the inhibitory properties of phenolic compounds against carbohydrates-hydrolyzing enzymes [29, 30, 37, 38]. Phenolic compounds have been receiving much attention for controlling the digestibility of starch [30], and flavonoids are widely studied as α-amylase inhibitors [37]. Tadera et al. [39] tested several flavonoid compounds for their inhibitory activity against α-amylase and showed that, in general, the potency of inhibition is correlated with the number of hydroxyl groups on the B ring of the flavonoid scaffold. The structural requirements for the inhibition of human salivary α-amylase by 19 flavonoids were studied by Lo Piparo et al. [40]. Author's findings demonstrated that the flavonols and flavones enzyme inhibitory capacity depend on hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site and formation of a conjugated α-system that stabilizes the interaction with the active site. Recently, Xiao et al. [37] revised the structure-activity relationship of polyphenols inhibiting α-amylase and concluded that the hydroxylation galloylation of flavonoids, including catechins, improved the inhibitory effects against α-amylase. Moreover, these authors also observed that the glycosylation of the hydroxyl group, methylation, methoxylation and the hydrogenation of the C2-C3 double bond on flavonoids decreased the inhibition of the enzyme.

There are also some reports describing the inhibitory properties of other classes of phenolic compounds, like hydroxycinnamic acids and tannins, against α-amylase and α-glucosidase; however, overall they are less effective than flavonoids [29, 37].

Phenolic compounds, in particular flavonoids, may provide a protective effect against hyperglycemia-induced chronic diseases through a dual protection: inhibition of starch digestion and antioxidant effect. The co-application of phenolics with synthetic enzyme inhibitors for controlling prosprandial glycemia may reduce the effective dose of synthetic inhibitors required [30].

4.3. Obesity: inhibition of pancreatic lipase

Obesity is considered a global epidemic problem by the World Health Organization (WHO) and is recognized as the main life style disorder in developing countries, being termed as the "New World Syndrome." It is associated with multiple chronic diseases and disabilities such as dyslipidemia, fatty liver disease, osteoarthritis, hypertension, obstructive sleep apnea, gallstones, type II diabetes, reproductive and gastrointestinal cancers, coronary artery disease, heart failure and stroke [10, 41, 42].

The methods used to reduce body weight include diet, exercise, drug therapy, bariatric surgery or combinations of several of these methods. Currently, orlistat (Xenical) is the only drug approved by FDA for long-term treatment of obesity [43, 44]. Orlistat reduces intestinal triglyceride absorption through inhibition of pancreatic lipase. Its long-term administration is associated with a small but statistically significant weight loss of about 3% more than diet alone in overweight and obese people [45]. Moreover, it can also decrease blood pressure, prohibit the onset of DM type II and improve oral glucose tolerance [44]. However, some adverse gastrointestinal effects of orlistat have been reported as steathorrhea, bloating, oily spotting, fecal

urgency and fecal incontinence, as well as hepatic adverse effects [2]. Thus, there has been an increase interest in the search for new natural substances that show potent inhibitory activity against pancreatic lipase with fewer side effects. As a result, many plant extracts and compounds have been screened for their capacity to inhibit pancreatic lipase [44, 46, 47]. Among plant compounds, proteins, polysaccharides, saponins, triterpenes and phenolic compounds have shown capacity to inhibit this enzyme [46]. Phenolic compounds have some potential efficacy for preventing obesity by inhibiting the activity of enzymes related to fat metabolism as pancreatic lipase, lipoprotein lipase and glycerophosphate dehydrogenase [48]. Examples of compounds with pancreatic lipase inhibitory capacity are the flavonoid hesperidin from the peels of *Citrus unshiu*, proanthocyanidins from *Cassia mimosoides* and tea catechins [47, 48]. A compilation of recent and significant results of phenolic compounds as pancreatic lipase inhibitors can be found in the review recently published by Buchholz and Melzig [47]. Flavonoids and phenolic acids are probably the most studied chemical classes of phenolics showing this effect [47]. The lipase inhibitory capacity has been documented for more than 70 different flavonoids, and the inhibitory effect depends on the number and position of phenolic hydroxyl groups [47]. In the class of phenolic acids, several hydroxybenzoic and hydroxycinnamic have shown capacity to inhibit the enzyme. However, hydroxybenzoic acids are less effective than hydroxycinnamic acids, and the influence of methoxy groups (less efficient) in the molecule and hydroxyl groups (more efficient) can be seen. The carboxy group takes part in the activity of these compounds, and the size of the molecule influences the activity [49].

4.4. Alzheimer's diseases (AD): inhibition of cholinesterases

Alzheimer's disease (AD), the most common form of dementia, is a progressive age-related neurodegenerative disorder. AD rates are predicted to increase enormously, especially in developing regions, considering the accelerated aging of human society and the increase in life expectancy worldwide. Although the exact pathogenesis of AD still remains to be fully elucidated, it is currently considered to be a multifactorial disease. In the early 1970s, post-mortem studies revealed that choline uptake and acetylcholine release are reduced in the brains of AD patients being these reductions associated with substantial presynaptic cholinergic deficits [50]. This led to the establishment of the "cholinergic-deficit hypothesis," which posits that the impairment in the cholinergic function is of critical importance in AD especially in the brain areas dealing with learning, memory, behavior and emotional responses that include the neocortex and the hippocampus. The levels of acetylcholine, a neurotransmitter responsible for the conduction of electrical impulses from one nerve cell to another nerve cell, are decreased due to its rapid hydrolysis by acetylcholinesterase (AChE) enzyme [3, 50]. Butyrylcholinesterase (BChE) is an enzyme closely related to AChE and serves as a co-regulator of cholinergic neurotransmission by hydrolyzing acetylcholine [51]. Some studies have shown an increased BChE activity in the most affected areas of the brain during the development of AD. The inhibition of both AChE and BChE has been documented as critical targets for the management of AD by an increase in the availability of acetylcholine and decrease in the Aβ deposition. However, the brain region contains a small quantity of BChE since it is mostly localized in the peripheral tissues. Therefore, the potential advantage of selective inhibition of AChE over BChE may include lesser degree of associated side effects

due to peripheral inhibition of cholinesterase enzyme [52]. The first drug developed for AD based on the cholinergic-deficit hypothesis was tacrine that was approved for the treatment of cognitive loss in patients with AD by the FDA in 1993 [3]. Later, other cholinesterase inhibitors, like donepezil (1996), rivastigmine (2000) and galantamine (2001), have been approved and used for the management of AD [3, 52]. Due to the limited efficacy and gastrointestinal side effects of these drugs, such as nausea and diarrhea, there has been a continuous effort to find more effective cholinesterase inhibitors to be used to prolong and improve the life of the AD patients [3]. In this sense, plant extracts and compounds have been investigated for their role in prevention and treatment of AD including as cholinesterase inhibitors. Several investigations described the anticholinesterase effects of many naturally occurring flavonoids and newly synthesized flavonoids analogues. Uriarte-Pueyo and Calvo [53] reviewed the acetylcholinesterase inhibitory capacity of 128 flavonoids and conclude that flavones and isoflavones are the compounds with higher activity, proving that the carbonyl group at C4 seems to be important in this activity. Later on, Anand and Singh [54] discussed the data on the effects of flavonoids in various enzyme targets that play an important role in the pathogenesis of AD. Some conclusions arising are that isoflavone analogues demonstrate high AChE inhibitory activity, as compared to chalcone, flavones and flavanone analogues, suggesting that the nature of flavonoid moiety affects AChE inhibition in a great extent. Moreover, different moieties capable of interacting with catalytic site of AChE, including benzyl piperidine, piperidine, pyrrolidine, have been linked to paraposition of ring B of flavonoid scaffold using appropriate spacer to obtain dual-binding AChE inhibitors. Flavonoids may be attractive lead compounds for the development of effective and safe anti-AD drugs by modulating the enzyme targets in the disease [54].

Other compounds with potent AChE inhibitory activity are coumarins [55], and similar to flavonoids, several coumarins analogues have been synthesized, and their enzyme inhibitory capacity evaluated. Data on the inhibitory effects of coumarins against cholinesterases have been reviewed by several authors [56–58]. Various coumarins obtained from plants particularly from the *Angelica* genus revealed potent cholineterases inhibitory capacity (**Figure 4**) [56–59]. Taking into consideration structure-activity relationships, coumarins that small moieties such as hydroxyl or methoxy attached at C-7 of the coumarin skeleton seem to have a lower AChE inhibitory effect, in comparison with coumarins of greater substitutions, such as benzyloxy, $O–CH_2–C_6H_5$, positioned at the same carbon [60]. Additionally, a cyclized isoprenoid moiety at C-6 greatly contributes to the increase in AChE inhibition of some coumarin derivatives [59]. Moreover, research suggests that furanocoumarins are selective BChE inhibitors [56].

Numerous studies showed that phenolic compounds not only exhibit cholinesterase inhibitory capacity as potent antioxidant and anti-inflammatory properties, acting to scavenge radicals and regulate inflammatory responses; moreover, some of these compounds readily cross the bloodbrain barrier to act on specific targets that have been implicated in the pathogenesis of AD.

4.5. Inflammation: inhibition of proinflammatory enzymes

Several diseases such as diabetes, obesity, cancer, osteoarthritis, atherosclerosis and Crohn's disease are associated with chronic inflammation. The mechanisms of inflammation involve
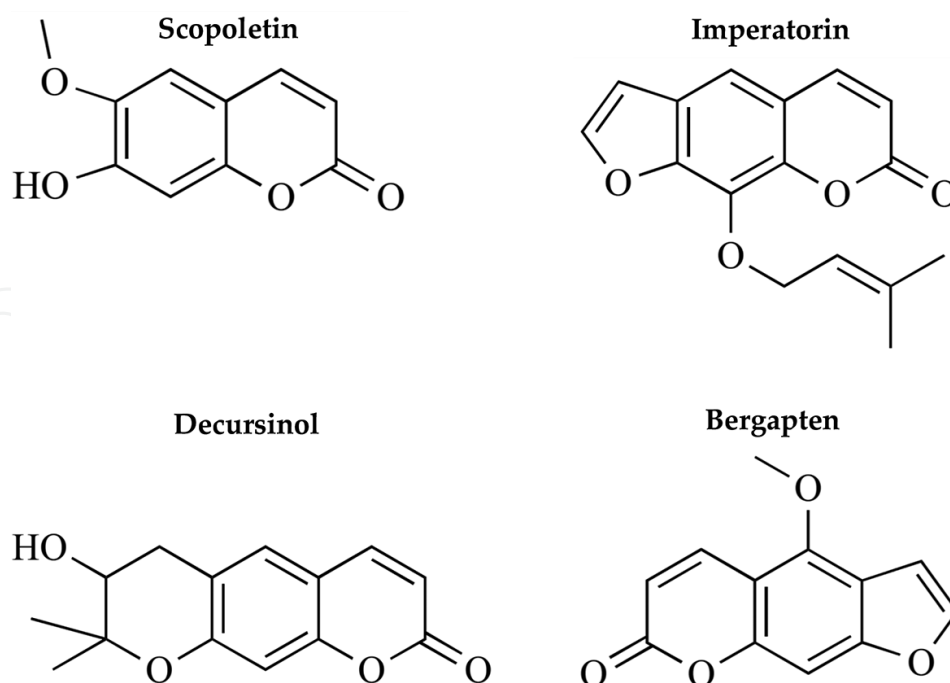
Figure 4. Chemical structure of some coumarins with cholinesterase inhibitory capacity.

a series of events in which the metabolism of arachidonic acid plays an important role [61] COX-1 and COX-2 enzymes catalyze the conversion of arachidonic acid to prostanoids. The second pharmacologically relevant metabolic pathway of arachidonic acid is mediated by 5-LOX. This enzyme is involved in the biosynthesis of inflammatory mediators named leukotrienes. COXs and LPXs are considered proinflammatory enzymes; the former affects platelet aggregation, vasoconstriction, vasodilatation and later, the development of atherosclerosis [62]. Both COXs and 5-LOX have received considerable attention because they are putative targets for cancer prevention. Nonsteroidal and steroidal anti-inflammatory drugs exert their action by inhibiting these proinflammatory enzymes through different mechanisms [63]. The nonsteroidal and steroidal anti-inflammatory drugs currently in use effectively manage the acute inflammatory reaction; however, in chronic inflammatory states, the long-term treatment with these drugs is followed by severe adverse effects. This justifies the search for new and safe anti-inflammatory agents being plant compounds good candidates. Recently, there has been interest in the antiinflammatory/immunomodulatory potential of flavonoids including their capacity to inhibit the activity of proinflammatory enzymes [64–66]. Li et al. [66] demonstrated the anti-inflammatory activity of the flavonoid baicalin. This compound inhibits COX-1, COX-2 and 5-LOX activities, decreases production of proinflammatory eicosanoids and attenuates edema in an *in vivo* model of inflammation. Butein, another flavonoid, decreased COX-2 expression in cancerous lung cells [67]. In addition, flavocoxid, a mixed extract containing the flavonoids baicalin and catechin, acts as a dual balanced inhibitor of COX-1 and COX-2 with a significant inhibition of 5-LOX [65]. It exerts beneficial effects in several experimental models of inflammation, and it has a significant efficacy in management of osteoarthritis and a good gastrointestinal tolerability [65].

Stilbenes are another important group of plant compounds with anti-inflammatory properties. Kutil et al. [61] recently demonstrated that several natural stilbenes are potent inhibitors of proinflammatory enzymes and not only the most extensively studied compound of this group, resveratrol (3,5,4'-trihydroxy-trans-stilbene) found in red wine. The same group [62] evaluated the inhibitory potential of several wines and 33 phenolic compounds commonly occurring in wine against COX-1, COX-2, and 5-LOX. Authors observed that red wines were potent inhibitors of all three tested enzymes but the results obtained with isolated compounds could not fully explain the overall activities of the wine. Although trans-resveratrol considerably inhibits both COX-1 and COX-2, the activity of this compound alone could not be responsible for the overall inhibitory activity. In addition, results also showed that piceatannol, luteolin, quercetin, and myricetin were potent inhibitors of 5-LOX, but considering the ratio between their IC_{50} values and their concentration in wine only piceatannol could substantially contribute to the overall activity of red wines. Authors hypothesize that wine proanthocyanidins could also contribute to its overall potential since their inhibitory capacity against these enzymes was previously described.

4.6. Skin hyperpigmentation: inhibition of tyrosinase

The color of mammalian skin is mainly determined by the degree and distribution of melanin pigmentation. Melanin plays an important role in protecting skin from ultraviolet UV damage; however, overproduction of melanin poses not only an esthetic but also a dermatological problem. Indeed, some dermatological disorders, such as melasma and age spots, result in the accumulation of an excessive level of epidermal pigmentation [68].

Melanin is formed by several oxidative reactions which involve tyrosine and tyrosinase [69]. Tyrosinase catalyses three reactions in the biosynthetic pathway of melanin in melanocytes: the hydroxylation of tyrosine to L-DOPA and its oxidation to dopaquinone. Following a series of oxidoreduction reactions, the intermediate dihydroxyindole DHI and dihydroxyindole carboxylic acid are produced and polymerized to form melanins [70]. The inhibition of tyrosinase is one of the major strategies used to treat hyperpigmentation; however, concerns over the toxicity and side effects of synthetic inhibitors have led to a search for new safe and effective tyrosinase inhibitors. Moreover, tyrosinase is also responsible for browning in fruits and vegetables, and thus, inhibitors of this enzyme are frequently applied to plant-based foods.

Search of tyrosinase inhibitors is crucial for the development of skin whitening agents but also anti-browning and insect control substances. A number of researchers have been dedicated to identify inhibitors from natural sources including plants. The largest group of phytochemicals with potent tyrosinase inhibitors belongs to phenolics ranging from the simple ones to polyphenolics [71]. Furthermore, flavonoids occupy the largest portion in newly discovered natural tyrosinase inhibitors, and their structure is compatible with roles of both substrates and inhibitors of tyrosinase [71, 72]. Some flavonoids, such as kaempferol, quercetin, and morin, show inhibitory activity against tyrosinase, while others such as catechin and rhamnetin behave as substrates and suppress tyrosinase activity by being a cofactor (catechin) or acting as a free radical scavenger (rhamnetin) [72]. For instance, steppogenin, a flavanone derivative isolated from *Cudrania tricuspidata*, showed tyrosinase inhibitory activity much

higher than kojic acid, a known inhibitor of this enzyme [73]. The presence of two hydroxyl groups located on the aromatic ring at positions 2 and 4 in flavonoids was concluded to be necessary for tyrosinase inhibitory activity [71].

Some stilbenes, such as resveratrol, oxyresveratrol, chlorophorin and andalasin, have also been reported as having tyrosinase inhibitory properties [72, 74, 75]. The most promising inhibitor appears to be oxyresveratrol (32-fold higher inhibitory activity than kojic acid). Some of these compounds are sensitive to photo-oxidation, which limits their use in cosmetic formulations; however, the acetylated resveratrol derivative triacetyl resveratrol is noted to be comparably effective and much more stable than resveratrol [75].

There are also a number of chalcones (the precursors of flavonoids and isoflavonoids), such as licochalcone A, kuraridin, kuraridinol, 2,4,20,40-tetrahydroxy-3-(3-methyl-2-butenyl) and morachalcone A, with remarkable tyrosinase inhibitory activity [72]. Additionally, due to the versatile bioactivity and unique structural motif of chalcones, a number of derivatives have been developed as effective tyrosinase inhibitor candidates.

Although a great number of phenolic compounds revealed tyrosinase inhibitory capacity, the necessity to clarify the viability of these inhibitors in terms of their skin-whitening efficiency has become an urgent task.

5. Concluding remarks

The control of some important human diseases includes the use of enzyme inhibitors; however, there is some concern about the use of synthetic inhibitors due to their side effects. Thus, the use of natural enzyme inhibitors, particularly from plant origin, is encouraged nowadays. Indeed, up to date, extensive research has been conducted to study the enzyme inhibitory properties of phenolic compounds. Data revised showed that different phenolic compounds are efficient inhibitors of the activity of a broad number of enzymes linked with important human conditions, such as hypertension, type II diabetes, obesity, Alzheimer's diseases, inflammation, and skin disorders. It is unmistakable that phenolic compounds are multifunctional compounds that provide a wide spectrum of biological actions beneficial for human health. These compounds exert their action by different mechanisms and have a huge potential in the prevention and treatment of several human diseases. Our bibliographic survey also indicates that flavonoids are probably the group of phenolic compounds with great capacity to inhibit the activity of all the human enzymes analyzed. Several structure-activity relationships studies can be found for some flavonoid compounds. In addition to the enzyme inhibitory capacity, several studies demonstrated the strong antioxidant and anti-inflammatory properties of flavonoids which highlights the relevance of these compounds for the prevention and control of diseases involving oxidative stress or inflammation.

For the future, standardized protocols to search potential inhibitors should be designed in order to minimize the differences among obtained results. Despite the vast number of phenolic compounds studied *in vitro*, few compounds have continued to *in vivo* tests. In addition, further studies should be performed to predict drug-likeness and drug ability.

Acknowledgements

Author details

Sandra Gonçalves and Anabela Romano*

*Address all correspondence to: aromano@ualg.pt

Faculty of Sciences and Technology, MeditBio, University of Algarve, Campus de Gambelas, Faro, Portugal

References

[1] Copeland RA. Evaluation of enzyme inhibitors in drug discovery: a guide for medicinal chemists and pharmacologists. New Jersey: John Wiley & Sons, Inc.; 2005. 271 p.

[2] Filippatos T, Derdemezis C, Gazi I, Nakou E, Mikhailidis D, Elisaf M. Orlistat-associated adverse effects and drug interactions: a critical review. Drug Safety. 2008;**31**:53–65. doi:10.2165/00002018-200831010-00005

[3] van Marum RJ. Current and future therapy in Alzheimer's disease. Fundamental and Clinical Pharmacology. 2008;**22**:265–274. doi:10.1111/j.1472-8206.2008.00578.x

[4] Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α-glucosidase and pancreatic amylase. Food Chemistry. 2009;**115**:1268–1273. doi:10.1016/j.foodchem.2009.01.042

[5] Li A-N, Li S, Zhang Y-J, Xu X-R, Chen Y-M, Li H-B. Resources and biological activities of natural polyphenols. Nutrients. 2014;**6**:6020–6047. doi:10.3390/nu6126020

[6] Fraga CG, Galleano M, Verstraeten SV, Oteiza PI. Basic biochemical mechanisms behind the health benefits of polyphenols. Molecular Aspects of Medicine. 2010;**31**:435–445. doi:10.1016/j.mam.2010.09.006

[7] World Health Organization. Noncommunicable diseases. Last update January 2015, accessed August 2016. http://www.who.int/mediacentre/factsheets/fs355/en/.

[8] Aguilera Y, Martin-Cabrejas MA, de Mejia EG. Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. Phytochemistry Reviews. 2016;**15**:405–423. doi:10.1007/s11101-015-9443-z

[9]   Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. The Lancet. 2005;**365**:217–223. doi:10.1016/S0140-6736(05)17741-1

[10] Vermaak I, Viljoen AM, Hamman JH. Natural products in anti-obesity therapy. Natural Produts Reports. 2011;**28**:1493. doi:10.1039/c1np00035g

[11] Dey TB, Chakraborty S, Kr. Jain K, Sharma A, Kuhad RC. Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: a review. Trends in Food Science and Technology. 2016;**53**:60–74. doi:10.1016/j.tifs.2016.04.007

[12] Martin KR, Appel CL. Polyphenols as dietary supplements: a doubleedged sword. Nutrition and Dietary Supplements. 2010;**2**:1–12.

[13] Manach C, Scalbert A, Morand C, Rémésy C Jiménez L. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition. 2004;**79**:727–747.

[14] Guerrero L, Castillo J, Quiñones M, Garcia-Vallvé S, Arola L, Pujadas G, Muguerza B. Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. Plos One. 2012;**7**:49493. doi:10.1371/journal.pone.0049493

[15] Sharma R. Enzyme inhibition: mechanisms and scope. In: Sharma R, editor. Enzyme inhibition and bioapplications. Rijeka: InTech; 2012. pp. 3–36.

[16] Nelson D, Cox M. Lehninger principles of biochemistry. 4th ed. New York: Worth Publishers; 1993. 1130 p.

[17] Copeland RA. Enzymes: A practical introduction to structure, mechanism and data analysis. 2nd ed. New York: Wiley; 2000. 416 p.

[18] Chalmers J, MacMahon S, Mancia G, Whitworth J, Beilin L, Hansson L, Neal B, Rodgers A, Ni Mhurchu C, Clark T. 1999 World Health Organization – international society of hypertension guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization. Clinical and Experimental Hypertension. 1999;**21**:1009–60. doi:10.3109/10641969909061028

[19] Al Shukor N, Van Camp J, Gonzales GB, Staljanssens D, Struijs K, Zotti MJ, Raes K, Smagghe G. Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: a study of structure activity relationships. Journal of Agriculture and Food Chemistry. 2013;**61**:11832–11839. doi:10.1021/jf404641v

[20] Ji Z, Huang C, Liang C, Chen B, Chen S and Sun W. Protective effects of blocking renin-angiotensin system on the progression of renal injury in glomerulosclerosis. Cellular and Molecular Immunology. 2005;**2**:150–154.

[21] Zhang RZ, Xu XH, Chen TB, Li L, Rao PF. An assay for angiotensin-converting enzyme using capillary zone electrophoresis. Analytical Biochemistry. 2000;**280**:286–290. doi:10.1006/abio.2000.4535

[22] Imig JD. ACE inhibition and bradykinin-mediated renal vascular responses: EDHF involvement. Hypertension. 2004;**43**:533–535. doi:10.1161/01.HYP.0000118054.86193.ce

[23] Sleiman O, Murin J and Ghanem W. Angiotensin-converting enzyme inhibitors: do we utilize our knowledge in heart failure patients? Journal of Clinical and Basic Cardiology. 2001;**4**:279–283.

[24] Hügel HM,Jackson N, May B, Zhang AL, Xue CC. Polyphenol protection and treatment of hypertension. Phytomedicine. 2016;23:220–231. doi:10.1016/j.phymed.2015.12.012

[25] Patten GS, Abeywardena MY, Bennett LE. Inhibition of angiotensin converting enzyme, angiotensin II receptor blocking, and blood pressure lowering bioactivity across plant families. Critical Reviews in Food Science and Nutrition. 2016;**56**:181–214. doi:10.1080/10408398.2011.651176

[26] Field D, Newton G. The anti-hypertensive actions of cocoa polyphenols–A review. Current Topics in Nutraceutical Research. 2013;**11**:113–128.

[27] Ojeda D, Jiménez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, Alvarez L. Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-*O*-sambubiosides from *Hibiscus sabdariffa*. Journal of Ethnopharmacology. 2010;**127**:7–10. doi:10.1016/j.jep.2009.09.059

[28] Eriz G, Sanhueza V, Roeckel M, Fernández K. Inhibition of the angiotensin-converting enzyme by grape seed and skin proanthocyanidins extracted from *Vitis vinifera* L. cv. País. LWT – Food Science and Technology. 2011;**44**: 860–865. doi:10.1016/j.lwt.2010.11.034

[29] Sales PM, de Souza PM, Simeoni LA, Magalhães PO, Silveira D. α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. Journal of Pharmacy and Pharmaceutical Sciences. 2012;**15**:141–183. doi:10.18433/J35S3K

[30] Asgar A. Anti-diabetic potential of phenolic compounds: A review. International Journal of Food Properties. 2013;**16**:91–103. doi:10.1080/10942912.2011.595864

[31] Zimmet P. Diabetes epidemiology as a trigger to diabetes research. Diabetologia. 1999;**42**:499–518. doi:10.1007/s001250051188

[32] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;**27**:1047–1053. doi:10.2337/diacare.27.5.1047

[33] Lopez-Candales A. Metabolic syndrome X: A comprehensive review of the pathophisiology and recommended therapy. Journal of Medicine. 2001;**32**:283–300.

[34] Rhabasa-Lhoret R, Chiasson JL. α-Glucosidase inhibitors. In: Defronzo RA, Ferrannini E, Keen H, Zimmet P, eds. International textbook of diabetes mellitus, UK: John Wiley & Sons Ltd. 2004. Vol. 1. pp. 901–914.

[35] Singh J, Dartois A, Kaur L. Starch digestibility in food matrix: A review. Trends in Food Science and Technology. 2010;**21**:168–180. doi:10.1016/j.tifs.2009.12.001

[36] Ríos JL, Francini F, Schinella GR. Natural products for the treatment of Type 2 diabetes mellitus. Planta Medica. 2015;**81**:975–994. doi:10.1055/s-0035-1546131

[37] Xiao J, Ni X, Kai G, Chen X. A review on structure–activity relationship of dietary polyphenols inhibiting α-amylase. Critical Reviews in Food Science and Nutrition. 2013;**53**:497–506. doi:10.1080/10408398.2010.548108

[38] El-Abhar HS, Schaalan MF. Phytotherapy in diabetes: Review on potential mechanistic perspectives. World Journal of Diabetes. 2014;**5**:176–197. doi:10.4239/wjd.v5.i2.176

[39] Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. Journal of Nutritional Science and Vitaminology. 2006;**52**:149–153. doi:10.3177/jnsv.52.149

[40] Lo Piparo E, Scheib H, Frei N, Williamson G, Grigorov M, Chou CJ. Flavonoids for controlling starch digestion: structural requirements for inhibiting human α-amylase. Journal of Medinal Chemistry. 2008;**51**:3555–3561. doi:10.1021/jm800115x

[41] Billington CJ, Epstein LH, Goodwin NJ, Hill JO, Pi-Sunyer FX, Rolls BJ, Stern J, Wadden TA, Weinsier RL, Wilson GT, Wing RR, Yanovski SZ, Hubbard VS, Hoofnagle JH, Everhart J, Harrison B. Overweight, obesity, and health risk. Archives of Internal Medicine. 2000;**160**:898–904. doi:10.1001/archinte.160.7.898

[42] Padwal R, Li SK, Lau DCW. Long-term pharmacotherapy for overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. International Journal of Obesity. 2003;**27**:1437–1446. doi:10.1038/sj.ijo.0802475

[43] George M, Rajaram, Shanmugam E. New and emerging drug molecules against obesity. Journal of Cardiovascular Pharmacology and Therapeutics. 2014;**19**:65–76. doi:10.1177/1074248413501017

[44] Seyedan A, Alshawsh MA, Alshagga MA, Koosha S, Mohamed Z. Medicinal plants and their inhibitory activities against pancreatic lipase: A review. Evidence-Based Complementary and Alternative Medicine. 2015;**ID 973143**:1–13. doi:10.1155/2015/973143

[45] Drew B, Dixon A, Dixon J. Obesity management: update on or list at. Journal of Vascular Health and Risk Management. 2007;**3**:817–821.

[46] Garza Al, Milagro FI, Boque N, Campión J, Martínez JA. Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta Medica. 2011;**77**:773–785. doi:10.1055/s-0030-1270924

[47] Buchholz T, Melzig MF. Polyphenolic compounds as pancreatic lipase inhibitors. Planta Medica. 2015;**81**:771–783. doi:10.1055/s-0035-1546173

[48] Yoshikawa M, Shimoda H, Nishida N, Takada M, Matsuda H. Salacia reticulata and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild anti-obesity effects in rats. Journal of Nutrition. 2002;**132**:1819–1824.

[49] Karamać M, Amarowicz R. Inhibition of pancreatic lipase by phenolic acids – examination *in vitro*. Zeitschrift für Naturforschung C. 1996;**51**:903–905. doi:10.1515/znc-1996-11-1222

[50] Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. Journal of Neurology, Neurosurgery and Psychiatry. 1999;**66**:137–147. doi:10.1136/jnnp.67.4.558

[51] Mesulam M, Guillozet A, Shaw P, Quinn B. Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. Neurobiology of Disease. 2002;**9**:88–93. doi:10.1006/nbdi.2001.0462

[52] Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. Archives of Pharmaceutical Research. 2013;**36**:375–399. doi:10.1007/s12272-013-0036-3

[53] Uriarte-Pueyo I, Calvo MI. Flavonoids as acetylcholinesterase inhibitors. Current Medicinal Chemistry. 2011;**18**:5289–5302. doi:10.2174/092986711798184325

[54] Anand P, Singh B. Flavonoids as lead compounds modulating the enzyme targets in Alzheimer's disease. Medicinal Chemistry Research. 2013;**22**:3061–3075. doi:10.1007/s00044-012-0353-y

[55] Changwong N, Sabphon C, Ingkaninan K, Sawasdee P. Acetyl- and butyryl-cholinesterase inhibitory activities of mansorins and mansonones. Phytotherapy Research. 2012;**26**:392–396. doi:10.1002/ptr.3576

[56] Pinho BR, Ferreres F, Valentão P, Andrade PB. Nature as a source of metabolites with cholinesterase-inhibitory activity: an approach to Alzheimer's disease treatment. Journal of Pharmacy and Pharmacology. 2013;**65**:1681–1700. doi:10.1111/jphp.12081

[57] Orhan IE, Gulcan HO. Coumarins: Auspicious cholinesterase and monoamine oxidase inhibitors. Current Topics in Medicinal Chemistry. 2015;**15**:1673–1682. doi:10.2174/1568026615666150427113103

[58] de Souza LG, Rennã MN, Figueroa-Villar JD. Coumarins as cholinesterase inhibitors: A review. Chemico-Biological Interactions. 2016;**254**:11–23. doi:10.1016/j.cbi.2016.05.001

[59] Kang SY, Lee KY, Sung SH, Park MJ, Kim YC. Coumarins isolated from *Angelica gigas* inhibit acetylcholinesterase: structure-activity relationships. Journal of Natural Products. 2001;**64**:683–685. doi:10.1021/np000441w

[60] Brühlmann C, Ooms F, Carrupt PA, Testa B, Catto M, Leonetti F, Altomare, C, Carotti A. Coumarins derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase. Journal of Medicinal Chemistry. 2001;**44**:3195–3198. doi:10.1021/jm010894d

[61] Kutil Z, Kvasnicova M, Temml V, Schuster D, Marsik P, Cusimamani EF, Lou J-D, Vanek T, Landa P. Effect of dietary stilbenes on 5-lipoxygenase and cyclooxygenases activities *in vitro*. International Journal of Food Properties. 2015;**18**:1471–1477. doi:10.1080/10942912.2014.903416

[62] Kutil Z, Temml V, Maghradze D, Pribylova M, Dvorakova M, Schuster D, Vanek T, Landa P. Impact of wines and wine constituents on cyclooxygenase-1, cyclooxygenase-2, and 5-lipoxygenase catalytic activity. Mediators of Inflammation. 2014;**ID 178931**:1–8. doi:10.1155/2014/178931

[63] Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. Inflammation Research. 1998;**2**:S78–S87.

[64] González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustin O, Sánchez de Medina F. Effects of flavonoids and other polyphenols on inflammation. Critical Reviews in Food Science and Nutrition. 2011;**51**:331–362. doi:10.1080/10408390903584094

[65] Bitto A, Squadrito F, Irrera N, Pizzino G, Pallio G, Mecchio A, Galfo F, Altavilla D Flavocoxid, a nutraceutical approach to blunt inflammatory conditions. Mediators of Inflammation. 2014;**ID 790851**:1–8. doi:10.1155/2014/790851

[66] Li BQ, Fu T, Gong WH, Dunlop N, Kung H, Yan Y, Kang J, Wang JM. The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. Immunopharmacology. 2000;**49**:295–306. doi:10.1016/S0162-3109(00)00244-7

[67] Li Y, Ma C, Qian M, Wen Z, Jing H, Qian D. Butein induces cell apoptosis and inhibition of cyclooxygenase-2 expression in A549 lung cancer cells. Molecular Medicine Reports. 2014;**9**:763–767. doi:10.3892/mmr.2013.1850

[68] Kim J, Uyama H. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cellular and Molecular Life Sciences. 2005;**62**:1707–23. doi:10.1007/s00018-005-5054-y

[69] Lin J-W, Chiang H-M, Lin Y-C, Wen K-C. Natural products with skin – whitening effects. Journal of Food and Drug Analysis. 2008;**16**:1–10.

[70] Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. Anais Brasileiros de Dermatologia. 2013;**88**:76–83.

[71] Orhan IE, Khan MTH. Flavonoid derivatives as potent tyrosinase inhibitors – A survey of recent findings between 2008–2013. Current Topics in Medicinal Chemistry. 2014;**1**:1486–1493.

[72] Lee SY, Baek N, Nam T. Natural, semisynthetic and synthetic tyrosinase inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry. 2016;**31**:1–13. doi:10.3109/14756366.2015.1004058

[73] Zehng ZP, Tan HY, Chen J, Wang M. Characterization of tyrosinase inhibitors in the twigs of *Cudrania tricuspidata* and their structure activity relationship study. Fitoterapia. 2013;**84**:242–7. doi:10.1016/j.fitote.2012.12.006

[74] Kim YM, Yun J, Lee CK, Lee H, Min KR, KimY. Oxyresveratrol and hydroxystilbene compounds. Inhibitory effect on tyrosinase and mechanism of action. Journal of Biological Chemistry. 2002;**277**:16340–16344. doi:10.1074/jbc.M200678200

[75] Park J, Park JH, Suh HJ, Lee IC, Koh J, Boo YC. Effects of resveratrol, oxyresveratrol, and their acetylated derivatives on cellular melanogenesis. Archives of Dermatological Research. 2014;**306**:475–487. doi:10.1007/s00403-014-1440-3