

# Handbook of **Antioxidants in Food**

Jacqueline Bach

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Edited by  
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# **Antioxidants in Maca (*Lepidium meyenii*) as a Supplement in Nutrition**

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Serol Korkmaz

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## **Abstract**

Maca plant belongs to Brassicaceae such as broccoli, cabbage and radish, and has a tuberous root. With the declaration of The Food and Agriculture Organization (FAO) that maca is a forgotten and disappearing plant, the fresh, dried, powder and organic forms of it take part in nutrition as a food supplement world-wide. Studies have focused on antioxidant effects depending on its bioactive components such as phenols, glucosinolates, alkaloids and polysaccharides. Antioxidant enzymes and their ability of inhibition the free radicals in blood and tissues were measured to determine the antioxidant effects. The research results have suggested that these compounds present the antioxidant effect by increasing enzyme activity and scavenging free radicals. Yet further experiments are needed to understand this relation between antioxidant activity and maca's antioxidants. The objective of this chapter is to carry out the possible antioxidant activity of maca in human and animal nutrition related to its active compounds such as: phenols, glucosinolates, alkaloids and polysaccharides.

**Keywords:** antioxidant, human and animal nutrition, *Lepidium meyenii*, maca, macamide, polysaccharide

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## **1. Introduction**

Antioxidant effects of plants used in daily nutrition are investigated, their bioactive contents are analyzed and its mechanisms are revealed. Recently, bioactive compounds with antioxidant effects have been found in many plants traditionally used. These plants cross their local region, cultivated in many parts of the world, and take place in markets as various supplement products. Plants are linked to bioactive compounds in which they contain antioxidant

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effects. These compounds act alone or synergistically and are consumed in plants or extractions in various forms. The antioxidant effect produced by plants in metabolism is measured by various methods. The most common methods are to determine the levels of antioxidant enzymes and free radicals. In addition, measurement methods such as physical performance and health score give information about antioxidant status. Like many plants known to have antioxidant activity, Maca contains antioxidant compounds. Due to its chemical composition, pharmacological effects and positive effects on various metabolisms, The South American maca plant has attracted both the consumers and the researchers in great demand all over the world recently. The aim of this chapter is to establish an analysis of the properties related to antioxidant activity of different kinds of maca plant and its contents from active compounds such as: phenols, glucosinolates, alkamides and polysaccharides.

## 2. Maca (*Lepidium meyenii*)

Maca is a plant which has tuber roots underground and belongs to Brassicaceae family including also plants such as broccoli, radish, turnip, cabbage. It is supplemented in pudding, jam, beverage and yogurt based on its aromatic flavor and odor. It is traditionally consumed in daily meals by local people because of its high nutritional value, aphrodisiac, energizer and increasing fertility of them and their farm animals. The root colors are varies such red, yellow, brown, purple, black etc. (Figure 1). Maca is endemic in the South America. It is cultivated at high altitudes of Andes Mountains and dried in the sun and freezing cold in the natural environment to store for a long time. The dried maca is boiled in water and softened, and the water is consumed with its roots. Maca powder are added to drinks as energy source and aromatic sweetener [1]. Besides increasing interest in maca [2], and the International Plant Genetic Resources Institute announced that this local plant is neglected, under the danger of disappearing and must be protected [3]. It has positive effects on various metabolisms in laboratory animals and clinical studies. In addition, these effects and the mechanism of action have been confirmed by in vitro studies. Studies suggest that its impacts originate from antioxidant compounds such as phenols, glucosinolates, alkamides and polysaccharides. These compounds are determined by different extraction and analysis methods. Their antioxidant effects is established with factors such as free radical scavenging and cell viability invitro. In



Figure 1. Some ecotypes of maca [2].



clinic nutrition studies, the effects of maca on antioxidant status was determined by measuring the antioxidant enzyme activity, free radical scavenging, anti-fatigue effect and health score.

### 3. Antioxidants in maca

Like many other plants, maca plant contains various antioxidant compounds. The quantities of these substances vary according to the soil composition, maca ecotype, the time of harvest, the drying process and the extraction method [4]. In spite of the quantity differences, maca contains several and substantial amount of antioxidant compounds. These are especially phenols, glucosinolates, alkalamides and polysaccharides. They have various functions on metabolism and antioxidant effects in scientific researches. In vitro studies, their antioxidant effects are mostly established by several methods such as the measurements of ferric reducing antioxidant potential (FRAP), hydroxyl radical scavenging ability (HRSA), lipid peroxidation inhibition ability (LPIA), 11,1-difenil-2-pikrilhidrazil (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging abilities of bioactive compounds.

#### 3.1. Phenols

Depending on the structure elements and phenol rings they contain, phenols are termed. Phenolic compounds are divided into groups such as phenolic acids, flavonoids, tannins, resveratrol and lignans. They are found in the structure of many plants as nature antioxidant. They represent an antioxidant activity by, breaking chains, chelating metal ion, decomposing products of oxidation and scavenging free radicals [5]. Maca contains phenols in different quantities based on ecotype and extraction method. Total phenolic compounds in maca were mostly analyzed according to the Folin–Ciocalteu method by using gallic acid standard. The results of measurement are expressed as mg gallic acid equivalent (GAE) per gram of dried maca.

The ecotypes (hypocotyl colors) of maca influence the phenol contents and composition. Black maca has more total phenols than red and yellow maca. Despite black maca shows more antioxidant activity than red maca, the methanol extract of yellow maca presents more DPPH scavenging activity than that of black maca [6, 7]. When compared to yellow, pink, violet and lead hypocotyls, total phenols are highest in yellow maca (**Table 1**) [8]. Besides the effect of ecotype, phenol contents are influenced by the extraction methods. Hydroalcoholic extract and its fractions (petroleum ether, chloroform, ethyl acetate, n-butanol, and aqueous) of yellow maca have various levels of total phenols. But there is a positive correlation between FRAP, HRSA, LPIA and total phenol contents [9]. Campos et al. [4] analyzed the total phenols of maca with several extraction methods and identified a standard and optimal extraction method. It has been shown that ethanol concentration is more effective on total phenol extraction than temperature, liquid/solid ratio and extraction time. In addition, cooking process affects the total phenols content and also antioxidant activity. It was reported that boiled yellow maca contains 13.6 mg GAE/g while raw yellow maca has 7.8 mg GAE/g of total phenols (**Table 1**) [10]. Some studies argue that maca has the lowest amount of total phenols (5.5–7.6 mg GAE/g) in used herbs, plants and spices in South American culinary. Because of low phenol content, its FRAP, DPPH and ABTS scavenging abilities and antioxidant activity might be seem limited when compared to the others [11, 12].

Ecotype	Form	Value (mg GAE/g maca)	Effect	Antioxidant activity	Reference
Black	Spray-dried	13.5–17.9	DPPH scavenging	15.06–18.52%	[6]
Red		11.6–13.6		14.11–16.23%	
Yellow	Methanol extract	1.85	DPPH scavenging	21.7%	[7]
Black		2.51		18.2%	
Yellow	Methanol extract	2.27–2.29	FRAP, HRSA, LPIA activities	Various	[9]
Yellow	Fresh Hypocotyl	5.65–5.85	Effects of ecotype	Nonmeasured	[8]
Pink		5.72			
Violent		4.61–5.21			
Lead		4.89–4.91			
NA	Ethanol extract	3.56–9.51	Effects of extraction, Antioxidant activity	Various	[4]
NA	Fresh Hypocotyl	5.5–7.6	Dose-dependent DPPH scavenging	>10%	[11]
NA	Aqueous	4.6	DPPH, ABTS, FRAP scavenging	0.434 mmol/100 ml	[12]
Yellow	Boiled	13.6	Effects of cooking process	Nonmeasured	[10]
	Non-boiled	7.8			

**Table 1.** Phenol content of maca and its antioxidant effects.

### 3.2. Glucosinolates

Glucosinolates (Gls) are the secondary metabolites with nitrogen and sulfur chains which many plants in Brassicaceae family contains. In the chemical structure of Gls, there are R and sulphate groups derived from amino asides. During the consumption, plant texture is damaged and myrosinase enzyme hydrolyses Gls to  $\beta$ -D-glucose and aglycone. By releasing sulphate, these metabolites reorganize to thiocyanate, isothiocyanate and nitrile which give the typical taste and smell of Brassicaceae plants. The main source of Gls is seeds, roots, stems and leaves of cruciferous vegetables in human diet [13–15]. The most of Gls in maca is aromatic type and glucotropaeolin. The Gls content varies by ecotype, part of maca plant, harvest time, cultivation region, drying and extraction process (**Table 2**) [8, 16–18]. Also, researchers have focused on antioxidant and anticarcinogenic effects of Gls in maca [4, 19].

The Gls content of maca is affected by harvest time, processes of drying and manufacturing. Total Gls content increases up to 90 days before harvest and 15 days after harvest. During traditional drying process in the open air, instable temperature and dehydration cause the tissue damage and decreasing myrosinase enzyme activity to generate Gls in hypocotyl [10, 20]. This process of freeze drying also decreases benzylglucosinolate and benzylisothiocyanate contents of maca (**Table 2**) [17]. Likewise, supplementing to food as a flavorant, encapsulating

Gls	Ecotype	Form	Value	Unit	Effect	Reference
Total glucosinolate	Yellow	Methanol extract	36.2	mmol/kg DW	Effects of harvest time and drying	[20]
	Red		34.9			
	Black		31.43			
Total glucosinolate	NA	Ethanol extract	4.06–17.81	mmol/kg DW	Effects of extraction method, ABTS scavenging	[4]
Benzyl glucosinolate	Yellow	Pulverized	126	mg/100 g DW	Protect the skin against UV	[10]
		Aqueous	302			
		Dried Hypocotyl	83			
Total glucosinolate	Yellow	Fresh Hypocotyl	28.42–37.23	μmol/g DW	Effects of ecotype	[8]
	Violent		33.22–34.30			
	Pink		44.1			
	Lead		54.78–56.00			
Benzyl isothiocyanate	Mix	Fresh Hypocotyl	475	μg/g DW	Effects of drying	[17]
		Dried Hypocotyl	21.5			
Benzyl glucosinolate	Mix	Fresh Hypocotyl	46.3	mg/g DW		
		Dried Hypocotyl	17.8			
Total glucosinolate	Mix	Fresh Hypocotyl	25.66	μmol/g DW	Effects of manufacturing process	[16]
		Dried Hypocotyl	4.45			
		Seed	69.45			
		Powder	4.06			
		Mayonnaise	2.69			
		Liquor Tonic	–			

**Table 2.** Glucosinolate and their derivates contents of maca (DW, dry weight).

or grinding influence adversely [16]. Boiling the dried maca hypocotyl before consumption increases total Gls content. Fresh hypocotyls have the highest level of Gls. The vast majority of Gls in maca is benzyl glucosinolate (>76%), also called glucotropaeolin. The other derivatives of Gls such as glucoalyssin, glucosinalbin, glucolimnanthin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and glucoraphanin are in trace amounts [4, 8, 20].

It is questionable whether or not there is a relation of Gls with ecotype of maca. Clement et al. [8] reported that there are differences in Gls between ecotypes and the lead color ecotype of maca has higher total Gls than that of yellow, pink and violent. Supporting to this, black maca has more benzyl glucosinolate than yellow and purple macas [18]. But, other researchers

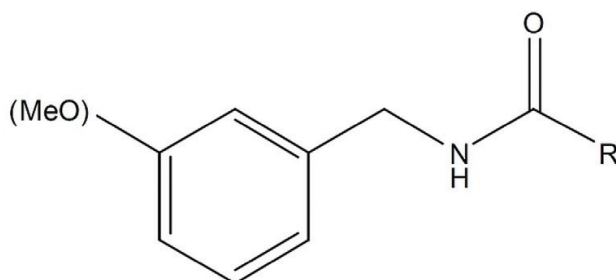
found out that various ecotypes have Gls in similar quantities and there is not an influence of ecotype on Gls content in maca [20].

### 3.3. Alkamides

Alkamides are formed by the different amine groups and the fatty acids and are natural components of many plants. Since their chemical structures are different from other alkamides, the alkamides of maca are called as the macamide. They which are maca-specific alkamides, are thought to have antioxidant effects. Chemical structures of macamides are formed by binding the phenylamine to fatty acid with an amide bond. These fatty acids range from 12 to 24 carbon atoms. In some macamides there is a methoxy group on the benzyl ring. The R group derives the macamides according to the number of carbons and chains they contain (**Figure 2**) [21, 22]. Day by day, a new macamide, its chemical structure and pharmacological effects are introduced in scientific publications. Despite their low levels, they are important markers to measure and standardize the maca's quality [22–25].

First, Muhammed et al. [23] identified N-benzyl-5-oxo-6E, 8E-octadecadienamide and N-benzylhexadecanamide which maca-specific alkamides and named them 'macamide'. The major amount of macamides in maca forms in N-benzylhexadecanamide. In addition to these two macamides, Zhao et al. [22] isolated five new macamides not reported in other *Lepidium* species before (**Table 3**). N-(3,4-dimethoxybenzyl)-hexadecanamide and N-benziltetracosanamide, commonly found in cultivated maca, were also detected in wild maca [25]. The cultivation region and the drying process affect the amount of macamides but the effect of ecotype is not clear [17, 26, 27]. Compared to maca grown in Peru, China and Czechia, the most of the N-benzylhexadecanamide is in that of China, then Peru and Czechia respectively. Further, macamide was not detected in maca grown in greenhouse [18, 27, 28]. However the above-mentioned studies argue that ecotype has no effect on macamide, they were reported that violent color of hypocotyl has higher total macamide than yellow, pink and lead colors of them [8]. Among black, purple and yellow hypocotyls of maca, black one contains the highest total macamide content [29].

Macamides are believed to be FAAH (fatty acid amide hydrolase) inhibitors, and also play a role like endocannabinoids in the cannabinergic synapses. Some derivatives of macamide have shown the inhibition activity of FAAH and to be a natural alternative to FAAH inhibitors to treat the neurological diseases, such as pain, epilepsy, anxiety, depression [30, 33, 53]. But some



**Figure 2.** The main structure of macamide [21].

Macamides	Ecotype	From	Effect	Reference
N-benzyl-5-oxo-6E,8E-octadecadienamide	NA	Petroleum ether extract	First report	[13]
N-benzylhexadecanamide				
5-oxo-6E,8E-octadecadienoic acid				
N-benzyl-9-oxo-12Z-octadecenamide	NA	Ethanol extract	First report	[22]
N-benzyl-9-oxo-12Z,15Z-octadecadienamide				
N-benzyl-13-oxo-9E,11E-octadecadienamide				
N-benzyl-15Z-tetracosenamide				
N-(m-methoxybenzyl)-hexadecanamide				
N-benzylhexadecanamide	NA	Petroleum ether extract	First report	[24]
N-benzyl-9Z-octadecenamide				
N-benzyl-(9Z,12Z)-octadecadienamide				
N-benzyl-(9Z,12Z,15Z)-octadecatrienamide				
N-benzyl-octadecanamide				
N-benzylhexadecanamide	Yellow	Petroleum ether extract	Antifatigue and antioxidant activities	[32, 34]
N-benzyl-5-oxo-6E,8E-octadecadienamide				
N-benzylhexadecanamide	NA	Pentane extract	FAAH inhibition	[21]
N-benzyl-octadecanamide				
N-benzyl-9Z-octadecenamide				
N-benzyl-5-oxo-6E,8E-octadecadienamide				
N-(3-methoxybenzyl)-9Z-octadecanamide				
N-benzyl-(9Z,12Z)-octadecadienamide				
N-(3-methoxybenzyl)-(9Z,12Z)-octadecadienamide				
N-benzyl-(9Z,12Z,15Z)-octadecatrienamide				
N-(3-methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide				
N-(3-methoxybenzyl)-hexadecanamide				
N-benzyl-15Z-tetraisocenamide				
N-(4-florobenzyl)-hexadecanamide				
N-(4-chlorobenzyl)-hexadecanamide				
N-benzyl-5-oxooctadecanamide				
N-(4-chlorobenzyl)-5-oxooctadecanamide				
N-pyridine-9Z-octadecenamide				
N-(3-methoxybenzyl)-6-phenylhexanamide				
N-(3-methoxybenzyl)-6-phenylheptanamide				
N-(3-methoxybenzyl)-7-oxo-7-phenylheptanamide				
N-(3,4-dimethoxybenzyl)-hexadecanamide	Wild maca	Hexan extract	First report in wild maca	[25]
N-benzyltetracosanamide				

Macamides	Ecotype	From	Effect	Reference
N-benzyl hexadecanamide	Mix	Methanol extract	Effects of drying process	[7]
N-benzyl-(9Z,12Z)-octadecadienamide				
N-benzyl-(9Z,12Z,15Z)-octadecatrienamide				
N-benzylinoleamide	NA	Petroleum ether extract	No effect	[31]
N-benzyloleamide			Antifatigue and antioxidant activities	
N-benzylpalmitamide			No effect	
N-benzylhexadecanamide	Black, Yellow, Purple	Petroleum ether extract	Effects of ecotype, Antioxidant activity	[29]
N-benzyl-5-oxo-6E,8E-octadecadienamide				
N-benzyl-9-oxo-(12Z,15Z)- octadecadienamide	Mix	Petroleum Ether Extract	Effects of region and greenhouse	[18, 27, 28]
N-benzyl-13-oxo-(9E,11E)-octadecadienamide				
N-benzyl-9-oxo-12Z-octadecanamide				
N-(3-methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide				
N-benzyl-(9Z,12Z,15Z)-octadecatrienamide				
N-(3-methoxybenzyl)-(9Z,12Z)-octadecadienamide				
N-benzyl-(9Z,12Z)-octadecadienamide				
N-(3-methoxybenzyl)-hexadecanamide				
N-benzyl-hexadecanamide				
N-Benzyl-9Z-octadecanamide				
N-Benzyl-octadecanamide				
N-Benzyl-heptadecanamide				
N-benzylhexadecanamide	NA	NA	Neuroprotective activity	[33]
N-(3-methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide				

**Table 3.** Macamides and macene content of maca and their effects.

macamide derivatives which has the carbonyl group do not produce the inhibition effect of the FAAH because of their interaction with the FAAH [21]. Wu et al. [30] have reported that the FAAH inhibition activity of macamides can be reversible or irreversible due to their chemical structures. When mice in exercise-induced stress was daily fed with the low (12 mg/kg) and high (40 mg/kg) doses of N-benzyloleamide, N-benzylinoleamide and N-benzylpalmitamide, antioxidant and antifatigue effects were recorded by increasing GPx and SOD (superoxide dismutase), decreasing MDA (malondialdehyde) lactic acid, blood ammonia, LDH (lactate dehydrogenase), liver glycogen and increasing non-esterified fatty acid (NEFA) specially in N-benzyloleamide high dose group (40 mg/kg). Thus, N-benzyloleamide influences the energy metabolism and reveals antioxidant and antifatigue activities (**Table 3**) [31].

### 3.4. Polysaccharides

Polysaccharides are found in the structure of many plants and their major components. They are high molecular weight carbohydrates and formed by linking monosaccharides together with glycoside bonds. They have nutritive value and some pharmacological activities such as anti-fatigue, antioxidant, immunomodulator and antimicrobial etc [34–36]. Thus, polysaccharides may take a main part of components in some drugs and some food supplement [37, 38]. As a food supplement, maca also has large amount of polysaccharides which influence significant metabolism (Table 4). Maca polysaccharides (MP) are mostly composed of rhamnose, arabinose, glucose and galactose. Dominant components are D-GalA (D-Galacturonic Acid, 35.07%) and D-Glc (D-Glucose, 29.98%) [35]. Although the composition, the yield and the purity of MP vary according to the extraction method, the water extraction method, simple and eco-friendly, is preferred in studies to isolate them. But there are disadvantages such as the need for additional applications (ultrasonic extraction, enzymes, centrifuge, deproteinization) to increase the yield or purity of polysaccharides in maca extract [39, 26]. For example, when increasing the concentration of solvent, MP yield increases but the purity of MP decreases from 69.4 to 39.5%. Amylase and glucoamylase enzyme applications decrease both amount and purity of MP. Contrary to filtration, centrifuge enhances the yield and decrease the purity of MP [39].

The antioxidant and antifatigue activities of MP are established by measuring some biochemical parameters in blood and tissues. When fed several doses of MP (79% of glucose), hypoxia tolerance, and exercise ability of mice and muscle glycogen were enhanced. But blood lactic acid (LA) lactic dehydrogenase (LDH) and urea nitrogen (BUN) were not affected [40]. Otherwise, it was reported that increasing in swimming time and antifatigue effects of MP are based on increasing liver glycogen and decreasing urea nitrogen, BUN, LDH, and LA of mice and rats with exercise-induced stress [35, 41, 42]. In addition to these results, MP has effects on the precursor enzymes of antioxidant status such as SOD, GPx (glutathione peroxidase) and CAT (catalase). While Tang et al. [35] have introduced that a daily dose of 100 mg MP/kg body weight of mice significantly increased GPx and decreased MDA (malondialdehyde),

Species	Yields (% DM)	Dose	Unit	Effect	Reference
Mice	0.2	20–100	mg/kg/d	Antifatigue activity	[34]
Mice	0.01	25–50–100	mg/kg/d	Antifatigue and Antioxidant activities	[35]
Alcoholic Mice	NA	200–800	mg/kg/d	Antioxidant activity	[43]
Cell culture	NA	0.125–2	mg/ml	Increasing viability, hepatoprotectant	[43]
Cell culture	6	62.5–1000	µg/mL	Immunomodulator activity	[44]
Rat	2.37	50–100–200	mg/kg/d	Antioxidant activity	[42]
In vitro	0.052–0.15	2	mg/ml	Antioxidant activity, Effect of extraction	[39]
Mice	NA	0.1–0.5–1	g/kg/d	Antifatigue activity	[40]
Mice	2.37	500–2000	mg/kg/d	Antifatigue and Antioxidant activities	[41]

**Table 4.** Polysaccharides content of maca and their antioxidant effects (DM, dry maca).

Li et al. [41] have reported that MP has occurred a dose depend antioxidant activity by increasing SOD, GPx and CAT enzymes and decreasing MDA in liver of mice. Also, He et al. [42] have reported that antioxidant activity with a correlation between the doses and enzymes levels in muscle against the exercise-induced oxidative stress. Similar to animal experiments, MP plays the crucial roles of antioxidant and free radical scavenger in cell cultures (**Table 4**) [39, 43].

In brief, the mechanism of antifatigue and antioxidant effects of especially aqueous polysaccharides in maca originates from improving hypoxia tolerance, eliminating metabolic wastes, serving energy source with high glucose contents and reducing oxidative damage by enhancing antioxidant enzyme [38, 41].

#### 4. Antioxidant effects of maca as a feed supplement in animal nutrition

Most scientific research has worked with laboratory animals to know the antioxidant effects of maca. Rarely, studies on farm animals have been published in recent years. In these studies, daily doses (mg /kg BW/d) of maca or its bioactive content were calculated on their body weights (**Table 5**). In order to demonstrate antioxidant effects, laboratory animals, they are exposed to exercise- induce stress and antioxidant enzyme levels of their serum and various organs (brain, liver, muscle etc.) and exercise performance is measured [31, 42]. In farm animal nutrition, the criteria such as feed efficiency, nutrition performance, viability were recorded besides antioxidant enzyme activities. Dried, milled powder form of maca is mostly used in animal experiments.

When used in rats and mice at various doses of maca or its antioxidant compounds, some effects are occurred against the stress factors. In particular, GPx, SOD and glutathione (GSH) levels in serum, liver and brain increase, MDA and ROS (reactive oxygen species) decrease [45, 46]. Choi et al. [32] determined that the lipid soluble extract of maca contained 7.8 mg/g DM of macamide and macaene while maca powder contained at the level of 0.3–0.4 mg/g DM. When this lipid soluble extract is given at 100 mg/kg BW per day, it reduces lipid peroxidation in muscles of rats, increases GSH and exercise duration (**Table 5**) [32]. Similarly macamides (N-benzylinooleamide, N-benzyloleamide, N-benzyloleamide), isolated from maca, reduce the oxidative stress induced by exercise and eliminate the waste products in serum [31]. But Qui et al. [29] argue that maca improves the antioxidant enzyme (CAT, SOD, GPx) activity both in blood and liver independently of macamide and macaene, and there is no correlation between antioxidant effect and these bioactive compounds. When rats are daily fed with maca polysaccharides at between 20 and 100 mg/kg BW doses, serum LA, BUN and MDA are decreased, GPx and creatine kinase are increased, especially in high dose (100 mg/kg BW) [34, 43].

In other animal species, there are effects on the criteria such as sperm quality, survival, feed conversion, nutritional performance as well as antioxidant effects of maca as a feed additive [47, 48]. When there is no effect on blood parameters in horses, Aspartate transaminase (AST) and gamma-glutamyl transpeptidase (GGT) increase [49]. In fish fed the fresh hypocotyl of maca, nutritional performance and feed conversion and viability enhanced [48, 50]. While



Species	Ecotype	Form	Dose	Unit	Effect	Reference
Fish	NA	Fresh Hypocotyl	5–10–15	% of feed	Improving growth rate and survival, decreasing magnesium (not significantly)	[48]
Poultry	NA	Powder	0.5–1	% of feed	Antioxidant activity, decreasing magnesium	[51]
Horse	NA	Powder	50–75	gr/day	Increasing AST and GGT, decreasing magnesium	[49]
Horse	Yellow	Powder	20	mg/d	Effects on sperm quality	[47]
Rat	NA	Aqueous	50–100–200	mg/kg/d	Antioxidant activity	[42]
Rat	NA	Powder	1	% of feed	Antioxidant activity	[52]
Rat	Yellow	Lipid soluble extract	30–100	mg/kg/d	Antioxidant and antifatigue activities	[32]
Rat	Black	Petroleum ether extract	100	mg/kg/d	Antioxidant activity (significantly)	[29]
	Yellow				Antioxidant activity (slightly)	
	Purple				Antioxidant activity (slightly)	
Mice	NA	Powder	500–1000	mg/kg/d	Antioxidant activity	[46]
Mice	NA	Macamide	40–12	mg/kg/d	Antioxidant activity	[31]
Mice	Yellow	Polysaccharide	20–100	mg/kg/d	Antifatigue activity	[34]
Mice	NA	Petroleum ether extract	125–250–500	mg/kg/d	Antioxidant activity	[45]
Mice	NA	Polysaccharide	25–50–100	mg/kg/d	Antioxidant activity	[35]

**Table 5.** Antioxidant effects of maca and its compounds in animal nutrition.

laying hens fed dry maca powder at the rate of 0.5 and 1% (w/w) no effect on nutritional performance, serum parameters and reproductive hormones was determined. But serum GPx level increased depend on the ratio of maca supplementation in diet of hens [51]. Day by day, the antioxidant effect of maca as feed additive in laboratory studies has been clarifying, but not yet on the other species and more nutrition experiment is needed.

## 5. Antioxidant effects of maca as a food supplement in human nutrition

Maca is being both at the meals of the indigenous people and exported to the whole world. Consumers around the world are taking it as a food supplement for improving their sexual and sportive activities and energy. So researchers have similarly given priority to these issues. However, studies on antioxidant status of human are limited [54].

Stress and inflammation affect human health score in the worst way, and interleukin-6 as a marker of inflammation increases in serum. People consuming regular maca have a lower interleukin-6 level and higher health scores than those not consume it [1]. Although macamides content is higher in black maca, red one enhanced the health score of human suffered from chronic mountain illness [55]. It has been shown in women that postmenopausal symptoms such as anxiety, depression and sexual dysfunction are reduced without being dependent on reproductive hormones [56, 57]. Similar effects were also observed in men. When consumed 1.5 and 3 g/day of maca powder, men's sexual desire increases and anxiety and depression are inhibited and sperm production and quality are improved (**Table 6**) [58, 59]. These reproductive effects of maca appeared independently of the hormones [28, 60]. In addition to sexual activity, when athletes got 2 g /day, performance improved and running time was reduced [61]. By scavenging DPPH and peroxy radicals, polysaccharides isolated from maca have protected human erythrocyte against hydrogen peroxide and inhibited the hemolysis [62]. Some studies suggested that maca consumption is well tolerated and has no adverse effect [55]. On the contrary, some studies have reported negative effects on blood pressure [57, 63, 64].

Species	Form	Dose (g/d)	Duration (day)	Effect	Reference
Men	Powder	1.5–3	84–120	Increasing spermatogenesis	[28, 58, 60]
Post menopausal women	Powder	3.5	42	Decreasing sexual dysfunction and depression	[56]
Depressed women and men	Powder	1.5–3	84	Improving sexual activities, Decreasing sexual dysfunction and depression	[65, 66]
Women and men	Powder	0.6	90	Decreasing metabolic syndrome symptoms	[64]
Sportsmen	Aqueous extract	2	98	Increasing sexual and sportive activities	[61]
Women and men	Dry hypocotyl	Consume and Nonconsume	—	Increasing interleukin-6 and health score	[63]
Post menopausal women	Powder	3.3	42	Decreasing depression, anxiety and health status	[57]
Women and men	Spray-dried	3	84	Increasing health score	[55]

**Table 6.** Effects of maca as a food supplement in human nutrition.

## 6. Conclusion

Phenols, glucosinolates, alkamides and polysaccharides, which are important antioxidant source of many plants, were above mentioned. Many scientific researchers are attempting to reveal the effects of these compounds on antioxidant metabolism. While some of these compounds are peculiar to maca, others are common in tuberous plants. The variety and proportion of the bioactive compounds in maca depend on lots of different factors, specially

ecotype, cultivation region, harvest time, production process and consumption preferences. Despite the wide-consumption of maca, its antioxidant effects are still being discussed in the academic circles. So the standardization of maca plant based on antioxidants is needed to take part as a safe supplement in nutrition and diets.

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# The Use of Iodine, Selenium, and Silicon in Plant Nutrition for the Increase of Antioxidants in Fruits and Vegetables

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## Abstract

Iodine, silicon, and selenium are considered elements not essential for the metabolism of plants. However, these elements are vital for humans, and their presence as traces in food is beneficial. The use of I, Si, and Se in the fertilization programs of the plants allows, on the one hand, the mineral biofortification of the crops and, on the other hand, through mechanisms not yet fully understood, the production and accumulation of more antioxidants in the edible organs. This chapter provides an overview about the use of I, Si, and Se both for mineral biofortification and for the increase in the concentration of antioxidants in plants, with an emphasis on redox metabolism adjustments and antioxidant chemical species studied. The scope of the chapter is on horticultural species in the open field and under greenhouse or tunnels.

**Keywords:** food and human health, functional foods, nutraceuticals, plant stress, trace elements

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## 1. Introduction

Elements I, Se, and Si are not considered essential for plants but are essential elements for humans and domestic animals. These three elements have been the objective of biofortification

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programs and projects, to increase their intake by improving their concentration and bioavailability in food. On the other hand, it has been found that by using I, Se, and Si in crop plants, applied in seeds, plants, or fruits, favorable responses are obtained such as increased growth and tolerance to stress. Tolerance to stress is associated with a higher concentration of antioxidants. Thus the use of these elements is a useful technique for the nutritional improvement of crop plants, both in antioxidant level and biofortification. This chapter presents the advances in the last 10 years about the use of I, Si, and Se both for mineral biofortification and for the increase in the concentration of antioxidants in plants, with an emphasis on redox metabolism adjustments and antioxidant chemical species studied. The scope of the chapter is on horticultural species in the open field and under greenhouse or tunnels.

## 2. Iodine, selenium, and silicon in agricultural systems

The contents of iodine, selenium, and silicon in the Earth's crust range from  $<0.1$  to  $150 \text{ mg kg}^{-1}$  and  $0.05$  to  $1.5 \text{ mg kg}^{-1}$  and  $540,000 \text{ mg kg}^{-1}$  (54%) [1, 2]. This data establishes iodine and selenium as trace elements and silicon as the second most abundant element. Although silicon reaches 23–46.5% of the mass of the parent rocks in the soil, most of this element is present in mineralized form as crystalline or amorphous  $\text{SiO}_2$  [3]. It has been found that the concentration of Si in the soil solution can be as low as  $0.09 \text{ mg L}^{-1}$ , reaching a maximum of  $23.4 \text{ mg L}^{-1}$ . The amount of bioavailable silicon depends mainly on the composition of the parent rock of the soil, as well as on factors such as the content of organic matter, the pH, and the oxidation-reduction potential (Eh) [4].

Regarding iodine and selenium, uniformly low concentrations have been found in most of the minerals in the parental rocks. However, there is a positive correlation between the amount of organic matter and the availability of I and Se in soils derived from sedimentary rocks [5].

The availability of iodine and selenium in the soil seems to be based on factors other than the geology itself. In iodine, the most significant influence is exerted by the distance to the ocean, because the ocean is the primary reservoir of iodine on the planet [6]. However, in the soil, the amount of organic matter is the most studied factor regarding the dynamics of the I and Se. In general, it has been established that in the presence of a high content of organic matter, low volatility of I and Se is found, and that the presence of metal oxides and hydroxides such as aluminum, iron, and manganese plays an essential role in retention, and this process is directly related to pH and Eh [7].

In the soil, the predominant chemical species with reducing conditions and low pH ( $<7$ ) are the iodides ( $\text{I}^-$ ) and selenite ( $\text{Se}^4$ ), species with a great affinity for organic matter; additionally under these conditions, the  $\text{Se}^4$  can be reduced to  $\text{Se}^0$  by precipitating and thus becoming less available [8, 9]. On the contrary, under basicity conditions (pH  $> 7$ ) and soils with oxidizing conditions, the predominant forms will be  $\text{IO}_3^-$  and  $\text{Se}^6$ , which have been shown to have binding affinity with the metal oxides and hydroxides present in the organic matter, through weak electrostatic attractions, thus allowing availability for mobilization and absorption by plants [10]. On the other hand, the Si available in the soil depends on the type of parent rock,

since this comes from the weathering of the original material. Greater solubilization has been found from granite rocks than from basalt rocks [11]. The bioavailable form of Si is monosilicylic acid ( $H_4SiO_4$ ) which is found in the liquid phase of the soil [12]; it has been established that it remains in a non-charged and bioavailable form in a pH range of 4.5 to <8, and it is de-protonated to  $H^+ + H_3SiO_4$  at pH > 9, forming polymers of different molecular weights [13, 14]. A high degree of polymerization of  $H_4SiO_4$  has been found under conditions of high concentration of aluminum in the mineral fraction of the soil, as well as during the processes of evaporation of soil water and freezing [15]. The application of acidic solutions in the soil favors solubilization, whereas liming reduces it [16].

There are differences with respect to the hydrological mobilization of these elements. For the Se and Si occur by the dragging of sediments or dissolved chemical species through continental aquatic flows, with an estimated 14,000 t per year toward the ocean in the case of Se [17]. Iodine mobilization presumably occurs in reverse to that of Se and Si, that is, from the ocean to continental waters, mainly through rainfall. The rainfall has an iodine concentration of 0.5–5  $\mu\text{g L}^{-1}$ , and probably such concentration is a reflection of the gaseous dynamics of iodine in the atmosphere [18]. In surface water, iodine has been reported in ranges of <20  $\mu\text{g L}^{-1}$ , while in groundwater the reported concentrations have been higher (from 430 to 4100  $\mu\text{g L}^{-1}$ ), probably due to the desorption of organic matter rich in iodine, sediment leaching, or concentration by evaporation in arid zones [19]. Silicon solubilized from the parent rock and converted to its bioavailable forms, both by the physicochemical processes and by the metabolism of different organisms such as plants, has been estimated to be  $240 \pm 40 \mu\text{g L}^{-1}$  [20].

The natural uptake of these elements by the plants will be conditioned to the different growth conditions. The low bioavailability is the typical situation; thus, the resulting level of I, Se, and Si in the plants will be similarly low (Table 1). In the case of soilless crops grown under protected conditions, both the availability of these elements and the resulting concentration in the plants will be very low, since in this case, the primary source would be irrigation water, which contains a small amount of I, Se, and Si.

Concentration in soils ( $\text{mg kg}^{-1}$ )	Concentration in plants ( $\text{mg kg}^{-1}$ )	Reference
<b>Iodine concentration in soils and agricultural plants</b>		
3.35 (Forest zone in Russian plane)	0.128 Gramineae, 0.121 leguminoseae	[21]
11.8 (Andosol upland in Japan)	20 in wheat and 7.7 in barley	[22]
0.66 (Agricultural soils in Pakistan)	0.01 in wheat grain	[23]
0.92 (Samples from the last 105 years in an experimental field in Rothamsted, UK)	$4.8 \times 10^{-5}$ in weeds	[24]
<b>Selenium concentration in soils and agricultural plants</b>		
0.06 (Se plant available in South Dakota)	0.63 in wheat grain	[25]
1.6 (samples from the last 105 years in an experimental field in Rothamsted, UK)	$5.5 \times 10^{-6}$ in weeds	[24]
58.7–304 (agricultural soil in Songpan, Tibetan Plateau)	0.009 in barley grains	[26]

Concentration in soils (mg kg <sup>-1</sup> )	Concentration in plants (mg kg <sup>-1</sup> )	Reference
<b>Silicon concentration in soils and agricultural plants</b>		
500 (rice fields in Chiba, Japan)	15.6 mg g <sup>-1</sup> leaves of rice plants	[27]
7.3 mg kg <sup>-1</sup> amorphous Si, 0.092 mg kg <sup>-1</sup> dissolved Si (Serengeti, North of Tanzania, and South of Kenia)	<i>Themeda triandra</i> 36.9 mg g <sup>-1</sup>	[28]

**Table 1.** Concentration of I, Se, and Si in soils, irrigation waters, and crop plants.

In regard to the concentration of I, Se, and Si, the use of soilless crops results in plants with a lower level of these elements than in soil crops. Hydroponic production of crops for human consumption has increased substantially in recent years, mainly due to the efficient use of water and nutrients from the crop. However, as far as commercial production is concerned, nutrition only considers the application of the elements deemed essential for plants [29], leaving aside those that are beneficial as I, Se and Si. These beneficial elements raise the antioxidant content in plants, giving an advantage against oxidative stress, in addition to its use allows obtaining biofortified crops with high nutritional value for human consumption.

### 3. The impact of iodine, selenium, and silicon on the antioxidant system of plants

The energy metabolism of aerobic organisms inevitably produces reactive oxygen species (ROS), which are free radicals that react with the different biomolecules in the cell causing damage. Additionally, when there is some stress, both biotic and abiotic, a substantial increase in ROS content is induced. As an adaptive response to neutralize these species, the synthesis of enzymatic and nonenzymatic antioxidants is used, granting tolerance to stress [30]. A partial explanation of the beneficial effect provided by I, Se, and Si is the stimulation of the increase in antioxidants. **Table 2** shows results obtained with the application of different chemical species and concentrations of I, Se, and Si on the antioxidant content in soilless crops.

Plant species	Chemical species and concentration	Effect	Reference
<b>Iodine application in soilless crops</b>			
Lettuce	KI ≤40 μM	Increase of 6, 2, 1.5, 1.2, and 1.2 times, respectively, in the total phenols, flavonoids, anthocyanins, ascorbate, and antioxidant potential	[31]
Lettuce	IO <sub>3</sub> 80 μM	Increase of ascorbic acid by 1.2 times in the leaves of plants	[32]
Lettuce	IO <sub>3</sub> < 40 μM	Increases the antioxidant potential by double in the leaves of plants	[33]
Tomato	KI 1 μM daily foliar application	Increase of ascorbic acid by 22% and glutathione by 85% in the leaves of seedlings	[34]
Tomato	KIO <sub>3</sub> 7.88 μM	Increase of 8% in the concentration of ascorbic acid and 6% in total phenols in tomato fruits	[35]

Plant species	Chemical species and concentration	Effect	Reference
Strawberry	I <sup>-</sup> ≤0.25 mgL <sup>-1</sup> or IO <sub>3</sub> <sup>-</sup> ≤0.50 mg L <sup>-1</sup>	Increase in vitamin C content of 80 and 30%, respectively, in fruits	[36]
Pepper	KI 1 mg L <sup>-1</sup>	Increase in ascorbic acid by 35% in fruits and 50% reduction in total acidity	[37]
Lettuce	KIO <sub>3</sub> 40 μM and salicylic acid 40 μM	Increase in vitamin C and phenylpropanoids by 50 and 14%, respectively, in the leaves	[38]
<b>Selenium application in soilless crops</b>			
Plant species	Chemical species and concentration	Effect	Reference
Lettuce	Na <sub>2</sub> SeO <sub>4</sub> 40 μM	Increase in glutathione and ascorbic acid by 38% and three times, respectively, in the leaves of the plants	[39]
Tomato	Na <sub>2</sub> SeO <sub>3</sub> 5 mg L <sup>-1</sup>	Increase in total antioxidant capacity by 38% in fruits	[40]
Tomato	Na <sub>2</sub> SeO <sub>4</sub> 1 mg L <sup>-1</sup>	Increase of seven times the quercetin content in fruits	[41]
Tomato	Na <sub>2</sub> SeO <sub>4</sub> 25 μM	Increase of three times the glutathione in leaves during 5 days of exposure to the treatment	[42]
<b>Silicon application in soilless crops</b>			
Plant species	Chemical species and concentration	Effect	Reference
Wheat	Na <sub>2</sub> SiO <sub>3</sub> 1 mM	Increase of 28% in the concentration of glutathione in leaves sensitive and resistant to salinity	[43]
Rice	H <sub>4</sub> SiO <sub>4</sub> 1 mM	Increase cysteine content by 78% in plants subjected to arsenic stress	[44]
Cucumber	Na <sub>2</sub> SiO <sub>3</sub> 1 μM	Increase in the activity of APX and GPX of four and two times in leaves relieving the stress by salinity in <i>super dominus</i> cultivar	[45]
Tomato	K <sub>2</sub> SiO <sub>3</sub> 2.5 mM	Increase in the concentration of ascorbate and glutathione to double, at 7 and 3 days of treatment, respectively, in the roots of tomato plants subjected to water stress	[46]

**Table 2.** Impact of I, Si, and Se on the antioxidants of various crop species grown in soilless cultivation systems.

#### 4. Proposed mechanisms of action of iodine, selenium, and silicon as inducers of the accumulation of antioxidants

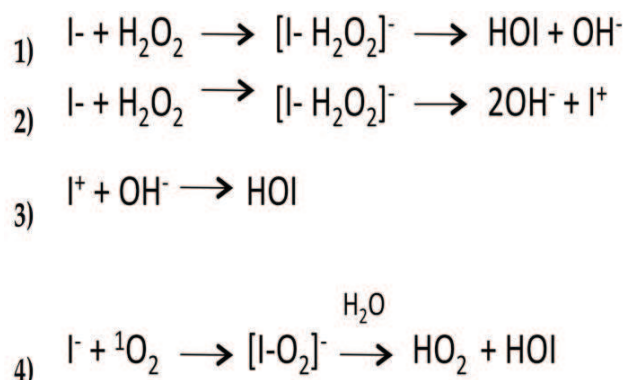
Iodine is considered the first inorganic antioxidant used by ancestral organisms when the concentration of atmospheric O<sub>2</sub> increased as a result of oxygenic photosynthesis [6]. This mechanism is widely elucidated in algae, where the direct neutralization of species such as superoxide (O<sub>2</sub><sup>-</sup>), hydroxyl (OH<sup>-</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [47] has been proven, mainly due to iodine oxidation–reduction power. **Figure 1** illustrates the possible mechanisms of reaction proposed by Luther et al. [48].

Subsequently, these organisms incorporated iodine as a cofactor in the reaction between the vanadium-dependent iodoperoxidase enzyme (IPO-V) and  $H_2O_2$ , thus becoming an essential element against oxidative stress, but not only directly but through a specialized enzymatic mechanism. In terrestrial plants neither of these two processes is fully established, but it has been shown that it exerts a direct function as an electron donor (inorganic antioxidant) at least on the superoxide radical [34], and it has been further verified that iodine can act as a moderate prooxidant, promoting the synthesis of nonenzymatic and enzymatic antioxidants, potentiating tolerance to stress [33, 49].

Selenium participates in antioxidant metabolism with different mechanisms, both directly and indirectly. An example of the direct effect is observed with the application of Se at low concentrations ( $\leq 2 \mu M$ ) in plants subjected to different stresses such as heavy metal toxicity [50, 51], low temperature [52], high temperature [53], or UV radiation [54], where a direct neutralization of the radicals  $O_2^{\cdot -}$  and  $H_2O_2$  occurs. Also among the direct mechanisms is the function of Se as a cofactor in the activity of the enzyme glutathione peroxidase [55]. The indirect relationship occurs with the overproduction of reactive oxygen species due to an excess of selenium ( $\geq 6 \mu M$ ). This process is attributed to the assimilation of Se and is dependent on the chemical species. An example of this was demonstrated by Paciolla et al. [56], in cinerary leaves, where the application of  $Na_2SeO_3$  showed an increase in the concentration of  $H_2O_2$ , while  $Na_2SeO_4$  did not show the same effect. The difference was probably due to the reduction to which  $Se^4$  must be subjected to L-selenomethionine for its subsequent transport through the plant; instead,  $Se^6$  is transported directly to the shoot of the plants, as has been shown in rice and broccoli [57]. The use of the reducing potential to assimilate  $Se^4$  causes an increase in the formation of ROS, which triggers a higher synthesis of antioxidants such as ascorbate, tocopherol, and glutathione as well as enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) [58].

The mechanisms through which Si reduces oxidative stress can be divided into three modalities: structural, reducing the absorption of heavy metals, and physiological mechanisms.

The structural mechanism is of a mechanical nature, attributed to deposition of stable Si in the form of biosilica ( $SiO_2$ ) in the cell walls, giving it rigidity and resistance [59].



**Figure 1.** Mechanism of reaction between iodide ( $I^-$ ) and reactive oxygen species such as hydrogen peroxide (1 and 2), hydroxyl (3), and singlet oxygen (4). Figure designed from data obtained from Medrano-Macías et al. [34].

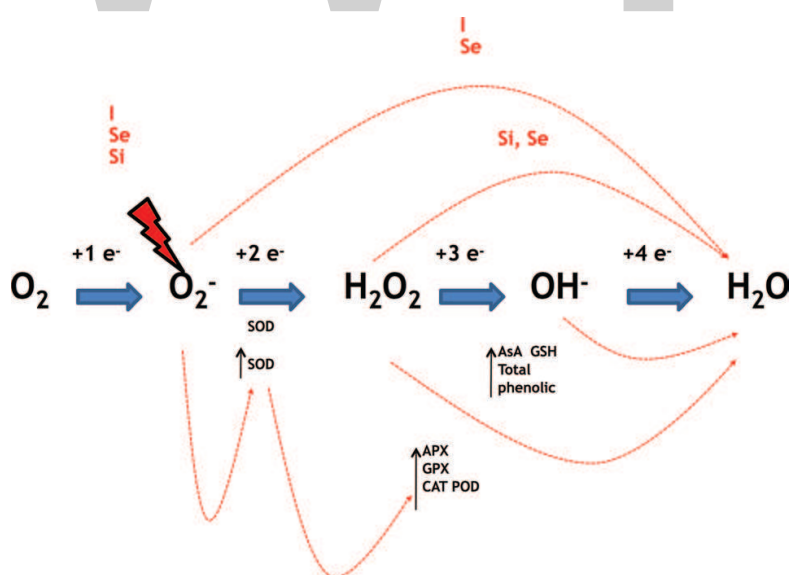
The ability of silicon to reduce the uptake of elements that cause toxicity in plants is well studied in the case of salinity stress, where there is a reduction in uptake and transportation from the root to shoot of  $\text{Na}^+$  and  $\text{Cl}^-$  [60]. A reduction in the absorption of heavy metals such as aluminum [61], cadmium [62], and chromium [63] has also been found. This beneficial effect has been attributed mainly to the reduction, by Si, of the impact that different stress factors have on the permeability of the plasma membrane, allowing it to retain selectivity in the ion flow [59].

The physiological mechanism is related to the induction of antioxidant metabolism. It has been proposed that this occurs due to a possible dual effect: decrease in ROS synthesis and increase in the activity of antioxidant enzymes [64]. Debona et al. [65], showed in wheat plants subjected to biotic stress (*Pyricularia oryzae*) a reduction in the activity of SOD, CAT, peroxidase (POD), APX, and glutathione-S transferase, explaining this phenomenon through a possible inhibition of Si on the fungus ability to colonize plant tissues.

Regarding the synthesis of antioxidants, Kim and collaborators in 2017 [66] made an extensive compendium of the effects of Si on antioxidant metabolism, evidencing that there is more information related to the increase of enzymatic antioxidants such as SOD, CAT, and APX in plants subjected to a variety of abiotic stresses such as heavy metal toxicity [44, 67], salinity [68, 69], and UV radiation [70], among others.

On the contrary, Ma et al. [71] conducted an experiment on soil with stressed wheat plants with water deficit, finding an increase in the concentration of nonenzymatic antioxidants (ascorbate, glutathione, total phenolic compounds, and total flavonoid content) as well as a decrease in the lipid peroxidation. Gong et al. [72] in a similar experiment found an increase in SOD activity, but not CAT or POD.

**Figure 2** shows the proposed mechanisms in which I, Se, and Si intervene in the antioxidant metabolism.



**Figure 2.** Mechanisms of action proposed for I, Se, and Si in the antioxidant metabolism of plants. In the three elements, there are two forms of action: (1) the direct form which is exemplified by the upper red arrows, where the I and Se can reduce the superoxide radical directly to water and Se and Si directly reduce the peroxide ( $\text{H}_2\text{O}_2$ ) to water. (2) The indirect form, which occurs by the influence of Se, I, and Si on ROS overproduction (big red ray) and consequently an increase in enzymatic and nonenzymatic antioxidants, represented by lower red arrows [34, 39, 43, 45, 47, 48, 53, 66, 71].

## 5. Perspectives and recommendations

Expanding the list of elements used in the fertilization of plants cultivated in soil and in soil-less systems will allow obtaining advantages for both agricultural producers and consumers. In particular, the use of the beneficial elements I, Se, and Si in crops for human consumption is expected to increase the functional quality of food, due to the increase in antioxidants that occur in response to the presence of these elements. On the other hand, the exogenous application of quantities as low as 50  $\mu\text{M}$  of  $\text{KIO}_3$ , 5  $\mu\text{M}$  of  $\text{Na}_2\text{SeO}_4$ , and 2 mM  $\text{Na}_2\text{SiO}_3$  in soilless crops provides enough to trigger an increase in antioxidants such as ascorbate, glutathione, and phenolic compounds, which give more reducing power useful to deal with various types of stress.

## 6. Conclusion

In the present chapter, the use of I, Si, and Se, as alternatives in plant nutrition, was described to increase the content of antioxidants, the tolerance to stress, as well as a mechanism of bio-fortification of crops. However, the information so far published presents the impact on the crop plants of only one element at a time, lacking information that describes the results of the use of two or the three elements simultaneously.

### Conflict of interest

The authors have no conflict of interest to declare.

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# Natural Beverages and Sensory Quality Based on Phenolic Contents

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## Abstract

Currently, consumers are demanding natural products, with low sugar content and high nutritional and sensory qualities. Natural beverages have taken an important place due to their mineral, vitamin, and antioxidant contents. Phenolic compounds have great relevance for food industries for their functional properties acting as good antioxidant molecules as well as aging retarders and preventing degenerative diseases. In this connection, design of functional beverages rich in phenolic compounds has been related to the acceptability, quality, and safety for consumers; however, sensory properties regarding influence of these compounds are still poorly investigated. Recent works have been conducted in order to highlight the impact of phenolics on sensory properties of natural beverages. This chapter discusses the relationship between sensory quality and the phenolic compounds in natural beverages. The antioxidant properties, methods, and statistical analysis for sensory evaluation are also reviewed.

**Keywords:** natural beverages, phenolic compounds, sensory evaluation

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## 1. Introduction

As described in the literature, development of a new product such as natural beverages with functional properties involves technical and market factors. Technical factors include ingredients, formulation, conservation process, and the resistance of functional compounds to thermal treatments as well as their shelf life. As market factors, it is important to consider the needs of the consumer and the acceptance of the new products. This is where the sensory

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evaluation of natural drinks with functional properties provides tools in the decision-making for the successful launch to the market of a new product as described below.

In general, market problems faced by natural drinks are:

- High prices currently prevailing in functional products, which limit their frequent consumption and adoption.
- The intensive marketing of sugar-sweetened beverages in contrast with the tiny natural beverage market strategy.
- The problems of industrial production of natural drinks such as: lower shelf life and degradation of functional compounds by light, temperature, and pH changes.
- Safety and consumer confidence in the functional compounds contained in natural drinks.

On the other hand, the development of a natural drink with antioxidant properties must consider the following:

- Development of beverage mix fruits, fruit extracts, herbal teas, and natural origin sweeteners containing functional benefits and lower price to consider in its formulation.
- Sensorially, these drinks should be slightly sweet and of a translucent color, with aromas and broad flavors that captivate consumers.
- Replace the dyes and flavor enhancers used with natural essences of herbs and spices.
- Assess the use of natural sweeteners such as stevia, honey, and agave syrup.
- Preferential presentation to 350 ml glass bottles (with little or no chance of release agents foreign to the drink) to facilitate its transport.
- Also ensure the availability at home of these drinks with presentations of 2–3 l.
- Advertising or marketing of natural beverages should be focused on creating healthy eating habits.

Natural drinks with antioxidant properties are considered functional beverages. According to the International Food Information Council (IFIC), functional foods and beverages are those products, characterized by source of physiologically active components, with beneficial properties for human health. Functional beverages can be made from concentrated juice, from 100% natural juice, from nectars, or teas. They can also be called healthy drinks.

Finally, this section is not intended to fully illustrate the process in which a new drink is developed, rather it is intended to provide the reader with relevant and current information about the ingredients, formulation, packaging, shelf life, and above all, the behavior of functional compounds in the process of conservation or heat treatment as well as through the time of storage, which are important factors in the development of new drinks with functional properties.



## 2. Antioxidant activity, total content of phenolic compounds in natural drinks

Natural drinks with functional properties include within its production mainly berries, apples, lemons, grapes, in mixtures of milk serum, infusions, or herbal extracts to enhance its beneficial effects to the health, and sweetening matter with substitutes of sucrose and fructose to preserve the image of “good for your health.” These substitutes focused on the use of honey and aspartame among others.

Functional properties usually studied in the development of natural beverages include antioxidant activity, contained phenolic, total phenols and anthocyanins, and vitamin C among others. Studies mainly focus on the study of their behavior during shelf life, after the heat treatments of conservation.

Thermal treatment for the conservation of natural beverages includes pasteurization, where the functional properties can be reduced by the effect of the treatment and the storage time. Cooling tends to preserve these properties during storage time; however, sedimentation of the compounds in the interior of the container may appear.

### 2.1. Drinks

Rubio-Perez et al. [1] developed an antioxidant drink from apple extract and green tea extract and observed changes in phenolic compounds and antioxidant activity during 8 months at a temperature between 4 and 25°C in a Tetra Brik packaging. They found that the color dropped 10.73%, becoming red-yellowish; drinks also decreased their ascorbic acid by 41.24% in samples stored at 25°C. On the other hand, an apple drink mixed with milk serum was developed by Jaworska et al. [2] and packaged in 330 ml glass bottles. The drink was pasteurized at 80°C for 18 min and was stored at 4°C for 12 months. The changes found after 6 months of storage was the decrease of 64% of phenolic compounds. The content of vitamin C, lactose, sucrose, polyphenols and antioxidant activity decreased between 6 and 93% during the period of storage. It also presented significant changes in the sensory quality of drinks. Color, glucose, and fructose increased during storage. Dominant polyphenols were derived of the cinnamic acid, flavonols, and quercetin.

Aonla juice (*Emblia officinalis*) is used as a mixture with extracts of leaves of custard apple (*Annona squamosa*) in order to enhance its nutraceutical value for the content of quercetin. Drinks are bottled in 750 ml bottles and refrigerated at 4°C. The quercetin was sedimented during the storage of juice at refrigeration temperature. Sensorially, drinks were rated high when they contained 10 g of custard apple leaf extract, and this concludes that you can prepare a juice of aonla containing quercetin with high value nutraceutical [3].

Lemon juice is also used as an ingredient of functional beverages, enhancing their health benefits and improving their sensory properties when sweetened with honey. Sharma et al. [4] prepared a ready to drink beverage in 200 ml glass bottles, used table sugar and honey as a sweetener, the drink was pasteurized at 77°C for 30 min and stored for 6 months at room

temperature (13–27°C) and then cooled (4–7°C). They found no physical-chemical and sensory changes during storage when samples were refrigerated. At room temperature, beverages sweetened with honey can be stored for a period of 6 months without microbial growth. Sensorially, lemon beverages sweetened with honey were rated higher on their sensory attributes than those where table sugar was used.

Blue-black grape juice was used to prepare a fermented beverage in which we studied the time effect and temperature of storage on the color and antioxidant properties [23]. When the drink was stored at 4 and 20°C for 60 days, the pH decreased 10 and 11%, respectively; this change is important to record since it determines the stability of the color of the drink. At the beginning of the test, samples recorded the highest proportion of red and as expected, the brown increased eventually reaching its maximum value at 60 days based on the storage temperature. The phenols total content significantly decreased, 21.4 and 24.1% at the studied temperatures. The anthocyanin content during storage at 4 and 20°C resulted in losses of 60 and 78%, respectively.

## 2.2. Infusions

Infusions are prepared drinks from very hot or boiling water and substances of vegetable origin like leaves, flowers, fruits, seeds or some barks of plants, in order to dissolve the soluble fraction of its components. The solution made with the leaves of *Camellia sinensis* is called tea infusion. When other plant material is used and properly processed, it is called infusion. **Table 1** summarizes main antioxidants in natural beverages and sources.

The needs of the consumer who seek greater functional properties and sensory attributes are best met with infusions, drinks based on mixtures of leaves, flowers, and roots and sweetened with stevia or sugar substitutes. Beverages containing *Camellia sinensis* are the most used due to the stimulant effect caused primarily by its contained caffeine and their antioxidant properties, however, according to the geographical context, other materials seem suitable for infusions [5].

Tzu-Ying et al. [6] prepared the drink kombucha from black tea leaves of *Camellia sinensis* and juice of germinated wheat (wheatgrass) WGT increasing the content of phenolic compounds and antioxidant capacity. Results showed that the content of phenolics, flavonoids, and antioxidant capacity was greater than in the traditionally prepared kombucha. The kombucha mixed with wheatgrass was characterized by having high contents of gallic acid, catechin, caffeic acid, ferulic acid, rutin, chlorogenic acid. The highest antioxidant capacity was presented at a ratio 1:1 WGT and black tea. They concluded that it is advisable to mix kombucha and WGT for their high and stable antioxidant capacity as a new drink.

Filipendula tea processing (Meadowsweet flower teas) is carried out starting from *Filipendula ulmaria*. Olennikov et al. [7] investigated photochemical profile and nutrition of three possible substitutes (*F. camtschatica*, *F. denudata*, *F. stepposa*) for functional beverages. It was found that *F. stepposa* produces the highest content of phenolic compounds and that the four investigated species in its essential oil contain 28 compounds including simple phenols, monoterpenes,

Plant or fruit	Product	Antioxidant	Reference
Sauco ( <i>Sambucus nigra</i> L. subsp. peruviana)	Zumo funcional	Anthocyanins Total phenolics Antioxidant activity	[9]
Date fruit <i>Phoenix dactylifera</i> L.	Jam	Total phenolics Antioxidant activity	[10]
Orange <i>Citrus sinensis</i> L., Osbeck	Minimally processed orange	Total phenolics Antioxidant activity Vitamin C	[11]
Black mulberry <i>Morus nigra</i> L.	Fresh mulberry Dried mulberry Mulberry wine	Total phenolics Flavonoids Anthocyanins Antioxidant activity	[12]
Blueberry <i>Vaccinium corymbosum</i> L. Cranberry <i>Vaccinium macrocarpon</i> Aiton	Fresh fruit at three maturities (white, turning, and fully colored)	Phenolics compounds Anthocyanins Antioxidant capacity	[13]
Date fruit <i>Phoenix dactylifera</i> L.	Jam	Total phenolics	[10]
Yacon <i>Smallanthus sonchifolius</i>	Yacon flour	Phenolic compounds	[14]
Grape <i>Vitis vinifera</i> L.	Table grapes	Total anthocyanins Total polyphenols Total hydroxycinnamic acid	[15]
Kiwifruit <i>Actinidia deliciosa</i> cv.	Wine	Phenolic compounds Total phenolic Antioxidant activity	[16]
Custard apple <i>Annona squamosa</i>	Custard apple leaf mixed with aonla juice	Quercetin	[3]
Chequén <i>Luma chequen</i> Murta Ugni molinae Arrayan <i>Luma apiculata</i> Chilean blueberry <i>Vaccinium corymbosum</i> Meli <i>Amomyrtus meli</i> Calafate <i>Berberis microphylla</i>	Fresh fruits: Chequén, Murta, Arrayan, Chilean blueberry, Meli, Calafate	Anthocyanins Total phenolics Flavonoids	[17]
Grape <i>Vitis labrusca</i>	Purple grape juice	Phenolic compounds Monomeric anthocyanins Antioxidant activity	[18]

Plant or fruit	Product	Antioxidant	Reference
Roselle <i>Hibiscus sabdariffa</i>	Blended with mango, papaya and guava juices	Monomeric anthocyanins Vitamin C Total phenol Antioxidant activity	[19]
Extract Green tea	Antioxidant beverage	Phenolic compounds	[1]
Extract Apple		Antioxidant activity	
Blackberry <i>Rubus fruticosus</i>	Fruits extract	Total phenol	[20]
Mulberry <i>Morus</i> spp.		Anthocyanins	
Blueberry <i>Vaccinium myrtillus</i> L.		Phenolic compounds Antioxidant activity	
<i>Filipendula camtschatica</i>	Meadowsweet floral teas	Flavonoids, tannins, catechins, proanthocyanidins	[7]
<i>Filipendula denudata</i>		WS polysaccharides	
<i>Filipendula stepposa</i>			
<i>Filipendula ulmaria</i>			
Plum fruit <i>Prunus domestica</i> L.	Cloudy juices	Anthocyanins Total phenolics Antioxidant activity	[21]

**Table 1.** Bioactive compounds in different natural beverages.

sesquiterpenes, and aliphatic components. Anti-diabetic activity and antioxidant properties caused by the presence of highly active ellagitannins were also found. It can be expected from formulations based on *Filipendula* beneficial effects due to its unique nutritional and photochemical profile. Potential applications are suggested as functional health promoting products.

A medicinal drink formulated with arjuna (*Terminalia arjuna*), Ginger (*Zingiber officinale*), safflower (*Carthamus tinctorius*) and stevia (*Stevia rebaudiana*) as an alternative to caffeinated beverages has been made. Verma and Singh [8] optimized and characterized ore and bromatological herbal mixture, finding that ascorbic acid content fluctuated between 35.66 and 37.64 mg /100 g of formulation. A drink was obtained with sensory characteristics of bright orange brown color and a strong aroma and pleasant taste. The microbial quality allowed a shelf life of 3 months of the herbal mixture packaged in foil pouches.

The preparation of teas from different species of *Artemisia* was studied. Jae et al. [22] included in his study *Artemisia princeps* Pamp. and *Artemis argy*; this last species was studied as a food resource in the preparation of teas. The preparation was performed by adding 5 g of dried leaves per liter of boiling water and was evaluated sensorially and compared between the two-species content of amino-free acids, fatty acids, vitamin C, and total phenolic compounds.

They conclude that *A. argy* is the best species for the production of tea since it exceeded *Artemisa princeps* in all measured variables, in addition to having higher content of volatile compounds when used as tea. As a result, they considered *Artemis argy* has potential for as an ingredient for industrial use.

### 2.3. Juices

Processing of fruit for juice preparation involves the following operations: grinding, pressing, and enzymatic treatment. When attempting to develop functional juices, it is necessary to extract and preserve most of the functional compounds. Flores [9] evaluated the extraction of antioxidants from elderberries (*Sambucus nigra* l. *subsp. peruviana*) by different techniques in order to obtain juice with these beneficial properties and to develop a functional drink. The author pointed out in his study that the maceration at 70°C for 20 min originated better antioxidant properties with which a juice with good antioxidant and sensorial characteristics was elaborated. Prune juice extraction was achieved with the use of 140 ppm of enzymes at 48°C for 1 to 2 hours. In general, the content of anthocyanins in the juice was increased with the concentration of enzyme, time, and temperature. There was also a growing trend in antioxidant capacity with the concentration of enzyme [21].

The beneficial effects of grape juice as ergogenic and antioxidant were investigated by Tavares et al. [18]; athletes were given 10 ml/kg/day of purple grape juice in two doses provided before and after the training. They found a significant increase in antioxidant capacity (38.7%), vitamin A (11.8%) uric acid (28.2%) and a possible anti-inflammatory effect (20.2%). These results contrast with the control group where whole grape juice was not supplied. Whole-grape grape juice shows an effect ergogenic in brokers to promote the increase in the time to exhaustion, accompanied by an increase in antioxidant activity and a possible anti-inflammatory effect. These results suggest that grape juice is a good component within a functional drink formula and that the antioxidant effect can be enhanced when combined with other functional ingredients such as citrus, pear, cinnamon, ginger, and chocolate among others.

The mixture of Apple juice with grape juice, pear, and peach juice was made by Chiusano et al. [23], who revealed that there are no technological problems for these mixtures. The general acceptance of juices was highly significant and positively correlated with °Brix/acidity relationship, where samples with high percentage of pear juice were preferred.

The mixture of *Hibiscus sabdariffa* flower extract (roselle) with tropical fruit juices such as mango, papaya, and guava was made to provide beverages with high nutritional content and functional activity [19]. The preparation of roselle mixtures with fruit juices was performed in the following proportions: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. The beverages thus prepared were packed in sterile 100 ml plastic bottles and pasteurized in a water bath at 82.5°C for 20 min. The mixture turned from red to yellow as the proportion of fruit juices increased. These combinations of extract with fruit juice are rich in essential minerals and vitamin C. The

consumption of anthocyanins in the mixtures studied (493.5–118.2 mg/l) can protect humans from the diseases attributed to the reactions of free radicals.

Juices with pulp or nectar which usually retain the nutritional value of the fruit from which they were made, however, settle during storage. Five types of juices with pulp obtained from apples, pears, carrots and tomatoes, as well as a mixture with ratio 1:1:1 of apples, pears and carrots were studied [24] according to their efficiency for the extraction of juice.

The efficiency of conversion to juice was high for all mixtures studied, the maximum value of 580 ml/kg of apples was recorded, and the minimum value was 25 ml/kg of carrot, which has a high content of vegetable fiber. The acidity, the presence of nitrates and nitrites, and the sugar content in the processed juices were lower than those found in the supermarket. Ascorbic acid was added to all the juices to correct the taste and to prevent deterioration by light oxidation.

### 3. Quality of natural beverages and their sensory attributes

The development of a new drink requires ensuring its quality in microbiological, physico-chemical, and sensory aspects; the first two relate to the sanitary and nutritional quality of the drink. The sensory aspects are the reason for this section; the properties and attributes that should be considered to assess and ensure the sensory quality of new natural beverages are presented.

The sensory quality of drinks considered the following properties:

- Organoleptic properties: Visual, olfactory, tactile; auditory properties in beverages do not represent a decision point for the consumer therefore not be addressed in this section.
- Digestive are those that are experienced after ingesting the drink: heaviness, fullness, and/or pleasure.

The parameters that define the quality of a drink are positive attributes such as: color and overall appearance, taste properties: flavor, mouth persistence, aftertaste; olfactory properties: aroma, odor, orthonasal and retronasal; tactile properties: mouth feel, body, and absence of contaminants (odors and strange flavors). Among the negative attributes are: discoloration, foaming, sedimentation, gas, unpleasant smells production (notes ketone or vinegary), bitterness and astringency [25].

Before releasing a drink to the market, its acceptability and availability of purchase by the consumer is valued. Positive and negative attributes are evaluated through hedonic scales; that can be verbal and applied in evaluations carried out with adults; the facial hedonic scale is used preferably with infants, the scales used can be from 5 to 11 points, which vary from the maximum level of displeasure to the maximum level of pleasure. The scales used in the tests of acceptance of a drink are according to their discriminative power, reliability and predictive value correlated with eating habits. The hedonic scale most commonly used in these tests is the

Product evaluated	Sensory evaluation	Sensory panel	Scale	Palate cleansing	Reference
Wine	Aroma, tactile attributes	8	Ten points	Water	[16]
Custard apple leaf extract fortified sweetened aonla juice	Color and appearance, flavor, taste and mouth feel, overall acceptability	Undefined	Nine points	Undefined	[3]
Cloudy plum juices	Color, odor, taste, consistency and overall sensory impression	10–15	Nine points	Undefined	[21]
Arjuna-ginger medicinal mix blended	Appearance, color, taste, flavor, brightness and strength	20	Nine points	Undefined	[8]
Grape juice mixed with apple, pear, and peach juices	Color, odor, aroma, sweet taste, persistence in the mouth, overall pleasantness	50 consumers	Five points	Natural water	[23]
Apple and apple- whey beverages	(a) appearance (color, sediments and suspension); odor desirability, odor intensity and flavor (b) Descriptive flavor analysis	(a) 15 (b) 18	Five points	Undefined	[2]
Drink lemon juice and honey ready to serve	Color/appearance, flavor/aroma, body, taste and overall acceptability	Undefined	Nine points	Undefined	[4]
Plum nectar ( <i>Prunus domestica</i> )	Quantitative descriptive analysis (QDA): color, odor, taste, consistency and overall sensory impression	15	Intensity scale 0–10	Salt-free bread and water	[26]
Mugwort tea ( <i>Artemisia argyi</i> H. Lev & Vaniot)	Color acceptability, flavor acceptability, saltiness, bitterness, sourness, astringency, sweetness and overall preference	15	Labeled affective magnitude (LAM) scale 15 points	Undefined	[22]
Amarone red wine	Development of sensory descriptors for aroma, taste, flavor and mouthfeel	12	Nine points	Undefined	[27]
Black cherry, concord grape, and pomegranate juices blend	(a) Consumers: overall liking, appearance, Just about right (JAR) attributes (b) Descriptive analysis: development of sensory descriptors for flavor, mouthfeel and strange flavors	(a) 100 consumers (b) 10	JAR scale nine points	Unsalted crackers and water	[28]

**Table 2.** Attributes commonly evaluated in natural drinks and the sensory tests used.

nine points, also can be three, five, or seven points. Acceptance tests are normally conducted with consumers; the number of participants will be based on the level of confidence that is desired for decision making. **Table 2**, presents a list of attributes commonly evaluated in natural drinks and the sensory tests used. You can call the reader's attention to the use of the attributes taste and taste as well as aroma and smell, for this reason, these qualities are pointed out here:

- Taste refers to those feelings that occur inside our mouth, including the tongue and focuses on sweet, salty, sour and bitter taste; lately umami taste has been included for deliciousness.
- Aroma refers to a perfume or fragrance very nice, usually is a mixture of pleasant olfactory sensations.
- Odor is usually used for unpleasant olfactory sensations and refers to a more specific concept than aroma.
- The oral sensation refers to the viscosity for example, the body of a drink.
- The flavor is the combination of aromatic sensations, taste and viscosity. The flavor is a synthesis that makes the brain express a general feeling of those combinations.

The scientists can also evaluate the persistence in the mouth of a flavor and refers to the time it takes for the stimulus to disappear from the oral cavity. Generally, it is used in wines, although with the new mixtures of juices and teas it is considered one more attribute to evaluate.

The cleansing of the palate aims to eliminate a taste in the mouth to give rise to a new sensory experience. As shown in the following table, the most widely used product is unsalted cookies. This product is used to eliminate sweet, spicy, bitter or fatty tastes. Water is the ideal complement to clean the mouth. To evaluate sweet beverages as natural beverages, it is common to use this combination of products, so common that researchers do not report them in their publications.

#### 4. Tests for sensory evaluation of natural beverages and their statistical analysis

The types of tests that are used in the sensory evaluation of beverages are acceptance tests, discriminative tests, and qualitative tests. In the first two types, can be used the hedonic scale, LAM and JAR, to evaluate attributes. The qualitative tests are used for qualitative descriptive analysis (QDA) as well as to determine the taste profile using the intensity grade scale.

**Figure 1** proposes a sequence of sensory tests that are used in the development of beverages (yellow box). In the tests of acceptance, optimization, substitution of ingredients and in the market study, alternative tests are presented; Through the review of literature in this section, the reader will notice that the sensory tests have different strength to accept or discriminate a



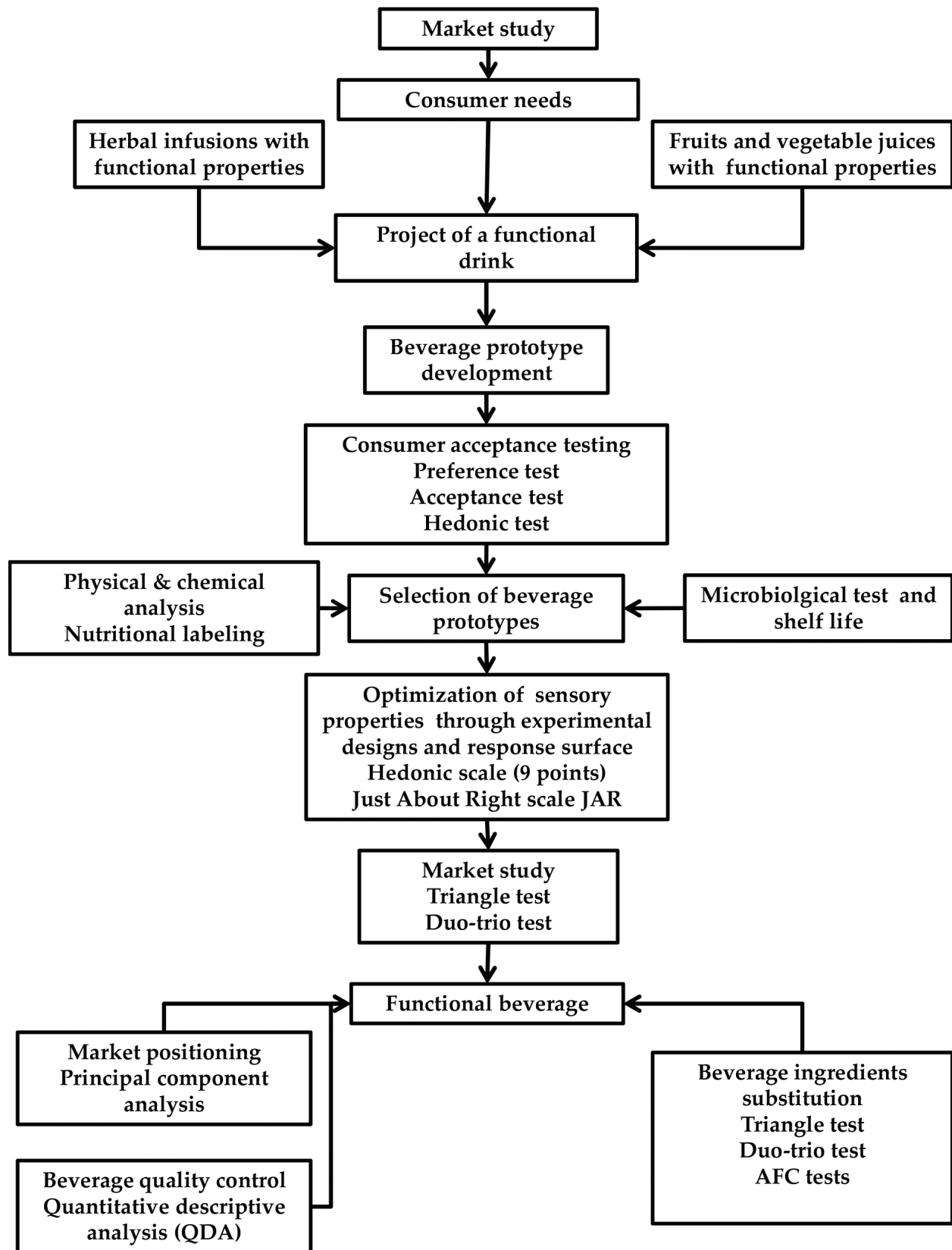


Figure 1. Sequence of sensory tests that are used in the development of natural beverages.

product; therefore, the choice of each of them will be based on the questions that you wish to answer during the process of developing the drink.

In the tests of acceptance with consumers, the hedonic scale is generally used with nine verbal categories, which represent different degrees of taste from “extremely disliked” to “extremely liked.” Then the verbal categories converted to numerical values are subjected to statistical analysis. Nicolas et al. [30], wondered if the words and numbers on the hedonic scale of nine points are interchangeable? The answer found after his research was that most consumers respond differentially to scales that use “only words” or “only numbers.” The percentage of consumers who give different results for “only words” or “only numbers” varied between 79 and 100%. They conclude that the numerical data derived from these two scales are not interchangeable, and if you want to compare results between them, you should be cautious.

Another scale used in consumer acceptance testing is the scale LAM (labeled affective magnitude) due to its higher discriminative power than the scale of nine points and the spacing of anchor words mainly foods or well-liked beverages [29].

A comparison between the hedonic scale of nine points and the LAM scale was made by Lawless et al. [29] in food acceptance tests and mentions that both scales behave well in the discrimination of products according to consumer tastes and reveal a strong relationship between consumption patterns and acceptance ratings. They suggest that there is no strong superiority of the LAM scale over the nine-point scale and that better scales can help to show differences between new products for the consumer preventing the type II error in which the true differences between the products were not detected.

Jae et al. [22] evaluated two species of *Artemis* for the elaboration of teas, through the acceptability of color and taste, salinity, bitterness, acidity, and general preference using the LAM scale. The scale ranged from 0 (greatest imaginable dislike) to 15 (greatest imaginable like). They did not find a significant difference for the evaluated attributes, except for taste acceptability and general preference and attribute the difference to the volatile compounds between the two species studied, particularly in the terpenic compounds.

When you want to optimize the level of an attribute in a product or when you want to identify attributes that need improvement, the JAR (Just About Right) scale is used. This implies that the ideal value of the attribute is equal to or very close to the value of the most liked attribute. Therefore, the products qualified as “just right” must be equivalent to the preferred or most liked products. However, the researches that used the JAR scale repeatedly report optimal values of the attributes very different from those of the products currently on the market.

Epler et al. [31] compared the hedonic scale and two types of JAR scale (boxes or lines) to optimize the degree of sweetness in lemonade. The predicted “optimal” level of sweetness for lemonade was determined, as well as differences in taste for formulations with different sugar content (6–14%). The two types of scales yielded similar results in terms of the predicted optimum value (9.2 and 9.4% sucrose), which was significantly lower than the result obtained by the hedonic scale (10.3% sucrose). In the preference test, consumers prefer lemonade with 10.3% sugar over that which contained 9.3%. These results indicate that the hedonic scale is better for predicting sweetness than the JAR scale.

JAR scale can be used with 3 and 9 points to determine the optimal concentration of sweetness in a drink. Some authors compared these scales by analyzing the data obtained through statistical analysis of survival, followed by a regression analysis. Three different ranges of concentration of sucrose were used in orange juice. Optimum sucrose concentration was 8.2% for the nine-point scale and 13.1% for the three-point scale. A later study showed that 70% of subjects preferred the sample with 13.1% of sucrose on the sample with 8.2%. The three points of JAR scale combined with statistical analysis provided a more real optimal level concentration than the nine-point scale.

Optimization of drinks is made through the methodology of Box and Wilson called response surface, which is a combination of the experimental design and regression analysis. It is an experimental strategy that allows you to find the optimal values of the independent variables (e.g., sugar level) that maximizes or minimizes the response variable (e.g., flavor). They used this methodology to optimize the ideal sweetness of a fermented beverage of extract of soybean to 11 g of sucrose per 100 mL and obtained predictive models of response with respect to the ideal flavor, sweetness, and generally accepted attitude of purchase.

The response surface methodology was applied to differentiate wines from different harvests by their aroma and taste by Pagliarini et al. [27]. There are few publications on this; however, these two publications illustrate the applications of the methodology in the development of beverages. The difference tests referred to in this section are presented in a comparative manner in the **Table 3**.

On the other hand, when you want to substitute ingredients or compare the attributes of the new beverage against a product already on the market, the difference tests are used. There are two types: when the cause of the difference is asked, focusing on a specific attribute (2AFC and 3AFC) and when it is not asked, focusing on others (duo-trio and triangular). These tests

Test	Samples	Presentation sequence	Judge instruction
Triangular	Encoded with three-digit numbers. Two samples are the same and are presented with different code. The third sample is different.	AAB ABA BAA BBA BAB ABB	Which sample is different from the other two?
Duo-trio	A sample is marked as R = reference and the other samples are marked as A or Bs	(a) constant reference: R AB, R BA (b) balanced reference: RA AB, RA BA, RB AB, RB BA	(a) select the sample similar to A (b) select the sample similar to A or B
2AFC	Encoded as A or B	AB and BA	What is the sweetest sample? (for example)
3AFC	Two samples are the same and are marked with three-digit codes. The third sample is the one that has the greatest strength in the attribute and is coded in the same way	ABB, BAB, BBA, BAA, ABA and AAB	What is the saltiest sample? What is the least salty sample?

**Table 3.** Comparison between different sensorial tests.

can work with trained judges and consumers, where the latter apply simpler tests and require more participants in order to increase the degree of reliability in the results.

These tests are applied with trained judges and with consumers; the statistical method used is Chi-square. Judge fatigue can occur when repeatedly testing the samples and consequently, the adaptation to the stimulus can occur; thus six maximum samples can be evaluated in a session. The interpretation is through the use of statistical tables according to the size of the sample, minimum number of correct answers and the level of significance required. Once the new drink is ready to go on the market, it is necessary to define their sensory properties to establish a flavor profile and define the properties that have to be monitored in the quality control process. Quantitative descriptive analysis (QDA), used for those purposes in this analysis, describes the sensory attributes (no more than seven) of products such as flavor, mouthfeel, aftertaste, and appearances through 10–12 trained judges. The objective of the QDA is to provide a quantitative specification of the sensory attributes of a product as well as to determine the nature and intensity of these.

Beverages are evaluated by the intensity of their attributes crossing the level of intensity found on a vertical line. These distances are converted into numerical values that will be analyzed by means of an ANOVA.

Hruškar et al. [26] evaluated nectars through quantitative descriptive analysis and generated ten descriptive terms related to color, smell, taste, consistency, and overall sensory impression. The analysis of variance showed significant differences in the color intensity, taste sour and sweet intensity, as well as for the overall sensory impression. There were no significant differences in the addition of sugar and acid.

The analysis of main components (PCA) is used to study the positioning of the beverage on the market. It uses the sensory attributes of beverage such as flavor, color, aroma, and body. This descriptive technique allows the study of the sensory attributes quantitatively through the correlation between them and calculates new variables by grouping attributes in such a way that it is possible to observe in a plane the distance between groups of attributes and define which product is better positioned to the consumer and in consequence on the market.

## 5. Conclusions

- The important factors facing the development of natural beverages with functional compounds are market and technological problems. The current cost of the functional drinks is very high and discourages consumption. A technological challenge is the conservation of the functional properties during conservation treatments and shelf life.
- The mixture of fruit juices and infusions is relevant in terms of the functional properties that contribute to the consumer, as well as provide new sensory experiences.
- Sensory methodologies and current knowledge about the functional properties of fruit and infusions do not constitute a limit for the development of natural drinks with functional properties.

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## Conflict of interest

The authors have declared that there is no conflict of interest.

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# Antioxidants from Natural Sources

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## Abstract

Antioxidants are the defense system of the body against the damage of reactive oxygen species, which is normally produced during the various physiological processes in the body. There are various sources of these antioxidants like endogenous antioxidant present in the body and exogenous food source. In recent decades, alternate of synthetic food antioxidants by natural ones has fostered interest on vegetable sources and the screening of inexpensive raw materials particularly from the agriculture for identifying new antioxidants. Polyphenols are the significant plant compounds with antioxidant activity, though not the only ones. Some but not only restricted to biological properties such as anticarcinogenicity, antimutagenicity, antiallergenicity, and antiaging activity have been reported for natural and synthetic antioxidants. Among the sources of natural antioxidants, the most important are those coming from routinely consuming vegetables and fruits; however, antioxidant from other plant and agriculture waste should not be ignored.

**Keywords:** antioxidants, vegetables, fruits, plants, herbs

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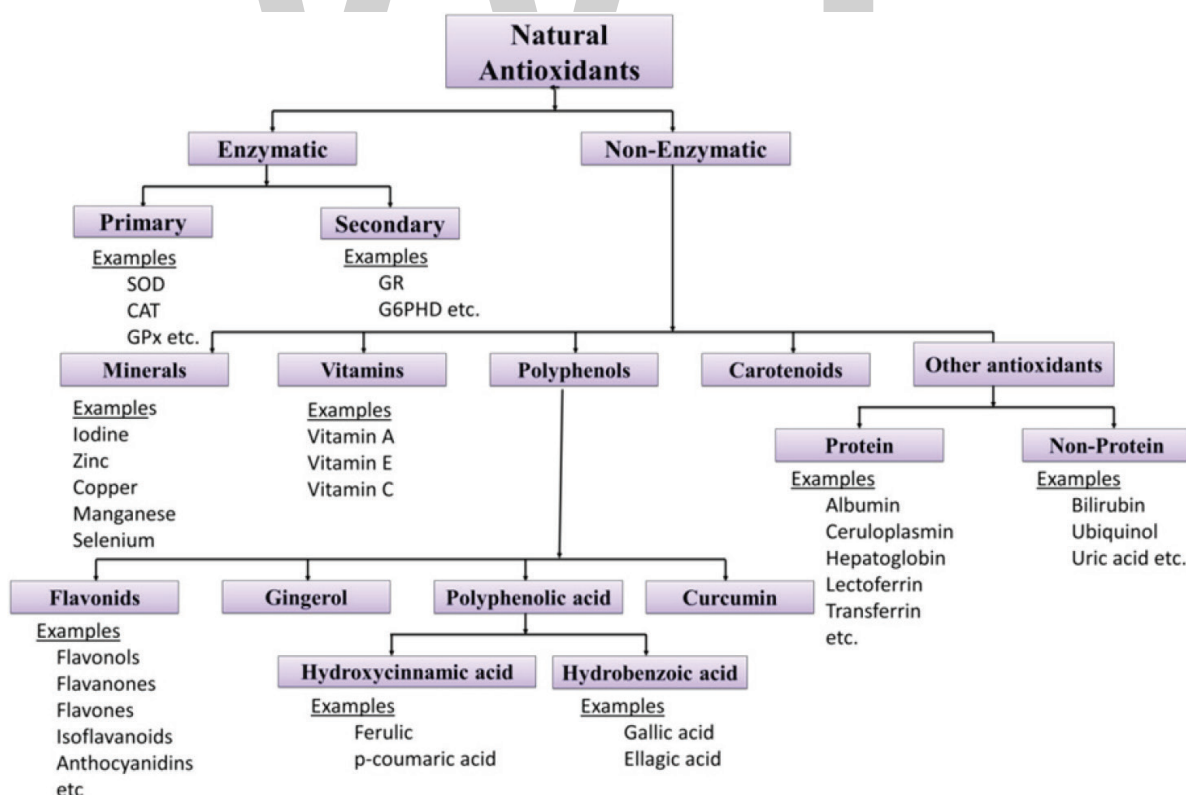
## 1. Introduction

The formations of oxygen reactive forms as a result of rigorous oxidative processes taking place in human organism are the potent precursors of systemic cells and tissues damage. Antioxidants being an inhibitor of the oxidation process remove these free-radical intermediates by oxidized themselves [1], even at quite diminutive concentration, and thus have assorted physiological function in the body to stop these oxidation reactions ultimately protecting the body from harmful chain reactions [2]. Thus, they have reviewed by many researchers as nature's answer to physiological and environmental stress, atherosclerosis, aging, and cancer [3]. Body's endogenous defense system against these free radicals plays



an imperative role, which can further supported by the supplementation of antioxidants in the diet. Generally, antioxidants can be divided into two major categories such as synthetic and natural. The main targeted site of these free radicals damage and defensive approach of antioxidants in the body is at the cellular level. Based up on this, these antioxidants can also be classified as enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants primarily include glutathione peroxidase, catalase, and superoxide dismutase. There are also several other enzymes in the body that contribute to the total antioxidant capacity, which reflects in the serum [4]. Nonenzymatic antioxidants contain several subdivisions mainly vitamins such as A, E, C, and to lesser extent vitamin D, enzyme cofactors (Q10), peptides and some minerals (zinc and selenium). The major ingredients from the natural sources are polyphenolic compounds, which are reported to have significant antioxidant potential [5]. A detailed classification and subclassification has been displayed in **Figure 1**.

Natural antioxidants are primarily phenolics that may occur in all parts of plants [6], such as fruits, vegetables, nuts, seeds, leaves, roots, and barks. In the recent past, some toxicological studies regarding the use of synthetic antioxidants have shown their unwanted or adverse effects. These reports have urged the researchers to focus their study on exploring the natural sources with reasonable antioxidant potential [7]. Moreover, the availability and economy are significant concerns too in the context of using these natural antioxidants. The antioxidants from the nature can be categorized into the various subclassifications. However, two major categories are like antioxidants from commonly consumed or routine natural diets (e.g., vegetables, fruits, cereals, and beans) and secondly from plant or herb source that have fair antioxidant



**Figure 1.** Classification and subclassification of antioxidants found in natural sources.

potential but are not the routine dietary source (e.g., medicinal plants and wild herbs). Among these, the routine dietary sources are very important as these can be easily available and more suitable for the dietary interventions. The need is to identify and generate awareness about these sources, which can be rated from top to down regarding antioxidant potential. The people who are habitual of consuming these vegetables and fruits in their routine meal are reported to be less affected by various chronic diseases [8], and studies have also endorsed the long-term healthy impact of consuming these nature-origin diets. Most common dietary supplements are comprised of vitamins C and E from synthetic as well as natural sources. Vitamin C is rich in the citrus fruits, which is a renowned fact, so fruits such as orange, lemon, blueberries, strawberries, grapes, prunes, and plums; red beans; spinach; kale; broccoli flowers; and alfalfa sprouts have good amount of antioxidants [9]. Fortunately, these are the part of our routine diet; however, their availability pertaining to the geographical distribution as well as cultivation is an important factor. Though international trading made them available throughout the world and even in the off season as well, the consumption of seasonal and fresh fruits is always encouraged. Vitamin E is a fat-soluble vitamin and exerts its antioxidant effect by reducing fat oxidation in the body [10]. Synthetic form of this vitamin is comprised of  $\alpha$ -tocopherol, which is widely used as food supplement. The natural form of this vitamin contains mixed tocopherols almost having eight isomeric forms of  $\alpha$ -tocopherol. The availability of these mixed tocopherols enhances the percent absorption of vitamin E from the natural sources in the human body.

An enormous growth has been observed in the vitamin supplement market during the past decade. It is estimated worth \$68 billion with the US renders to be major shareholder, i.e., around 30%. A \$421 million for just vitamin E and \$361 million for vitamin C business has been recorded from Europe. Moreover, according to a report from Business insight, an annual growth of 4.5% in US vitamin and mineral market, i.e., almost \$30 billion in the first half of this decade, was forecasted [11].

The targeted approach of the researchers around the world to date is to find natural sources of antioxidants, which will be inexpensive and closer to the nature. These findings will be the superior substitute of synthetic supplements in food, pharmaceutical, and cosmetic industries [12]. Though significant harmful effects of synthetic supplements have not been reported to date, a common concept of closer to nature is obviously a better approach in the supplementation. The coming decade will be the era of natural products, and exploration of natural antioxidants will be the prime focus of the researchers [11].

The main objective of the current chapter is to overview and summarize the natural sources with antioxidant potential. The summarized data will be a useful information for the professional and nonprofessional readers to gather a fair information regarding dietary and non-dietary sources in the nature, which can be the part of the routine diet to enhance the antioxidant capability of the body.

## 2. Natural antioxidants

The nature is always a significant and rich source of countless ingredients that can be served as health-promoting agents. Many of these natural sources include routinely used fruits, vegetables,

herbs, spices, and edible mushrooms that can be the part of routine diet. In addition to that, there is a huge list of medicinal plants reported to have extensive health-boosting potentials. One of the most beneficial effects from these natural sources is due to their potential antioxidant properties. Regarding the antioxidant capability, the researchers have focused their studies to explore the most potential sources along with their active ingredients. The researchers have added some marine sources such as algae and seagrass as well in the list of these natural sources. The recent studies have also explored the role of naturally occurring microbiome in the gut in the body antioxidant pool denoted as good bugs. These good bugs can also be used as supplement called as probiotics. Thus, generated literature is a big data bank for the researchers of the field and beneficial information for the general reader as well. There is a need to summarize this enormous data bank based upon the identification of most potential sources, which will make the literature feasible for the professional and nonprofessional readers at the same time. This chapter will elaborate the various natural sources of antioxidants that hopefully help to prioritize the beneficial amendments in dietary composition.

Fruits and vegetables are highly recommended dietary contents, widely known for their health-promoting effects and nutritious values. They got an essential place as conventional foods in the history because of their excessive amount of minerals, specifically electrolytes; vitamins, specifically vitamins C and E; while various current studies are revealing their phytochemical contents, having antioxidant capabilities [13]. These antioxidants scavenge the oxidants or free radicals produced as a result of several degenerative and disease processes such as diabetes, cancers, and cardiovascular disorders. So, regular consumption of fruits and vegetables may reduce the risk of mortality associated with these diseases [14]. Most of the natural antioxidants convert the lipid radicals into more stable products by breaking the chain. Antioxidants obtained from vegetables and fruits are mostly of phenolic structure, which may include vitamins, minerals, and polyphenols [15]. Antioxidant minerals, such as iron, zinc, selenium, copper, and manganese, act as cofactor of many antioxidant enzymes, absence of which may certainly disturb the activity of their enzymatic scavenging activity [16]. The antioxidant capability of different fruits is being presented in **Table 1**.

### **2.1. Types of antioxidants from fruits and vegetables**

Polyphenols, present in fruits and vegetables, is a group of several low- and high-molecular-weight compounds having antioxidant properties that prevent lipid oxidation [17]. Most of them are conjugates of mono and polysaccharides connected with one or more groups of phenol rings or may also present as functional derivatives such as esters and methyl esters [18]. This major class of natural antioxidants can be obtained from teas, particularly green and red teas, as well as fruits such as grapes [19]. However, polyphenols from teas have more significant than in fruits because of their bioavailability in blood. Approximately 15–20% polyphenols are absorbed in human blood from their consumed amount. This absorption is enhanced when there are no sugar molecules attached with them. So, teas have more absorption of polyphenols than in fruits because of high sugar content in fruits [20].

Flavonoids, another important antioxidant content, is a subclass of polyphenols present abundantly in most of the foods, such as potatoes, wheat, tomatoes, red berries, peaches,

No	English name	Antioxidant contents	Concentration (ORAC value)	References
1.	Beet root	Betalains		[146]
2.	Guava	$\beta$ -Carotene, lycopene, vitamin C, ellagic acid, anthocyanin		[147]
3.	Pears	Ascorbic acid, flavonoids (quercetin, isorhamnetin, myricetin, kaempferol, and luteolin), betalains, taurine, total carotenoids and total phenolics	135 (mmol TE/g)	[148]
4.	Pomegranate	Vitamin C and polyphenols	1245 (mmol TE/g)	[149]
5.	Papaya	Quercetin and $\beta$ -sitosterol	300 (mmol TE/g)	[150]
6.	Water melon	Lycopene, $\beta$ -carotene, vitamin C	100	[151]
7.	Apple	Proanthocyanidins, flavonoids (kaempferol, quercetin, and naringenin derivatives); phenolic acids (protocatechuic, caffeoylquinic, and hydroxycinnamic acid derivatives); hydroxychalcones (phloretin and 3-hydroxyphloretin derivatives); and isoprenoid glycosides (vomifoliol derivatives)	16.78 a $\pm$ 0.25 (mmol TE/g)	[152]
		Flavanols, flavonols, dihydrochalcones, and hydroxycinnamates	1508 $\pm$ 44 ( $\mu$ mol/100 g)	[153]
8.	Plum	Proanthocyanidins, flavonoids (kaempferol, quercetin, and naringenin derivatives); phenolic acids (protocatechuic, caffeoylquinic, and hydroxycinnamic acid derivatives); hydroxychalcones (phloretin and 3-hydroxyphloretin derivatives); and isoprenoid glycosides (vomifoliol derivatives)	14.55 c $\pm$ 0.21 (mmol TE/g)	[152]
			94.8 (mmol TE/g)	[154]
9.	Guava	$\beta$ -Carotene, lycopene, vitamin C, ellagic acid, anthocyanin		
10.	Beet root	Betalains, vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	4100 (dry extract)	[146]
			115 ( $\mu$ mol TE/g)	[155]
11.	Pea	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	19 ( $\mu$ mol TE/g)	[155]
12.	Carrot	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	60 ( $\mu$ mol TE/g)	[155]
13.	White cabbage	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	61 ( $\mu$ mol TE/g)	[155]
14.	Tomato	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	67 ( $\mu$ mol TE/g)	[155]
15.	White onion	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	85 ( $\mu$ mol TE/g)	[155]
16.	Cauliflower	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	102 ( $\mu$ mol TE/g)	[155]
17.	Spinach	Vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds	152 ( $\mu$ mol TE/g)	[155]

**Table 1.** Some important fruits having antioxidant constituents.

and almonds [21]. Anthocyanin is a subcategory of flavonoids (**Figure 1**) which is present in berries and red wine. It is a potent antioxidant with decreased bioavailability as compared to other flavonoids [19]. Polyphenols show their antioxidant properties by preventing the oxidation of low-density lipoproteins (LDL), thus preventing plaque formation [22]. Some types of polyphenols have also been found to inhibit the oxidation of some important enzymes and thus preserve their proper functioning [23]. Carotenoids are another major class of phytochemicals antioxidants from fruits and vegetables after polyphenols. They mostly found in vegetables, such as potatoes, carrots, papayas, and apricots [21].

Among the vitamins obtained from fruits and vegetables, acting as antioxidants, vitamin C, also known as ascorbic acid, is a very potent water-soluble antioxidant commonly found in citrus fruits and vegetables such as oranges, lemons [24], and tomatoes [21]. It is recommended that the fruits and vegetables containing vitamin C should be taken in small divided doses instead of having a large dose simultaneously because vitamin C shows less absorption when given in large quantities [25].

Another vitamin with antioxidant properties is vitamin E, which is related to tocopherol family of antioxidant. It is a fat-soluble, nonpolar vitamin found naturally in lipid-rich fruits and vegetables, such as olives, sunflower, and nuts [21]. Vitamin E shows higher bioavailability than vitamin C, which is perhaps due to its fat solubility and can be further enhanced when taken with fatty foods [25].

## 2.2. Antioxidants from fruits and vegetables wastes

Fruits and vegetable waste material is produced during their cultivation, industrial management, processing, preservation, and distribution [26]. In the past few decades, researchers have been struggling to devise the methods to recycle these wastes to get therapeutic benefits [27]. The vegetables and fruit waste material includes peelings, trimming, shells, seeds, stems, and pulp residues that remained after extraction of juices and starch or sugar preparation. This waste constitutes about 25–30% [28]. According to a research, greater amounts of phenols and ascorbic acids have been reported in these waste scalps than their pulp [29] and in unripe form than their ripened form [30]. Most of the fruit peels contain 2–27 times greater amount of antioxidants than their pulp [31]. According to Someya et al., banana pulp possesses 232 mg/100 g of phenolic components, and this amount is just 25% of the amount present in banana peels [32]. *Cucumis sativus* (Cucumber) peel has been reported as a good source of flavonoids, which is considered as a potential antioxidant [33]. Many bioactive substances may be recovered from these wastes to prepare foods in food processing and therapeutic preparations [34]. A significant amount of bioactive phytochemicals, having strong antioxidant properties, can be obtained from the tomato wastes, which include carotenes, tocopherols, terpenes, sterols, and polyphenols. These natural antioxidants obtained from food waste may be used to formulate functional foods or can be used as food additives [35].

A lot of antioxidants, such as carotenoids, phenolic compounds, vitamin C, and dietary fibers, are found in mango peel. These compounds have reported activity against many degenerative diseases, such as Alzheimer's disease, cataracts, cancer, and Parkinson's disease [36]. The

wastes of wine-making industry include degradable solids such as skin, stem, and seeds, which contain many antioxidants that have shown to prevent many degenerative processes and possess health-promoting effects [37]. Pujol et al. [38] reported that the coffee wastes from the coffee industry contains approximately 6% polyphenols and about 4% tannins. Antioxidant ingredients from various sources of fruit waste have been presented in **Table 2**.

No	Fruit	Residue	Antioxidant	Reference
1.	Banana	Unripe (green) fruit and peel	Phenols and flavonoids	[156, 157]
2.	Mango	Peel, kernel	Gallic acid, ellagic acid, gallates, gallotannins, condensed tannins	[158, 159]
3.	Water melon	Peel, rinds	Citrulline, lycopene, flavonoids, and phenols	[160, 161]
4.	Cucumber	Peel	Flavonoids and phenols	[33]
5.	Potato	Peel	Chlorogenic acid, caffeic acid, ferulic acid, and phenols	[162, 163]
6.	Coffee	Coffee ground and residue	Polyphenols, tannins, and gallic acids	[164, 38]
7.	Apple	Peel	Epicatechin, catechins, anthocyanins, quercetin glycosides, chlorogenic acid, hydroxycinnamates, phloretin glycosides, and procyanidins	[165]
8.	Grapes	Skin and seeds	Coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, cinnamic acid, neochlorogenic acid, p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid, proanthocyanidins, quercetin 3-o-gluuronide, quercetin, and resveratrol	[166, 167]
9.	Guava	Skin and seeds	Catechin, cyanidin 3-glucoside, galangin, gallic acid, homogentisic acid, and kaempferol	[168]
10.	Pomegranate	Peel and pericarp	Gallic acid, cyanidin-3,5-diglucoside, cyanidin-3-diglucoside, and delphinidin-3,5-diglucoside	[169, 170]
<b>Vegetables</b>				
11.	Carrot	Peel	Phenols, $\beta$ -carotene	[171]
12.	Cucumber	Peel	Phenols, flavonoids, pheophytin, phellandrene, caryophyllene	[33, 160]
13.	Potato	Peel	Gallic acid, caffeic acid, vanillic acid, chlorogenic acid, ferulic acid, and phenols	[160, 162, 163]
14.	Tomato	Skin and pomace	Carotenoids	[172]

**Table 2.** Antioxidants from some fruits and vegetable wastes.

### 2.3. Mushrooms as antioxidant

In the nutrition world, mushrooms are delegated vegetables; however, they are not actually plants. They have a place with the kingdom of fungi. In spite of the fact that they are not vegetables, mushrooms give imperative supplements. Mushrooms are considered as healthfully utilitarian sustenance and also source of valuable medicines [39, 40]. Numerous consumable mushrooms (for the most part Basidiomycetes) are great wellsprings of important nutritive components including carbohydrates, for example,  $\beta$ -glucans; lipids; B-vitamins, such as niacin, flavin, and pyridoxine; phenolics, like tocopherols; organic acids, for example, malate ascorbate, fumarate, and shikimate; monoterpene and diterpene; proteins, for example, hydrophobins; and trace components, for example, selenium [41–43]. These components are established as to be responsible for immunomodulatory, antimicrobial, antitumor, antihypertensive, hepatoprotective, and antioxidant activities of mushrooms [44, 45].

The amount of mushrooms on Earth is assessed at 140,000 yet might be just 10% (approximately 15,000 species) are known [43, 46]. Out of approximately 15,000 known species, 2,000 are found safe for human utilization, and around 650 of these contain therapeutic properties [47]. There are a number of mushrooms including *Agaricus bisporus* [48] *Lentinus edodes* [49], *Armillaria mellea* [50], *Auricularia auricula* [51], *Boletus edulis* [52], *Ganoderma applanatum* [53], *Grifola frondosa* [54], *Hypsizigus marmoreus* [55], *Pleurotus* sp. [56], *Schizophyllum commune* [57], *Termitomyces* sp. [58], and *Tricholoma* sp. [59] that possess antioxidant properties. The antioxidant properties of mushrooms are mainly due to their phenolic compound [52, 48]. Phenolic acid is the chief phenolic component present in the mushrooms. There are an assortment of phenolic compounds recognized in wild mushrooms, including cinnamic acid, protocatechuic, *p*-hydroxybenzoic, *p*-coumaric acids, gallic acid, vanillin, rutin, and quercetin [60]. Polysaccharides are one of the major components in the mushroom. In recent studies, it has been revealed that they contain the antioxidant property [61]. Scavenging properties of polysaccharides are impacted by chemical properties such as atomic weight, level of branching, monosaccharide types, and proportion of monosaccharides, intermolecular relationship of polysaccharides, and alteration of polysaccharides. Among the monosaccharides, rhamnose is the most critical determinant factor related to scavenging properties of mushroom [62].

#### 2.3.1. *Agaricus bisporus*

*A. bisporus*, also known as white button mushroom native to grasslands in Europe and North America, cultivated in more than 70 countries [63], possesses polysaccharides and antioxidants including vitamin C, D, B<sub>12</sub>; folates; and polyphenols [48]. The phenolic composition of methanolic extract from *A. bisporus* is investigated by HPLC. Polysaccharides and phenolic compounds such as gallic acid, rutin, caffeic acid, and catechin found in the mushroom are responsible for its scavenging activity [64]. Mushrooms being routinely used as dietary ingredient made them a significant natural antioxidant source.

#### 2.3.2. *Armillaria mellea*

*A. mellea* is a culinary-medicinal honey mushroom, used as an admired element in the traditional Chinese medicine. The mushroom is pathogenic and is found worldwide in temperate,

boreal, and tropical forests [65]. It grows on living trees and on dead and rotting sustenance material [66]. Polysaccharides obtained from wild sporophores and refined products of *A. mellea* have scavenging properties [67]. Two polysaccharides, AkPS1V-1 and AkPS1V-2, from the alkaline extract of the mushroom have been isolated and reported for their antioxidant activity [68]. Moreover, their ascorbic acid and phenolic components have been reported for their scavenging impact on superoxide anions [69]. Overall, it is reported to have a fair antioxidant capacity to be used as food oxidation-reducing substance.

### 2.3.3. *Auricularia auricula*

*A. auricula* is an edible mushroom, found worldwide, belongs to the family Auriculaceae, commonly known as tree-tea or wood-ear. The mushroom contains high quantity of carbohydrates including polysaccharides, proteins, minerals, and phenolic substances [69, 70]. Polysaccharides of the mushroom have antioxidant activity [71] by the inhibition of lipid peroxidation and have effective hydroxyl radical scavenging activity [72]. In the case of lipid peroxidation,  $IC_{50}$  values of ethanolic, crude, and boiled extracts of *A. auricula* are 398, 310, and 572  $\mu\text{g/ml}$ , respectively, and in the case of hydroxyl radical scavenging activity, 373, 403, and 510  $\mu\text{g/ml}$ , respectively [72]. Khaskheli et al. isolated two polysaccharides from fruiting body of *A. auricula* and evaluated potential antioxidant activity of these polysaccharides [71]. Among its various extracts, the boiled extract, which is also convenient, proves to be more effective antioxidant.

### 2.3.4. *Boletus edulis*

*B. edulis* belongs to the Boletus species of mushroom widely distributed in the holarctic across Asia, Europe, and North America, commonly known as porcino and penny bun [73]. Other edible mushrooms of boletus species including *B. aereus*, *B. reticulate*, and *B. edulis* have good antioxidant properties. Total phenol contents of *B. edulis* is higher that shows the scavenging activity [74]. *B. auranticus* ( $EC_{50}$  0.016 mg/ml) exhibits higher total phenol contents as well as hydroxyl radical-scavenging activity than *B. edulis*. However, reducing power of *B. edulis* is higher. *B. edulis* extricate averts lipid peroxidation [75]. Antioxidant activity of *B. edulis* is attributed to its polysaccharides found in the fruiting bodies that are reported to have chelating action and inhibitory impacts on superoxide radical and hydroxyl radical [67]. Vieira et al. [76] investigate the *B. edulis* antioxidant activity with the combination of another edible mushroom *Marasmius oreades*. They investigated different parameter to observe the antioxidant capacity of the both mushroom species mixture, and they observed the good antioxidant activity in synergism. These results show that on various occasions, the addition of more than one mushroom as a combination gives more effective results than alone.

### 2.3.5. *Ganoderma lucidum*

*Ganoderma lucidum* is also commonly known as Lingzhi, a basidiomycete fungus, native to China and grows in mountain woods with humid and dim-light conditions, in the rotten bark or root of tree. The mushroom is well known as medicinal mushroom and has been prescribed to prevent and treat different diseases [77]. *G. lucidum* contains polysaccharides, sterols, triterpenoids,



nucleosides, and alkaloids [78]. *G. lucidum* is called as marvelous mushroom of immortality because it shows that the consumption of the mushroom can prolong life [77]. Shi et al. [79] separated four polysaccharides from *G. lucidum* and investigate their antioxidant property. They demonstrated that these four polysaccharides have scavenging activities in a concentration-dependent manner [79]. *G. applanatum* is known as shelf fungus and also belongs to *Ganoderma* species of the mushroom. *G. applanatum* exhibits the higher antioxidant property over *G. lucidum* and other two edible mushrooms including *L. edodes* and *Trametes versicolor* [80].

### 2.3.6. *Grifola frondosa*

*G. frondosa*, also known as Maitake, is a culinary as well as medicinal mushroom native to China, North America, and northeastern part of Japan but cultivated worldwide in several countries because of its useful effects [81]. The mushroom is progressively being perceived as a powerful wellspring of polysaccharide with sensational well-being and advanced potential. Total phenols, ascorbic acid,  $\alpha$ -tocopherol, and flavonoids are the major scavenging agents present in the different *G. frondosa* extricates [82]. *G. frondosa* polysaccharides have critical inhibitory impacts on hydroxyl radical, superoxide radical, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [83]. Their ferrous particles chelating activity is also strong [84]. Fan et al. [85] extracted five polysaccharides from the natural product group of *G. frondosa* by various separating techniques. Hot alkali extract of *G. frondosa* has the better antioxidant activity as compared to partially purified polysaccharides. It has the lower  $EC_{50}$  values of DPPH scavenging ability [86]. The study results enforce the variation of their antioxidant ingredients due to the seasonal and geographic displacements.

### 2.3.7. *Hypsizigus marmoreus*

*H. marmoreus* is an edible mushroom commonly found in East Asia including Korea, China, and Japan, widely known due to its antioxidant activity [87]. Phenols [88] and polysaccharides are the major bioactive components present in *H. marmoreus* that show the antioxidant activity by scavenging reactive oxygen species and strengthening its reducing power [55]. Liu et al. investigated the intracellular polysaccharides of *H. marmoreus* and revealed that these polysaccharides can be utilized as an antioxidant agent that improves adaptive immune reactions [89].

### 2.3.8. *Lentinus edodes*

*L. edodes* also known as shitake mushroom is the second most well-known consumable mushroom in the worldwide market and is usually cultivated in Indonesia, Taiwan, China, and Japan [90]. The mushroom is credited to its wholesome incentive as well as to conceivable potential for therapeutic applications [91]. *L. edodes* contains some important polysaccharides that have therapeutic activity. The mushroom is also a good source of vitamins, particularly vitamin B, including B1, B2, B5, B12, and provitamin D2. *L. edodes* extract has potent antioxidant effect due to the presence of bioactive compounds ergothioneine [92]. *L. edodes* has the ability to increase the total antioxidant capacity and reduce the total oxidative stress [91]. UV-C radiation can improve scavenging capacity of *L. edodes* [93]. Chen et al. [83] separated three types of polysaccharide from fruiting bodies of *L. edodes*, and they demonstrated the polysaccharides as potent antioxidant agents that can produce healthy immune response. The treatment of mushroom crude powder or its extract may also helpful to enhance its antioxidant capability.

### 2.3.9. *Pleurotus ostreatus*

*Pleurotus ostreatus* is the third most cultivated mushroom worldwide after *A. bisporus* [94]. Its mycelia as well as fruiting bodies have well-known therapeutic effects due to its various biologically active compounds including phenols, flavonoids, and carotenoids [95] having strong antioxidant activity [96]. The methanol extract demonstrated the most grounded  $\beta$ -carotene-linoleic acid restraint when contrasted with alternate extracts. On the other hand, acetone has the strong reducing power than alternate concentrates.

### 2.3.10. *Schizophyllum commune*

*S. commune* is a standout among the usually discovered fungus and can be separated from all landmasses, aside from Antarctica. *S. commune* has been accounted for to be a pathogen of people and trees; however, it principally receives a saprobic way of life by causing white rot. The antioxidant activity of *S. commune* is due to polysaccharides and polyphenols components [97].

## 2.4. Medicinal plants and spices having antioxidants

### 2.4.1. *Allium sativum*

*A. sativum* commonly known as garlic is a species belongs to family Alliaceae commonly cultivated in India [98]. It is a perpetual herb with a tall, erect blooming stem that grows up to 3 feet. Garlic has been utilized all through history for both culinary and therapeutic purposes. *A. sativum* is an adaptable herb that contains various trace elements, vitamins, and minerals. The total phenolic compound of the garlic has the antioxidant activity [99]. As an antioxidant, garlic has the strongest DPPH-scavenging ability [100]. Aged garlic extract has significantly eminent total phenolic substance than raw garlic extract [101]. It has been noticed that as the plant gets older, more the antioxidant potential it will gain.

### 2.4.2. *Capsicum annuum*

*Capsicum annuum* (red pepper) is native to southern North America and northern South America and was introduced in Asia in sixteenth century from South America [102]. It contains a wide cluster of phytochemicals with their radical-scavenging properties [103]. The spice contains carotenoids, flavonoids, tocopherols, free sugars, capsaicinoids, L-ascorbic acid, and organic acids [104]. At the ripe stage, hot-dried peppers have a high bioactive substances that show huge free radical-scavenging properties such as polyphenols and carotenoids [103].

### 2.4.3. *Curcuma longa*

*Curcuma longa* is a well-known spice that has a place in the Zingiberaceae family and is a lasting herb that measures up to 1 m high with a short stem. It is circulated all through tropical and subtropical locales of the world, being generally developed in Asiatic nations [105], primarily in India and China. In Pakistan and India, it is prevalently known as Haldi. As a powder, called turmeric, it has been in continual use as a flavor enhancer in both veggies lover

and non-vegan foods. Essential oil of fresh rhizomes has higher scavenging properties [106]. The phenolic compounds of *C. longa* are the primary contributor of antioxidant activity [107].

#### 2.4.4. *Eugenia caryophyllus*

*Eugenia caryophyllus* commonly known as clove is a medium-size tree (8-12 m) that belongs to family Myrtaceae. *E. caryophyllus* has been utilized for a considerable length of time as nourishment additive and for some therapeutic purposes as well [108]. Clove is local of Indonesia yet these days also cultivated in some other countries including Brazil in the province of Bahia. This plant is one of the wealthiest sources of phenolic compounds, for example, gallic acid eugenol and eugenol acetate [108]. *E. caryophyllus* leaf essential oil and its main constituent eugenol possess high antioxidant activity [109]. Among various extracts, the methanolic extract has higher scavenging activity than acetone and chloroform extracts [110].

#### 2.4.5. *Geranium sanguineum*

*Geranium sanguineum*, usually called as bloody cranesbill, is a herbaceous plant that belongs to family Geraniaceae. It is local from Asia and Europe and is developed as a garden subject. In Pakistan, India, Sri Lanka, Indonesia, and Zanzibar, it is cultivated on large scale. It is found naturally in Madagascar, Brazil, Sri Lanka, Tanzania, and West Indies [111]. Methanol extract of *G. sanguineum* has the free radical-scavenging property [112].

#### 2.4.6. *Pistacia lentiscus*

*Pistacia lentiscus* is extensively used in folk medicine by rural populations in Algeria. The herb is imperative due to its therapeutic uses. Ethanol, ethyl acetate, aqueous, hexane, aqueous/hexane, and chloroform extracts from the leaves of *P. lentiscus* have the radical-scavenging activity [113]. *P. lentiscus* have exceptional reducing power and strong radical-scavenging activity against DPPH [113, 114].

#### 2.4.7. *Salvia officinalis*

*Salvia officinalis*, also known as garden sage, belongs to family Lamiaceae and possesses strong antioxidant property [115]. The plant is grown and cultivated in some parts of Iran. The leaves of the plant are utilized as a part of Iranian folk medicine. The antioxidant activity of the plant is due to the presence of polyphenol constituents [116]. Dried sage leaves infusion with boiling water (sage tea) is the most typical form of preparation. Sage tea contains polyphenolic constituents that possess antioxidant property and other therapeutic effects [117].

#### 2.4.8. *Uncaria tomentosa*

*Uncaria tomentosa* is generally known as cat's claw and belongs to the family Rubiaceae. Its native is Amazon rainforest and other tropical territories of Central and South America. For centuries, the plant has been utilized as a part of customary practices in South America particularly in Peru. Due to its anti-inflammatory and radical-scavenging activities, the plant has been used to treat rheumatic diseases and cancer [118]. Decoctions prepared from the bark

of *U. tomentosa* are generally utilized as a part of the conventional Peruvian medicine for the treatment of many diseases [119]. The bark decoctions have strong ability to decrease the free radicals diphenylpicrylhydrazyl, hydrogen peroxide, and hypochlorous acid [119].

#### 2.4.9. *Leea indica*

*Leea indica* belongs to the family Vitaceae and has been traditionally used as natural folk medicine in Malaysia. In the leaves of *L. indica*, 23 known chemical compounds are identified [120]. The identified compounds include 11 hydrocarbons, 3 phthalic acid esters, phthalic acid, gallic acid, ursolic acid, solanesol, farnesol,  $\beta$ -sitosterol, lupeol, and 1-eicosanol [120]. Among these, total phenolic compounds possess the antioxidant activity [121].

#### 2.4.10. *Polyalthia cerasoides*

*Polyalthia cerasoides* belongs to the family Annonaceae and is a medicinal plant used in Thai native medicine. The roots of *P. cerasoides* are used for therapeutic purposes that contain alkaloid, bidebiline, three known sesquiterpenes, four known isoquinoline, and other compounds such as laudanoline, codamine, laudanidine, and reticuline [122]. The extract has the highest phenolic compound and high reactive oxygen species-scavenging activity [123, 124].

### 2.5. Antioxidants from marine sources

Marine ecosystem has been reported as a potential source of biodiversity and chemical activities. The organisms living in marine environment are gaining the attention of industries such as pharmaceuticals, nutraceuticals, and cosmetics because of possessing various interesting and useful chemical compounds [125]. Marine biotechnologists are trying to produce the tool for the utilization of marine biodiversity for the production of cheap source of pharmaceutical products and functional foods [126]. Seaweeds and sponges are considered as the richest source of bioactive compounds having the antimicrobial and antioxidant activities [127]. Seaweeds and sponges with their associated bacteria have been found to possess various health-promoting and disease prevention effects due to their phenolic compounds, polysaccharides, and useful organic acids [128]. These are supposed to be the most protective group of foods against environmental pollutants and radiation [129]. Among various other useful compounds, the marine organisms also contain polyphenolic compounds that are responsible for antioxidant activity including flavonoids, benzoic acid, cinnamic acid, gallic acid, quercy, and phlorotannins [130]. Nonanimal sulfated polysaccharides are reported to have antioxidant activities [131], which can be obtained from marine algae and other marine organisms from the phaeophyta group [132].

A large number of different species of algae and microalgae have been studied for the use of their bioactive contents as functional food components. Algae comprised of a huge and complex group of photosynthetic organisms with simple reproductive organs, which can be multicellular, known as macroalgae and unicellular called as microalgae. Algae grow in extremes of environmental conditions such as light, temperature, and salinity, which results in the production of a large number of reactive oxygen species (ROS). To cope with these ROS, algae produce various secondary metabolites with many antioxidant activities such as phycobilins, polyphenols, carotenoids, and vitamins [133].

People living in coastal areas use many types of seaweed, both as fresh and dry forms, as a natural source of food, and from the research, it is known that these seaweeds contain a large amount of proteins, minerals, and vitamins. Although the composition of these seaweeds varies according to their species, geographical distribution, temperature, and seasonal variation, the overall nutritional value remains the same. Many compounds from marine algae possess anticancer activity, and recently, seaweeds have gained attention as a rich source of antioxidants [134]. Many of the secondary metabolites produced by marine organisms reflect the presence of chloride and bromide ions in seawater. Marine halogenated compounds assemble a large number of other useful compounds such as indoles, peptides, terpenes, phenols, acetogenins, and volatile halogenated hydrocarbons. This protective effect suggests the presence of antioxidant compounds that show their antioxidant activity as free radical scavengers, hydrogen-donating compounds, single oxygen quenchers, and metal ion chelators. Many biological compounds have previously isolated from some other marine organisms such as fish, crustaceans, and their byproducts [135].

Seaweeds also create a suitable environment to a large number of bacteria that live on their surface having much more diversity of microorganisms as compared to other multicellular organisms [136]. These associated microorganisms have a protective effect on the seaweeds from pathogen, and they produce a large number of bioactive compounds of biomedical importance [137]. Exopolysaccharides produced by these bacterial species are used as an ingredient in food, petroleum, and pharmaceutical industries and emulsification of crude oil, vegetables, mineral oils, and bioremediation agents in environment management systems [138].

Fish protein hydrolysate (FPH), which is prepared from various marine organisms such as mackerel, tuna, Alaska Pollock, and yellowfin sole, has also been reported to have antioxidant activity [139–141]. Many types of peptides are obtained from fish muscle, bone, skin, and other tissues. All of these amino acids can scavenge free radicals, but the most powerful scavenging activity attributes to those who can easily donate hydrogen atoms. These amino acids are cystine and methionine, which have nucleophilic sulfur-containing side chains or tryptophan, tyrosine, and phenylalanine, which have aromatic side chains. Peptide size and amino acid composition are important for the FPH because it determines its antioxidant nature [142].

An in vitro study on phycocyanin, a pigment obtained from blue-green algae, reveals its antioxidant activity. It was evaluated in vitro by the use of luminol-enhanced chemiluminescence (LCL). Luminol reacts with oxygen ( $O^{\cdot 2}$ ), alkoxyl ( $RO^{\cdot}$ ), and hydroxyl ( $OH^{\cdot}$ ) radicals and shows a luminous signal measurable before and after antioxidant addition. This antioxidant activity was also confirmed in vivo by induction of inflammation in mice paw with glucose oxidase. The edema caused by inflammation was reduced, and the luminous signal indicated that the phycocyanin can scavenge  $OH^{\cdot}$  and  $RO^{\cdot}$  [143]. Algal antioxidants are also used in the cosmetic industries as antiaging agents [144]. A carotenoid pigment known as astaxanthin, found in microalga *Haematococcus pluvialis*, is reported to have anti-inflammatory, immunomodulatory, and antioxidant activities [145].

### 3. Conclusion

An increasing interest has been observed from the past decade in exploring the natural ingredients to be used in the food and food products. The researchers from all over the world are

focusing on the alternate sources other than the synthetic one, which will be more safe and convenient as dietary component. Although there are no such harmful reports have been observed regarding the use of synthetic antioxidants however the consumer's interest is also compelling toward the nature close products. Moreover, the synthetic antioxidants and preservatives in the food may lead to lipid peroxidation and deterioration of food flavor and quality. The use of natural herbs, spices, and plant ingredients is in practice from the ancient times and still practiced in the traditional food preparation as preservative, aroma, and flavor. This chapter is an effort to overview the potentials of various natural sources having reasonable antioxidant potential. The literature reports compiled here will be beneficial to identify the significance of various natural sources based on their antioxidant capacity, active ingredients, and geographic availability. This chapter reveals that people can prioritize their dietary habits based on the antioxidant potential and cost-effectiveness of the available source because 70–80% of the world population cannot afford the modern supplement and medicines.

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## Traditional Foods as Putative Sources of Antioxidants with Health Benefits in Konzo

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### Abstract

Konzo is a toxico-nutritional neurological disease associated with oxidative damage induced by cyanide poisoning through the ingestion of poorly processed bitter cassava. Dietary uses and patterns, determined using food frequency questionnaires, structured interviews and direct observation in consenting households to Kahemba, the rural area most affected by konzo in the world, showed that the diet of affected population is not varied and largely dependent on cassava (*Manihot esculenta* Crantz) products. Commonly consumed foodstuffs include herbal teas, mushrooms, spices, vegetables and yams. Phytochemical composition of extracts revealed that they contained flavonoids and phenolic acids as major compounds. All extracts of investigated traditional foods at the concentration range of 0.25–20 µg/mL, displayed high radical scavenging and cellular antioxidant activities using lucigenin on equine neutrophils, related to their phenolic content. The leaves of *Manihot esculenta* and *Manihot glaziovii* exhibited the highest antioxidant activity among vegetables. *Lippia multiflora* is the most active of the herbal teas, *Auricularia delicata* of mushrooms, *Dioscorea alata* of yams and *Ocimum basilicum* of spices. Traditional foods showed more efficient effects on extracellular ROS production and MPO activity. Traditional foods have interesting antioxidant, anti-inflammatory properties and could putatively be used as functional foods or nutraceuticals in the prevention of oxidative damage associated with konzo.

**Keywords:** anti-inflammatory activity, brain damage, cassava, cyanide, functional foods, nutraceuticals, oxidative stress

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## 1. Introduction

Konzo is a permanent and non-progressive paralytic disease that affects thousands of children and women of child-bearing age among millions of people who rely on cassava as their main source of food, mainly in Sub-Saharan Africa [1]. We recently showed that cognition may also be affected and subtle or pre-clinical forms of the paralytic disease may exist. Thus, the global burden of the disease may therefore have been underestimated, which raises serious concerns for the public health of millions of people for whom cassava is the main subsistence crop [2, 3].

The mechanisms underlying konzo remain unclear, although epidemiological studies have consistently shown an association between the occurrence of konzo and chronic dietary reliance on foodstuffs from insufficiently processed bitter cassava, with poor protein intake. Our recent studies suggest that disease development may be mediated through oxidative damage, findings that appear to be consistent with the putative effects of cyanide intoxication and/or chronic undernutrition [4]. Konzo is a permanent and irreversible condition with no treatment available. Improved processing methods to remove cyanogens from cassava prior to human consumption and enhancement of human cyanide detoxification capabilities perhaps through dietary supplementation may be critical to the prevention of the disease [5]. However, because of the chronic and heavy dietary reliance on bitter cassava as the main food source and the potential for continuous exposure to residual amounts of cyanogens due to variations in processing methods, chronic low-dose exposure to cyanide may persist, which may, possibly, lead to oxidative damage and neurocognitive deficits. It is therefore important that preventive measures are embedded in daily food practices and dietary habits to avoid the unnecessary burden of toxicity related to cyanide. The promotion of traditional or ethnic foods with potential health benefits may be useful in konzo-affected areas.

In this chapter, we report findings from a survey of food consumption and a subsequent phytochemical composition of relevant foods, aiming to identify foods with interesting antioxidant properties that could be used as functional foods or nutraceuticals in the prevention of chronic cassava cyanogenic poisoning, including konzo.

## 2. Konzo

Konzo is a distinct neurological entity with selective upper motor neuron damage, characterized by an abrupt onset of an irreversible, non-progressive, and symmetrical spastic para/tetraparesis. The first description of the disease was done by the Italian doctor Trolli eight decades ago in the Democratic Republic of Congo (DRC), epidemics have been reported from many cassava-consuming areas in rural Africa such as Angola, Cameroon, Mozambique, Tanzania, the Central African Republic, the DRC and recently in Zambia [1, 2, 6]. The common feature of these affected areas is that Cassava (**Figure 1**) is the staple food associated with food and social insecurity, poverty and malnutrition. Cassava (*Manihot esculenta*) forms part of the staple diet for more than 600 million people across the world. The plant grows in poor soil and is relatively drought resistant. Cassava roots and leaves are a good source of carbohydrates



**Figure 1.** Leaves and roots of cassava (*Manihot esculenta*).

and some minerals and vitamins (vitamin C). The roots are a poor source of lipids and proteins. *Manihot* leaves contained anthocyanins, flavonoids and other polyphenols. All parts of the plant contained the cyanogenic glycosides (linamarin and lotaustralin) that constitute the antinutrient factors [7, 8].

In DR Congo, the dependence on cassava is particularly strong and it is estimated that cassava (*Manihot esculenta*) is “all good enough” for the Congolese people because they receive “the bread of the roots and the meat of the leaves [7].

Cassava contains cyanogenic glucosides (linamarin and lotaustralin) that are released as hydrogen cyanide, which are thought to protect the plants from insects and other animals. For human consumption, the plants need to be detoxified, usually by soaking, drying in the sun, boiling, fermentation, or grating with roasting. These processes allow the cyanogenic glucosides to be released, but depend upon traditional practices, time taken, and the availability of water. Major food crises following drought or war are the cause of konzo. In these situations, the traditional systems of processing cassava roots into flour and other derived products are completely modified by: (i) The reduction of cassava retting time that is achieved not in the river as practiced in traditional methods but within households in closed containers (ii) Reducing the drying time of roasted cassava, ... (iii) Drying of cassettes or roasted cassava under a wood fire [8]. These changes in cassava root transformation expose the population to cyanide intoxication through the consumption of flour and other by-products with cyanide levels that exceed the WHO standards (maximum 10 parts per million: ppm). A 2011 survey of 123 households in Kahemba showed that the average cyanide level in cassava flour was  $92.2 \pm 56.2$  ppm [9]. Neurotoxicity is associated with incompletely detoxified cassava, although the exact mechanisms by which these compounds cause neurological damage is unclear. Two neurological conditions are mainly associated with bitter cassava: a myeloneuropathy and konzo. The myeloneuropathy called tropical ataxic neuropathy (TAN) manifests as a slowly evolving bilateral sensory polyneuropathy, optic atrophy and sensorineural deafness, and sensory ataxia, is seen in adults (particularly elderly) who have a solely cassava diet. The toxicity of cyanide is reduced by its transformation to thiocyanate or cyanate, which requires sulfur donors, often limited in malnutrition. However, it has been shown that oxidative damage plays a crucial role in the pathogenesis of konzo [10].

Konzo is defined by World Health Organization (WHO), as a visible symmetric spastic abnormality of gait while walking or running in a formerly healthy person with a history of onset of less than 1 week. After onset, a non-progressive course follows and bilaterally exaggerated knee or ankle jerks without signs of disease of the spine. WHO definition dedicated konzo as a pure upper-motor neuron disorder, cognitive effects were originally deemed absent or minimal [11]. Recently, Boivin et al. showed that motor proficiency is dramatically affected, and both children with and without konzo have impaired neurocognition compared with control children from a no outbreak area. Therefore, konzo is associated with a subclinical neurocognitive form, extending the human burden of konzo with dramatic public health implications [2]. Dietetic macronutrients and micronutrients play a crucial role in the control of brain physiology, and food intake is known to stimulate the activity of neurotrophic factors regulating synaptic plasticity. In recent years, epidemiological studies have shown that the regular consumption of fruits, vegetables, and spices... had a lower incidence of cardiovascular, neurological disorders and others. Functional foods and nutraceuticals have been proven beneficial for the prevention or amelioration of cognitive impairments in degenerative diseases [12].

### 3. Traditional foods in diet of Kahemba's population

Kahemba in province of Kwango, has a special significance due to recurring outbreaks of konzo disease over the past 20 years and is severely affected by konzo [2]. Food frequency questionnaires (FFQ) were used to identify the most common food items consumed by the local population. The Ministry of Health for the Congo for the ethical conduct of human participant research provided study approval (MD/125/2013). Interviews were conducted with 30 consenting households (families) of which 13 had at least one child affected by konzo (konzo households). Direct observations were also made to document dietary habits adopted by the aforementioned families.

Dietary habits of Kahemba's population are centered on cassava, the main staple food. Common foodstuffs include cassava bread-like items known as *chikwange* and *fufu*, a stiff paste made from cassava flour (**Figure 2**). There are different processing techniques to detoxify cassava. These include soaking (retting) for 4 days minimum in water and drying in the sun outside or inside the house under firewood. In practice, the retting of cassava tubers is done in closed containers with little water. Sun drying usually takes several days, except certain households in konzo areas have reduced processing times mainly due to famine (**Figure 2**).

The number of meals per day varies between 1 and 3 depending on household income. These meals do not necessarily correspond to breakfast, lunch and dinner. For konzo or non-konzo households, the basic meal (cassava flour paste + condiment) is consumed 2–3 times and sometimes the condiment varies. Variability of meals per day is weak especially for konzo households due to lack of financial resources. In the majority of cases the same dish is split and eaten morning, midday and evening; consequently the food is not varied in spite of the availability of various traditional foods. For many households, the breakfast meal was cassava porridge, often cooked the day before.



**Figure 2.** Cassava processing and diet for children of Kahemba. Sun drying of cassava roots soaked in a container by a konzo household of Kahemba city (A) and the structure of a meal for households characterized by a high quantity of *fufu* accompanied by few vegetables and insects (B).

Few households consumed tea, coffee or herbal teas. Other households consumed boiled sweet cassava roots accompanied by peanuts, voandzous (*Vigna subterranea*) and avocado fruits. Konzo households mostly had one meal per day, consumed preferably in the evenings.

Readily available ethnic food items in the city of Kahemba, other than starch sources, included wild edible mushrooms, herbal teas, spices, vegetables (legumes), and yams. Among the mushrooms, *Auricularia delicata*, *Lactarius edulis*, *Lactarius symoensi* and *Schizophyllum commune* were the most abundant and readily available on the market all year round in dried form. For vegetables, leaves of *Manihot esculenta*, *Manihot glaziovii*, *Hibiscus cannabinus*, *Hibiscus sabdariffa*, *Ipomea batatas* and *Cucurbita maxima* are the most consumed by the two types of household. Households cultivate some sweet and bitter local varieties of cassava roots. Mwambu variety is the bitterest variety of cassava and was introduced to Kahemba in the year 1937 from Angola. This variety is the most cultivated because its yield of tubers is the highest after 6 months [8].

Leaves of *Abelmoschus esculentus*, *Abelmoshus moshatus*, *Amaranthus viridis*, *Gnetum africanum*, *Pteridium acquilium*, *Psophocarpus scandens*, *Sesamum angustifolium*, *Solanum gilo* and *Solanum aethiopicum* (fruit) are largely consumed by non-konzo households.

Fruit consumption by households was low despite the variety of fruits cultivated by households in their family plots. Fruit production was rather intended for sale and not consumption by the members of the households.

Caterpillars, larvae, and red meat, especially pig meat and fish, are largely consumed by the households but in small quantities.

Our findings indicate that konzo households mostly rely on cassava derived products as their main source of food and have limited access to other types of food including vegetables, fish and meats. These findings are consistent with previous studies that suggest that poor nutrition is a risk factor for konzo. This dietary pattern based principally on cassava flour paste exposes the consumer to intoxication with cyanide, especially in children for whom the quan-

tity of cassava flour paste represents more than 90% of the consumed meals. Previous studies showed that this dietary pattern is responsible for the persistence of konzo [9]. The Flora of province of Kwango is rich in traditional foods that are mostly unexploited. For this reason, Mbemba et al. assessed the nutritional value of some traditional foods by determining their relative amino acid composition, in order to contribute to the equilibration of the diet in the population of this area severely affected by malnutrition and konzo. Yams such as *D. alata* and *D. cayenensis* are the most widespread. Mbemba et al. reported that some dried yams are richer in proteins than cassava root and can be used with success to prepare porridge for children who represent the most at-risk population for konzo [13]. They are an alternative substitute for cassava especially as they are abundant during the dry season, a period corresponding to high outbreaks of konzo. Among the sources of animal proteins consumed, the fish *Chanmellabes tupus* (known locally as Misombi) and larvae are the foods economically accessible for the majority of Kahemba's population.

#### 4. Traditional foods as functional foods and nutraceuticals

Functional foods are in fact products that may look like or are a conventional food and be consumed as part of a usual diet, but apart from supplying nutrients they can reduce the risk of chronic diseases. Nutraceuticals are health promoting compounds or products that have been isolated or purified from food sources and they are generally sold in a medicinal (usually pill) form [14, 15]. Considering these definitions, we noticed that these products contain bioactive compounds called phytochemicals that are capable of modulating metabolic processes and resulting in the promotion of better health. By having antioxidant, anti-inflammatory, immunomodulatory, adaptogenic, anticancer, and several other health benefits, functional foods and nutraceuticals are used worldwide for the prevention and treatment of chronic diseases such as diabetes, arthritis, cardiovascular and respiratory disorders, neurodegenerative diseases, and cancer [12].

##### 4.1. Phytochemicals

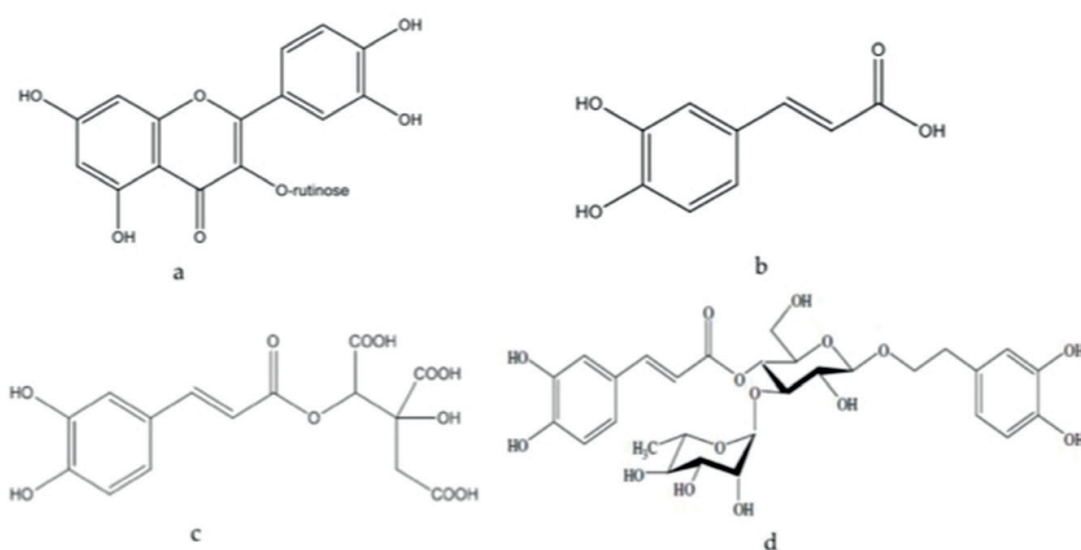
Phytochemicals are found in fruits, herbal teas, mushrooms, spices, vegetables and whole grains. They include an extremely heterogeneous class of compounds (polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organo-sulfur compounds) with different chemical structures (hydrophilic or lipophilic).

TLC and HPLC-DAD analysis of methanolic extracts of the most consumed foodstuffs of Kahemba, have shown the presence of polyphenolic compounds in all extracts. TLC fingerprints of extracts showed the presence of glycosylated flavonoids (yellow, orange and green fluorescent spots) and phenolic acids (blue fluorescent spots) as major compounds especially for vegetables and herbal teas (**Figure 3**). Cassava flours, mushrooms and yams contain mainly phenolic acids. Flavonoids identified were derivatives of quercetin (rutin, hyperoside, isoquercitrin, quercitrin) and kaempferol (Kaempferol 3-O-glucoside, kaempferol 3-O-rutinoside). Phenolic acids were caffeic acid and its derivatives such chlorogenic acid ferulic acid and verbascoside.



**Figure 3.** TLC chromatogram of methanolic extracts from *M. esculenta* (varieties: *Chamusuku*, *Disanka*, *Kamonji*, *Mwambu*, *Tshibombi*, *TEM 419*), *M. glaziovii* with astragalol (ast), caffeic acid (caf), chlorogenic acid (chl), ferulic acid (fer), luteolin (lut), quercitrin (que) and rutin (rut) as standards; developed with ethyl acetate/formic acid/methanol/water (20:0.5:2.5:2; v/v/v/v) and visualized at 365 nm with natural products-PEG reagent. Flavonoids are detected as yellow-orange fluorescent spots and phenolic acids as blue fluorescent spots.

Leaves of *Manihot esculenta* and *Manihot glaziovii* contained amentoflavone, quercetin, quercetin-3-rutinoside, quercetin-3-glucoside, and kaempferol 3-rutinoside as flavonoids and caffeic acid, ferulic acid, gallic acid as phenolic acids. Cassava roots, cossertes and cassava flours contained phenolic acids, such as ferulic acid, as major compounds [8]. *Hibiscus acetosella* contained 2-O-trans-caffeoyl-hydroxycitric acid as major phenolic compounds and flavonoids such as quercetin-3-galactoside. *Hibiscus cannabinus* and *Hibiscus sabdariffa* contained neochlorogenic acid as major phenolic compounds and flavonoids such as quercetin-3-rutinoside, quercetin-3-glucoside and kaempferol 3-rutinoside (**Figure 4**) [16].



**Figure 4.** Structures of phytochemicals (a) quercetin-3-O-rutinoside, (b) caffeic acid, (c) Caffeoyl-hydroxycitric acid, and (d) verbascoside.



Verbascoside is found to be abundant in herbal teas [17] and we first report here its presence in leaves of *Sesamum angustifolium*. Chlorogenic acid is the main phenolic acid found in extracts of *Raphia sese*, *Solanum aethiopicum* and *Solanum gilo*. Rosmarinic acid was found to be a major compound of *Ocimum* species of DRC [18]. Phytochemical screening of mushrooms and yams showed that phenolic acids are their major compounds. Phytochemicals derived from various sources target inflammatory and oxidative stress pathways and retard or delay the onset of neurological diseases.

Protective effects of phenolic compounds have often been ascribed to their direct antioxidant effect and/or to their anti-inflammatory action [12].

#### 4.2. Antioxidant properties

Oxidative stress is recognized as an important factor in a variety of neurodegenerative diseases, as a mediator of the adverse effects of a number of neurotoxins, and as a mechanism for age related degenerative processes [12]. Finding alternative and complementary ways to reduce the redox processes might have a beneficial interest in the context of developing countries.

Traditional foods contained a considerable amount of phenolic compounds expressed as total polyphenol contents as described in **Tables 1–3**. Total polyphenol contents of Kahemba's ethnic foods varied significantly between the samples of each group of foods. *Lippia multiflora* had the highest total polyphenol content among herb-teas, *Manihot glaziovii* among vegetables, *Cantharellus rufopunctatus* among mushrooms and *Dioscorea alata* among yams. Antioxidant activities significantly varied also between the samples of each group of foods ( $p < 0.05$ ). All methanolic extracts had significant radical scavenging effects with increasing concentrations in the range of 1–40  $\mu\text{g/mL}$  for herb teas, 10–80  $\mu\text{g/mL}$  for spices and vegetables, and 10–250  $\mu\text{g/mL}$  for mushrooms. This antiradical activity is connected to their ability to scavenge free radicals according to their  $\text{IC}_{50}$  values (**Tables 1–3**).  $\text{IC}_{50}$  values ranged from  $7.56 \pm 0.87 \mu\text{g/mL}$  (*L. multiflora*) to  $653.13 \pm 51.25 \mu\text{g/mL}$  (*D. cayenensis*) for the ABTS assay and from  $10.44 \pm 1.13 \mu\text{g/mL}$  (*L. multiflora*) to  $17179.09 \pm 1150.25 \mu\text{g/mL}$  (*Cantharellus* sp.) for the DPPH assay. Cassava flours also exhibited a good scavenging activity with  $\text{IC}_{50}$  values ranging from  $99.54 \pm 9.60$  to  $974.99 \pm 94.01 \mu\text{g/mL}$ .

Beside conventional cell-free antioxidant assays, it can be pertinent to evaluate the anti-oxidant and anti-catalytic potential of plant extracts in cellular models involved in ROS production and inflammatory responses [16, 19]. The addition of extract solutions at increasing concentrations resulted in a dose dependent decrease of the ROS-induced lucigenin-amplified chemiluminescence. All tested extracts induced a significant inhibition ( $p < 0.0001$ ) of the ROS production by neutrophils compared to controls at the concentration range of 0.05–10  $\mu\text{g/mL}$  for herbal teas and vegetables; of 5–20  $\mu\text{g/mL}$  for mushrooms (**Figure 5**).

Aqueous and methanolic extracts of Herbal teas showed also the best cellular antioxidant activity using DCFH-DA on HL-60 monocytes assay at 1–20  $\mu\text{g/mL}$  [17]. The lucigenin-dependent chemiluminescence (CL) and the intracellular fluorescent probe DCFH-DA were used to evaluate the extra- and intracellular ROS production resulting mainly from NADPH oxidase activity by stimulated neutrophil and HL-60 cells [16].

Vegetables	TPC	AOX IC <sub>50</sub> (µg/mL)	
		ABTS	DPPH
<i>Abelmoschus esculentus</i> Linn	32.94 ± 0.93	86.27 ± 9.2	nd
<i>Abelmoschus moshatus</i> Medik	36.32 ± 1.05	52.36 ± 2.1	71.45 ± 14.44
<i>Amaranthus viridis</i> L.	23.31 ± 0.92	88.9 ± 11.1	762.08 ± 155.34
<i>Dioscorea praehensilis</i>	34.09 ± 4.46	106.44 ± 17.36	230.14 ± 31.07
<i>Hibiscus cannabinus</i> L.	89.05 ± 11.92	44.98 ± 0.87	73.79 ± 17.20
<i>Hibiscus sabdarifa</i> L.	82.97 ± 3.27	64.72 ± 6.17	86.04 ± 4.32
<i>Ipomea batatas</i> L.	76.78 ± 3.20	47.76 ± 3.25	233.35 ± 63.53
<i>Manihot esculenta</i> Crantz var. Chamusuku	41.51 ± 0.26	23.28 ± 1.11	nd
<i>Manihot esculenta</i> Crantz var. Kamonji	45.18 ± 0.79	17.65 ± 1.13	nd
<i>Manihot esculenta</i> Crantz var. Mwambo	86.4 ± 2.99	15.10 ± 1.13	20.15 ± 1.07
<i>Manihot esculenta</i> Crantz var. Tshibombi	57.56 ± 6.19	19.45 ± 1.11	40.93 ± 1.91
<i>Manihot esculenta</i> Crantz var. TEM 419	36.70 ± 4.16	23.07 ± 1.11	37.93 ± 2.25
<i>Manihot glaziovii</i> Müll. Arg	107.71 ± 7.80	12.42 ± 2.08	20.5 ± 1.06
<i>Megaphrynium macrostachum</i>	32.69 ± 3.65	79.25 ± 10.29	503.5 ± 10.29
<i>Sesamum angustifolium</i> auct*	63.76 ± 3.76	31.19 ± 1.07	48.3 ± 1.02
<i>Solanum aethiopicum</i> L	32.24 ± 4.13	123.89 ± 16.15	282.49 ± 27.81
<i>Solanum gilo</i> Raddi (leaves)	72.04 ± 1.70	29.51 ± 0.94	163.68 ± 30.41
<i>Solanum gilo</i> Raddi (fruits)	24.19 ± 0.37	81.97 ± 5.17	349.95 ± 19.03

\**Sesamum angustifolium*, one of banned vegetables for konzo households by local traditional medicine [2], is the most active (antioxidant activity) among the vegetables after *Manihot* species. nd = not determined.

**Table 1.** Total phenolic content (TPC) of vegetables, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC<sub>50</sub> (µg/mL) values of organic extracts in ABTS and DPPH assays (means ± SD, n = 6).

For the antioxidant activity, *L. multiflora* is the most active for herbal teas, *M. glaziovii* for vegetables and *A. delicata* for mushrooms related to their hydrophilic and lipophilic compounds [20]. The vegetable *D. praehensilis* exhibited higher cellular antioxidant activity than *I. batatas* and *S. gilo* (leaves) whereas these showed a superior activity to *D. praehensilis* for radical scavenging activity. López-Alarcón and Denicola showed that a good antioxidant is not just a good radical scavenger [21].

According to the scientific data, our study is the first to evaluate the antioxidant capacity of local traditional foods in an area severely affected by konzo disease in order to establish scientific basis of their use in the prevention of chronic cassava cyanogenic poisoning.

Eight species of edible mushrooms used in this study showed an interesting antioxidant activity compared to results reported in previous studies [22, 23]. There are few reports regarding the antioxidant activities of the studied mushrooms. *A. delicata* exhibited the highest antioxidant activity in comparison to other vegetables. However, Kabuyi et al. (2017) assessed

Mushrooms	TPC	AOX IC <sub>50</sub> (µg/mL)	
		ABTS	DPPH
<i>Amanita loosii</i>	8.82 ± 0.01	45.65 ± 1.00	1862.1 ± 425
<i>Auricularia delicata</i>	9.53 ± 0.12	39.31 ± 1.04	252.4 ± 15.5
<i>Cantharellus</i> sp.	4.73 ± 0.02	220.3 ± 17.40	1717.09 ± 522
<i>Cantharellus symoensii</i>	6.4 ± 0.02	144.9 ± 21.80	1367.73 ± 364
<i>Cantharellus rufopunctatus</i>	10.32 ± 1.09	41.1 ± 1.02	1815.52 ± 418
<i>Lactarius tenellus</i>	5.96 ± 1.47	43.51 ± 1.04	1603.25 ± 294
<i>Lactifluus edulis</i>	5.12 ± 0.11	262.4 ± 20.74	1318.3 ± 259
<i>Schizophyllum commune</i>	9.77 ± 0.40	169.8 ± 23.80	307.61 ± 25.05

Mushrooms exhibited a relatively interesting activity similar to some vegetables despite their low total phenol content.

**Table 2.** Total phenolic content (TPC) of mushrooms, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC<sub>50</sub> (µg/ mL) values of organic extracts on ABTS and DPPH assays (means ± SD, n = 6).

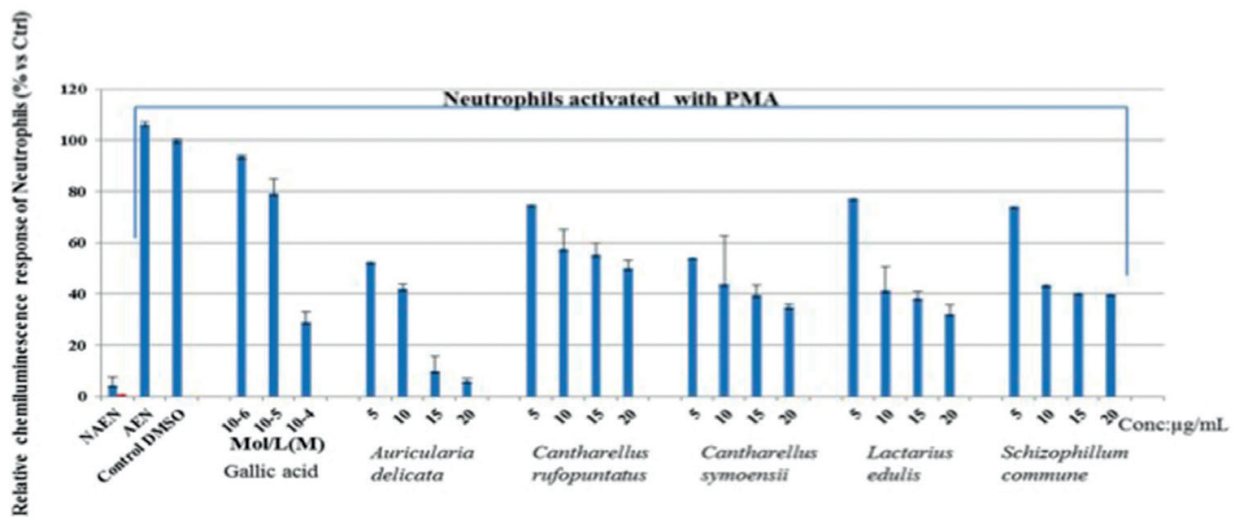
Herb-teas	TPC	AOX IC <sub>50</sub> (µg/mL)	
		ABTS	DPPH
<i>Lantana montevidensis</i> (Spreng.)	87.74 ± 1.66	21.11 ± 1.68	27.15 ± 3.50
<i>Lippia multiflora</i> Moldenke	110.35 ± 3.89	7.56 ± 0.87	10.44 ± 1.13
<i>Ocimum gratissimum</i> L.	71.88 ± 1.16	12.07 ± 0.84	21.76 ± 2.92
<b>Spices</b>			
<i>Aeollanthus suaveolens</i>	26.30 ± 3.72	41.82 ± 3.99	nd
<i>Ocimum basilicum</i>	6.52 ± 0.18	38.37 ± 3.13	136.77 ± 15.64
<i>Raphia sese</i> De Wild	10.08 ± 0.51	40.71 ± 1.05	518.8 ± 95.16

nd = not determined. Herbal teas exhibited considerable antioxidant activity.

**Table 3.** Total phenolic content (TPC) of herb-teas and spices, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC<sub>50</sub> (µg/mL) values of organic extracts in ABTS and DPPH assays (means ± SD, n = 6).

the selenium content and the antioxidant capacity of wild edible mushrooms from Kenge, another rural area of Kwango (DRC) with a high prevalence of malnutrition. They reported that *Auricularia delicata*, *Lentinus cf. cladopus*, *Pleurotus tuberregium*, *Marasmius buzungolo* and *Schizophyllum commune* showed the interest antioxidant activity and moderate quantity of selenium, and *L. cf. cladodus* had the highest concentration [24]. The radical scavenging activity of mushrooms found by ABTS assay is significantly higher than that obtained with the DPPH assay. This great difference could be explained by synergistic effects of mushroom hydrophilic and lipophilic compounds on the ABTS<sup>+</sup> chromogen [16].

Tested vegetables showed high antioxidant activity and leaves of *Manihot* species exhibited a strong radical scavenging capacity. A significant difference was found between antioxidant



**Figure 5.** Effects of gallic acid and organic extracts of mushrooms on the CL response produced by PMA activated equine neutrophils (means  $\pm$  SD, n = 6). The intensity of CL results from the reaction between lucigenin and the ROS produced by the non-activated (NAEN) and activated equine neutrophils (AEN). The chemiluminescence response observed in the presence of solvent (DMSO), used to solubilize the extracts, and was defined as 100%. For non-activated (NAEN) cells, 10  $\mu$ L of PBS was added to the cell suspension instead of PMA.

activities of different varieties of *M. esculenta* ( $P < 0.05$ ). Few reports exist on the antioxidant activity of leaves of *M. esculenta* and *M. glaziovii*. However, to the best of our knowledge, leaves of *M. glaziovii* are only consumed in the west of Democratic Republic of Congo. This species is particularly used elsewhere as biomass for bioethanol and bio-gas production [25]. The antioxidant activity of the other vegetables is also considerable and comparable to results reported in similar studies on ethnic foods from Kwango. *Entada gigas* (L.) Fawc. & Rendle, *Psophocarpus scandens* (Endl.) Verdc, *Salacia pynaertii* De Wild, and *Tetrorchidium congolense* J. Léonard, four unconventional green leafy vegetables with high nutritive value consumed to Kenge, showed important antioxidant activities [26]. Interestingly, these vegetables have high protein content specially *S. pynaertii* that constitute the richest vegetable in methionine and cysteine [13]. Methionine and cysteine are sulfur amino acids, essential for the detoxification of cyanogen glycosides implicated in the occurrence of konzo [27].

*S. angustifolium* showed a remarkable antioxidant activity which can be attributed to the presence of verbascoside, which is also responsible for high antioxidant activity of herbal teas. Herbal teas exhibited antioxidant activity according to the order *Lippia multiflora* > *Ocimum gratissimum* > *Lantana montevidensis* > *Ocimum basilicum*. Herbal tea is a commonly consumed beverage prepared from different parts of plant species other than *Camellia sinensis*. Particularly, extracts of *L. multiflora* are known to have an excellent antioxidant activity related to their abundance of phenylpropanoids such as verbascoside [17, 28].

Antioxidant activity of studied yams was considerable and *Dioscorea alata* was the most active. Bukatuka et al. studied *Dioscorea alata*, *Dioscorea bulbifera*, *Dioscorea dumetorum*, *Dioscorea burkilliana* and *Dioscorea praehensilis* from Kenge, known to be relevant in traditional medicine for diabetes mellitus treatment. These authors reported that they displayed highest radical-scavenging activities and a good antihyperglycemic activity related to their appreciable

amount of total phenolic contents [29]. Interestingly, cassava flours exhibited higher antioxidant activity than cassava roots. This could be explained by possible chemical modifications during processing before cassava flour is traded. Nevertheless, it is probable that the antioxidant capacity of cassava flours is not sufficient to counteract oxidative damage induced by cyanogenic glycosides. In this context, the mixture of cassava flours with maize, proposed to reduce the ingestion of cyanogenic glycosides from cassava and to improve amino acid intake, could be interesting to promote [8].

Altogether, the antioxidant activities measured with cell-based assays were in good accordance to radical-scavenging capacities. Mushrooms exhibited a considerable cell-based antioxidant activity comparable to certain vegetables. To the best of our knowledge this is the first report regarding the potential inhibitory effect on intracellular ROS production by inflammatory cells of the studied mushrooms and some vegetables such as *D. praehensilis*, *M. glaziovii*, and *S. angustifolium*. Globally, herbal teas showed the highest antioxidant and radical scavenging capacities, followed by vegetables, yams, mushrooms, spices and cassava flours. TLC and HPLC fingerprints of extracts of investigated foods revealed that they contain a diversity of phenolic compounds (Flavonoids, phenolic acids...) [8].

Flavonoids are a group of phenolic compounds or secondary metabolites that are widely distributed in higher plants and are part of our daily diet. It has been reported that flavonoids exhibit a wide variety of biological effects, including anti-inflammatory, anti oxidant, antiviral, antibacterial, anticarcinogenic, antituberculosis, vasodilatory, and antiallergic activities [12]. They are also cytoprotective in various organs and promote intracellular signals that enhance cell survival, among other benefits. However, interest in flavonoids stems mainly from their antioxidant activities, resulting from the catechol group in the B ring, which confers free radical-scavenging activity. Additionally, they act as electron donors or chelators of metal ions (e.g., iron, copper), inhibiting the oxidation of low-density lipoproteins (LDLs). Flavonoids have thus become key compounds. When ingested in the diet, they may prevent and combat neurodegenerative diseases such as Alzheimer disease (AD). Studies have reported that the oral administration of some flavonoids (apigenin, rutin, myricetin...) to mice prevents the development of Alzheimer disease [12]. Rivadeneyra-Domínguez et al. reported that *G. biloba* extract exert a protective effect against behavioral and neuronal damage associated with consumption of cassava juice in the rat and these effects are possibly related with flavonoids [30].

Traditional foods studied contained glycosylated flavonoids mainly the derivatives of quercetin. Quercetin is the major flavonoid in our daily diet and its estimated daily intake is between 5 and 40 mg. After absorption, quercetin is mainly metabolized in the intestine and liver. The plasma concentration of quercetin is normally in the nanomolar range, but it can reach the micromolar range after consumption of quercetin- rich foods [12]. Quercetin is the most extensively studied flavonoid that has been shown to exhibit antioxidant, antiviral, antibacterial, anti-inflammatory, and anticarcinogenic properties. Quercetin modulate several cellular signaling pathways involved in regulating the antioxidant response, cell survival, apoptosis, and inflammation [12, 31, 32]. Others compounds such as some biflavonoids founded in the seeds as *Garcinia kola* largely consumed to Kahemba, may have anticancer, antimicrobial, anti-inflammatory, antiviral,

and antimalarial activities [33]. Biflavonoids are compounds with therapeutic potential against AD and other neurodegenerative diseases [12].

Verbascoside is well known for its numerous biological activities including anti-oxidative, anti-apoptosis and anti-inflammatory effects. The *in vivo* effects of verbascoside could also be assigned to its metabolites such as caffeic and ferulic acids [34]. Verbascoside is able to reverse some of the cognitive impairment and to prevent the neuronal apoptosis due to oxidative stress. For this, previous results support the use of traditional medicinal herbs containing acteoside for neuroprotection [35].

Typical preparation methods applied to these food items before consumption include a strong heating process to prepare different sauces with spices and palm oil, which are consumed together with a maize or cassava preparation. Our results are limited to non-cooked traditional foods. The literature reports controversial effects of heat treatment on the antioxidant capacity depending on the analyzed plant and the applied heat treatment. Tsumbu et al. reported that the moderate heat treatment of the green vegetables did not modify their antioxidant and anti-inflammatory capacities [36]. Cooking could lead to the loss of phenolic compounds due to their good solubility in water [37]. However, Ola *et al.* (2009) demonstrated that almost all the phenolic constituents of *M. esculenta* leaves are stable even after heating processes such as boiling [38]. Abdullah *et al.* (2012), reported that selected culinary-medicinal mushrooms extracted by boiling in water for 30 min, showed a good antioxidant activity related to synergistic effects of entire water-soluble fractions [39]. Nevertheless, this aspect should be examined in further studies.

### 4.3. Anti-inflammatory activity

Motor and cognitive performance continues to be significantly impaired in konzo are associated in part with exposure to poorly processed cassava as measured by urinary thiocyanate [11]. Presence of very high concentrations of thiocyanate ( $\text{SCN}^-$ ), the major metabolite of cyanide, in the bodily fluids of konzo subjects is a consequence of dietary exposure to cyanide. Besides chloride, myeloperoxidase (MPO) also uses the thiocyanate as a major physiological substrate. The  $\text{SCN}^-$  concentration is a powerful driver of the extent of thiol proteins oxidation in induced by MPO [40, 41] and leading to carbamylation of proteins [42]. Cassava consumption is associated with increased protein carbamylation and neurological complications. Prevention of carbamylation may protect against the neuropathic effects of cyanate [43, 44]. MPO generates a battery of highly diffusible reactive oxidants such as hypochlorite, tyrosyl radicals and aldehydes, which instigate oxidative damage in the host tissues at the inflammatory sites exacerbating tissue damage. In some acute and chronic pathologies, the uncontrolled stimulation of neutrophils could contribute to amplify or maintain the inflammatory response with the release of MPO, a pro-oxidant enzyme involved in secondary cell damage and considered as a marker of inflammation [19]. Indeed, recent investigations have increasingly revealed the cause-effect relationship between MPO and the development of diverse inflammatory diseases supporting MPO and its metabolites as a promising biomarkers not only for infectious diseases but also for a wide array of non-infectious and neurodegenerative disorders [45]. Malle et al. (2007), suggested that the inhibitors of MPO activity are promising therapeutic agents [46].

All plant extracts tested and isolated phenolic acids exhibited a dose-dependent inhibitory effect on MPO activity performed with SIEFED (Specific Immunological Extraction Followed by Enzymatic Detection) technic. The SIEFED method used to measure MPO activity allowed the detection of compounds that have a direct interaction with the MPO. For the Hibiscus, dichloromethane extracts showed a stronger inhibition of MPO in comparison to methanolic extracts in the following order: *H. cannabinus* > *H. acetosella* > *H. sabdariffa*. The dichloromethane allowing a better extraction of lipophilic molecules may allow a better interaction of these molecules with the hydrophobic pocket at the entrance of the active site of MPO [16]. Tsumbu et al. evaluated the antioxidant, anti-radical, anti-inflammatory, and modulating properties of in “inflammation like” conditions of green vegetables from Bas Congo in DRC [36]. These authors showed that *Abelmoschus esculentus*, *Hibiscus acetosella*, *Manihot esculenta* and *Pteridium aquilinum* were active to inhibit MPO activity and the best effects were observed for *Pteridium* which contains the highest amount of total polyphenols and tannins, and *Manihot*, which has a high content of flavonoids [36]. Caffeoyl-hydroxycitric acid and neochlorogenic acid isolated from Hibiscus species, are less efficient MPO inhibitors in comparison to gallic acid compared to gallic acid, caffeoyl-hydroxycitric acid and neochlorogenic acid are larger molecules that cannot enter easily into the active site of MPO and thus inhibit the enzyme [16]. Gallic acid and caffeic acid were less active than quercetin. Quercetin shown the best activity than his glycosylated flavonoids [8].

Although many phytochemicals present in plant foods are poorly absorbed and undergo rapid excretion, they exert anti-inflammatory, antioxidant, and anticarcinogenic effects at realistic doses. Consumption of phytochemicals may also mediate neurohormetic response through the modulation of adaptive stress-resistance genes, which are responsible for encoding protein chaperones that favor resistance to cellular stress and modulate immune function. Thus, regular consumption of phytochemicals from childhood to adulthood may reduce the risk of age related neurological disorders [32].

Polyphenols are promising neuroprotective agents for the treatment of neurodegenerative diseases and act by different mechanism including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning and cognitive function. Evidence for neuroprotection has been provided by *in vitro* studies showing that various polyphenols protect neuronal cells from damage due to oxidative stress, and by *in vivo* animal studies that have shown their ability to protect neurons against oxidative insults [12].

Traditional foods are good source of essential amino acids and minerals especially for children who are exposed to many diseases. The high nutritive value of these traditional vegetables associated with their important antioxidant activities could contribute to a diversification of the diet in konzo’s population, and could then provide benefits leading to a protection against oxidative damage under different conditions including konzo.

## 5. Conclusion and perspectives

The diet of the population of Kahemba is largely dependent on cassava. Biodiversity of the flora of Kahemba constitutes an untapped reserve of traditional food resources that have

considerable potential for antioxidants. However, *in vitro* findings, such as the antioxidant activities we have measured, are of uncertain relevance to the *in vivo* situation in healthy humans. Further studies are needed to evaluate the *in vivo* activity of these traditional foods and particularly in their cooked forms. This could lead to the valorization of traditional foods as functional foods or nutraceuticals with high antioxidant, anti-inflammatory capacities and high quality protein. This may provide benefits to protect the population of Kahemba against oxidative damage under different conditions, including konzo.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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## **Plant Extracts as Antioxidant Additives for Food Industry**

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### **Abstract**

Plants have phenolic compounds with antioxidant activity. These compounds are distributed in tissues and cells of plants and their abundance depends on the species, the part of the plant used, maturity stage, light hours, among others. On the other hand, the profile and quantity of phenolic compounds extracted from plant matrices changes depending on the species, cultivar, climate, and other factors. Plant extracts do not present a unique phenolic component, they correspond a mixture and its antioxidant activity will be affected by the concentration of each one and their action depends on this composition. In this chapter, some generalities about the phenolic compounds with antioxidant activity present in plant matrices will be exposed, also the principal methods for their extraction and quantification will be described and this information will be complemented with a review on applications of these compounds in food industry. In that sense, the reader can infer the importance of continue to studying and developing techniques to obtain, extract, and characterize this kind of compounds, also they can identify possible application of them, the most important, they can recognize them as an alternative to replace chemical synthesized antioxidants used in food industry improving the market of natural products.

**Keywords:** extraction, food additives, natural products, phenolic compounds, vegetal species

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## 1. Introduction

Over the years, benefits have been attributed to fruits and vegetables consumption associated with their content of phenolic compounds with antioxidant activity that may contribute to reduce the risk of cardiovascular diseases and cancer [1]. The antioxidant behavior of phenolic components is related to their ability to chelate metals, inhibit lipoxygenase, and capture free radicals, although sometimes they can also act as promoters of *in vitro* oxidation reactions [2]. In this way, phenolic compounds act as antioxidants by delaying or preventing autooxidation or oxidation and free radicals sequestration, forming more stable compounds that cannot undergo subsequent oxidations, which allows them to protect low density lipoproteins (LDL) of the human body from oxidation [3]. Antioxidant activity of different foods research, such as fruits, vegetables, and plants in general, have indicated the positive effects of this on diseases control related to oxidative stress [4–6].

Phenolic compounds are chemical substances that have an aromatic ring attached to one or more hydroxyl groups, including functional derivatives such as esters, glycosides, among others. They have an acidic behavior, since the oxygen of the hydroxyl group is strongly bounded to aromatic ring, while the oxygen and hydrogen bond is relatively weak, which have allowed the proton dissociation that can be released into the medium, causing a phenolate ion negatively charged [7]. They are secondary metabolites distributed in the plant kingdom, from which more than 8000 compounds have been identified that differ in chemical structures and in activity. Its distribution in tissues and cells varies among different fruit and vegetable species and its abundance depends on the species, the type of crop, the part of the plant used, the type of soil, maturity stage, light hours, fertilization, part of the vegetative cycle, among others [8].

These compounds participate in various functions of plants, such as nutrient absorption, protein synthesis, enzymatic activity, photosynthesis, structural components formation, allelopathy, and defense against adverse environmental factors [9]. They are substances responsible for providing fragrances, colors, and flavors to various plants. As an example, anthocyanins are responsible for the red, blue, and violet color of red fruits such as cherries, blackberries, or currants, while flavones are attributed the yellow color of fruits such as lemon or banana. This type of compounds in addition to giving color also contribute by providing characteristics to taste (astringency) of some foods and affecting the sugar/acid ratio of some fruits, as well as being used as a criterion to determine the overall quality of fresh fruits and derived products of these [10]. Among the polyphenols that are considered important in food, are the acids such as gallic, synapic, ferulic, caffeic, *p*-coumaric, and their derivatives; as well as the flavonoids and their glycosides [11]. In that sense, phenolic compounds are usually classified into three groups such as flavonoids, phenolic acids, and polyphenols, the most important being the benzoic acid derivatives for their therapeutic use and specifically the flavonoids for having the greatest chemical diversity with approximately 6000 different structures. The profile and quantity of phenolic compounds extracted from plant matrices changes depending on the species, cultivar, season, climate, and other factors such as the cultivation method. However, the extraction performance depends mainly on the solvent polarity used, the extraction method, the duration of the process, and the quantitative and qualitative distribution of the compounds present [3, 11, 12].

The plant extracts, in their majority do not present a unique phenolic component, but, on the contrary, correspond to the mixture of several of these. In that sense, its antioxidant activity will be affected by the concentration of each one in the analysis matrix [3]. This is how there is no single methodology to know the antioxidant capacity of an extract and, therefore, to obtain more comprehensive and complete information, different methods must be used with different mechanisms of action. In addition, due to the heterogeneity of the analytical conditions used, among which are: wavelength, radical generator, time of analysis and how to express the results, is possible to reach values of antioxidant capacity that are not comparable. In that way, in this chapter some generalities about the phenolic compounds with antioxidant activity present in plant matrices will be exposed, also, the principal methods for their extraction and quantification will be described and this information will be complemented with a review on applications of these compounds in food industry.

## 2. Classification and properties of phenolic compounds

Phenolic compounds can be classified as extractable and non-extractable, as explained below:

*Non-extractable polyphenols:* these are compounds with high molecular weight, or polyphenols linked to dietary fiber or proteins that can be found in extraction wastes. Include hydrolysable tannins and condensed tannins with a high number of units in the polymer chain. The hydrolysable tannins are polymeric structures that can derive from gallic acid or its condensation dimer product hexahydroxydiphenic acid. The condensed tannins or proanthocyanidins, on the other hand, are polymeric structures, formed by the union of flavan-3-oles, and can be procyanidins, prodelfinidins, and propelargonidins [13].

*Extractable polyphenols:* these are low or medium molecular weight compounds that can be extracted with aqueous or aqueous-organic solvents. They are classified according to their chemical structure in: flavonoids, which are much more complex structures, which in turn, are subdivided into flavones (chrysin and rutin); flavonols (quercetin and myricetin); flavanols or catechins (epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate); flavanones (hesperidin and naringenin), anthocyanins (delphinidin, malvidin, and cyanidin) and condensed tannins with a low number of monomers, etc. [13]. On the other hand, non-flavonoid compounds are formed by an aromatic ring substituted by an alcohol in one or more positions and are classified according to the number of carbons they have, within this group can be found: simple phenols, benzoic acids, hydrolysable tannins, acetophenones and phenylacetic acids, cinnamic acids, coumarins, benzophenones, xanthenes, stilbenes, chalcones, lignans, and secoiridoids [14].

Showing up next a description of some of the phenolic compounds present in plant species, including some of their properties and possible applications.

*Simple phenolic:* these are compounds to which antioxidant, antibiotic, antiparasitic, and cytotoxic properties have been attributed. In general, foods such as cereals are found as resorcinol derivatives [15].

*Aromatic carboxylic acids and their derivatives:* they occur naturally in many plant species [16] and have been linked to immunostimulant, choleric, anti-inflammatory, analgesic, antipyretic, and protective properties of the cardiovascular system [11]. The antioxidant properties of phenolic acids have been associated to their capacity to purify free radicals avoiding lipids peroxidation, inhibiting LDL plasma oxidation, and purifying reactive oxygen, which plays a very important role in the promotion of tumors, carcinogenesis. They also act preventively on diseases of the coronary arteries, by decreasing platelet aggregation [17]. Among phenolic acids, antioxidant properties have been attributed as follows: caffeic acid, chlorogenic acid and its isomers, such as 4-O-caffeyl-quinic [18], being the chlorogenic acid the most abundant in plants and the most active antioxidant in this group. It is also known that benzoic acid plays important roles in plant metabolism by regulating their growth and chemically modifying the rhizosphere forming part of the radical exudates; it also increases the capacity to capture minerals when it accumulates in soil in a great concentration [19, 20]. This acid is also a mediator of stress responses, as it is incorporated into numerous secondary metabolites associated with plant-herbivore or plant-pathogen interactions [21].

*Acetophenones and phenylacetic acids:* they are used as sunscreens in sun creams and o-hydroxybenzophenones are used to protect fibers from photodegradation in the textile industry [11].

*Phenylpropanoids:* these are products that have a specific role in the response to pathogens or protection from ultraviolet rays, through their antioxidant capacity and energy dissipation, as well as their function as structural components of the cell wall [22]. Due to the phytotoxicity of these compounds, they are stored in their glycosylated form in the vacuoles or conjugated with other components of the cell wall [23]. The cinnamic acid derivatives are abundant in nature in free form (coumaric acid and caffeic acid) or esterified with sugars (caffeyl-tartaric acid), quinic acid (chlorogenic acid), etc. While the benzoic acid derivatives are in free form, such as acids (vanillic acid and gallic acid) or aldehydes (vanillin and anisaldehyde).

*Coumarins and related:* in plants, these compounds can be present in seed cover, fruits, flowers, roots, leaves and stems, but in general, the highest concentrations are found in fruits and flowers. Its function is related to plant defense, given its antimicrobial, antifeedant, protective properties of UV radiation and inhibiting germination. The pharmaceutical interest for coumarins is due to their vitamin properties, their ability to decrease capillary permeability and an increasing in the strength of capillary walls. They present a wide range of physiological effects in animals, ranging from the less complex element of this group known as simple coumarin, which is toxic to mammals, to the last in the scale that is noboviocin, recognized as a commercial antibiotic [17].

*Flavonoids and derivatives:* these compounds are widely distributed in plants, especially in leaves, flowers and pollen, as well as in woody parts, such as peduncles and barks. It is known that plants that grow in full sun exposition have more flavonoids than those that grow in the shade, which makes interpreting their presence as a mechanism of plant defense to oxidations promoted by UV light. Among its plant functions, antifungal and bactericidal role is highlighted. Flavonoids have an enormous scientific interest due to the protection they exert against chronic diseases such as cancer and cardiovascular diseases [24, 25]. Flavonoids contain a variable number of phenolic hydroxyl groups in their chemical structure, have excellent chelation properties



of iron and other transition metals, which gives them a high antioxidant capacity and therefore, play an essential role in protection against the phenomena of oxidative damage [26, 27].

The growing interest in flavonoids as antioxidants is due to the appreciation of their broad pharmacological activity, since they can bind to biological polymers, such as enzymes, hormone transporters and DNA; chelate transient metal ions, such as  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , catalyze the transport of electrons and purify free radicals. So, these type of compounds have an interest from a nutritional point of view, since the obtaining and food preparation with a high content of these, supposes a decreasing in the use of antioxidant additives, at the same time as obtaining healthier foods, which can even be considered functional foods [28].

The activity of flavonoids as antioxidants depends on the redox properties of their hydroxyphenolic groups and the structural relationship. For example, it has been determined that chemical criteria that are related to antioxidant capacity of the flavonoids are as follows: (a) the presence of O-dihydroxy structure in the B ring; that gives greater stability to the radical form and participates in electrons delocalization, (b) the double bond in conjunction with 4-oxo function of the C ring and (c) the hydroxyl groups in the three and five positions. Given these concerns, quercetin flavonol is the one that exerts an effective antioxidant function, with a Trolox value of 4.7 mM, which is five times greater than that shown by vitamins E and C and has a water-solubility similar to vitamin E. In addition, the antioxidant function of quercetin shows synergistic effects with vitamin C, since ascorbic acid reduces the quercetin oxidation, in such a way that combined with flavonoid can maintain its antioxidant functions for longer [29]. Another flavonol is kaempferol, a bleaching inhibitor of illuminated chloroplasts, due to the inhibition of the reactions promoted by the triplet of oxygen inside the chloroplast and the protection against thermal autooxidation of palm oils, corn, sunflower, soy, olive, peanut, coconut, butter and margarine [30]. In that sense, polyhydroxylated chalcones, such as butein, have shown considerable antioxidant activity for shortening, being twice as active as quercetin or  $\alpha$ -tocopherol. Chalcones with two adjacent hydroxyl groups are almost as effective as butein, however, the presence of additional hydroxyl only slightly increases the inhibitory activity of rancidity, while hydrogenation of the chalcone double bond increases the antioxidant power, is found that pentahydroxydihydrochalcone is 2–3 times more active than the corresponding unsaturated chalcone [31].

Other examples of this type of metabolites are the catechin, epicatechin, galocatechin, compounds that act as OH' radical sequestration generated in a Fenton system ranged from 100 to 300 times higher than the effects of mannitol, a typical sequestration of the most toxic from all reactive oxygen-ERO species *in vivo* generated [32]. In this, there is a positive correlation between phenolic compounds and antioxidant capacity, in this case, the antioxidant capacity depends on secondary metabolites, especially those of phenolic nature such as flavonoids [33].

*Tannins*: antioxidant activity study of condensed tannins *in vitro* and *in vivo* shows that they are effective scavengers of free radicals and they inhibit the tissues oxidation better than vitamin C, vitamin E and  $\beta$ -carotene. *In vitro* conditions has been shown that condensed tannins have a preference for neutralizing the hydroxyl free radical ( $\cdot\text{OH}$ ), as well as it has been demonstrated that they have the capacity to act as noncompetitive inhibitors of the enzyme xanthine oxidase, one of the biggest generators of free radicals in cellular metabolism [34]. The biological properties of tannins are linked to their capacity to form complexes with macromolecules,

particularly with proteins (digestive proteins, fungal or viral enzymes). This explains the problems that its presence can cause in industrial processes associated with food and beverages (cloudiness of beer) production or in agriculture (formation of humic acid, which decreases soybean nutritional value) [15]. Due to tannins affinity with proteins, they can be used as follows: antidiarrheals, in products for skin protection and mucous membranes, vasoconstrictors, skin regenerators, antiseptics, antibacterials and antifungals. Additionally, they act as scavengers of free radicals and inhibitors of superoxides formation, enzymatic inhibitors of 5-lipoxygenase, angiotensin-converting enzyme (ACE), elastase and protein kinase C.

*Xanthonnes, benzophenones and stilbenes:* xanthonnes and benzophenones are present in roots and exotic fruits, while stilbenes have been found in different types of fruits and foods. Its major representative is resveratrol (3,5,4'- trihydroxystilbene), which occurs in grapes, red fruits and peanuts. This compound has an anticancer effect and inhibits reactions that increase the risk of coronary heart disease [35]. Additionally, it has been found to be useful in treatment of various chronic diseases, such as inflammation, arthritis, cardiovascular diseases and delaying aging, while xanthonnes have shown antiprotozoal activity including activity against *Leishmania* [15].

*Lignans and lignins:* these are the most abundant organic substances in plants, after cellulose. These are part of the lignin of the cell wall and participate in plant growth. They also belong to the group of phytoestrogens acting as antioxidants, decreasing the effects of free radicals. There are simple lignans and cyclic lignans that fight against the damaging effects of free radicals and whose compounds like enterodiol and enterolactone have anticancer potential that mimics the functions of human hormones, in addition to inhibiting the growth of breast and prostate tumors [36].

*Phenolic diterpenes:* this type of compounds are widely known to be potent inhibitors of the linoleic acid oxidation. Its mechanism of action is related to metals chelation by the central beta-diketone group [37].

It can also be included in this review, ubiquinol, which is a product of the ubiquinone (vitamin Q) reduction, a potent antioxidant *in vivo* conditions of low oxygen concentration, such as those found in many cellular environments, also inhibits the peroxidation of arachidonic acid in emulsion, which have hemoglobin as an initiator and prevents lipids photooxidation in mitochondria. The observed reactivity of ubiquinol with the free radical diphenyl-picrylhydrazyl suggests that is an antioxidant that breaks the chain reaction and probably reacts *in vivo* with peroxy radicals [38].

In **Table 1** some of these compounds isolated from different plant matrices are presented [39–41].

## 2.1. Extraction and quantification of phenolic compounds

Among the techniques used in phenolic compounds extraction are as follows: maceration, digestion, decoction, infusion, percolation, soxhlet, countercurrent extraction, ultrasound-assisted extraction, microwave extraction, extraction with supercritical fluids, extraction with hydrofluorocarbon solvents, micro-extraction in solid phase, partition, chromatography, hydrolytic maceration followed by distillation, hydro-distillation, micro-distillation,

Group	Number of carbons	Plant matrix	Compound or type
Simple phenolic	C6	Banana	Transcinnamic acid
Benzoic acid	C6–C1	Strawberry	<i>p</i> -Hydroxybenzoic
Acetophenones and phenylacetic acids	C6–C2	Blueberry	
Phenylpropanoids	C6–C3	Grapes	
Coumarins	C6–C3	Tangerine, orange, lemon	Caffeine acid, scopolin
Flavonoids	C6–C3–C6	Cherry, orange, grape, soybeans	Isoflavones, quercetin, cyanidin
		Tangerine, orange	Flavanones
		Parsley, celery, oreganum	Flavones
		Apple, pear	Flavonols
		Grapes	Flavanols
Benzophenones and stilbenes	C6–C1–C6	Grapes	Resveratrol
Xanthones	C6–C2–C6	Mangosteen, mango	Mangiferine
Lignans and lignins	(C6–C3) <sub>n</sub>	Passion fruit, lime, fruits with bone	Daidzein

**Table 1.** Classification of phenolic compounds.

molecular distillation, thermo-micro-distillation, among others. In these methods, the solvents used depend on the hydrophilic or lipophilic character of the compounds of interest, thus, for the lipophilic extracts preparation, hexane, diethyl ether and methylene chloride can be used, while for the extraction of hydrophilic compounds, methanol has been used, ethanol, mixtures of ethanol/water, acetone/water and methanol/water and extraction in acidic medium (pH = 2) with methanol/water, followed by acetone/water and water/acetonitrile in acidic medium [14].

Showing up next applications of some solvents according to the polarity:

- Water: extraction of anthocyanins, lectins (carbohydrates bound to proteins), polypeptides, saponins, starch, terpenoids and tannins.
- Methanol: extraction of antoncyanins, lactones, phenols, polyphenols, saponins, tannins, terpenoids and xanthoxillins.
- Ethanol: extraction of alkaloids, flavonols, polyphenols, polyacetylenes, terpenoids, steroids and tannins.
- Acetone: extraction of alkaloids, coumarins, fatty acids and terpenoids.
- Chloroform: extraction of flavonoids and terpenoids.
- Ether: to extract flavonols and phenols [42].

To minimize the generation of organic waste from extractions and reduce the environmental impact caused by the disposal of toxic solvents used in some extractions, in recent years, the development of sustainable extraction methods has been studied, such as those in which they use acidified water and ethanol as solvents, in addition to the developments in the area of extraction with sub-critical water [14].

## 2.2. Methodologies for the quantification of phenolic compounds

Depending on equipment availability and the needs of the measurement in terms of costs, precision and speed, quantification techniques can range from simple spectrophotometric analysis to complex chromatographic analysis [43]. Among the spectrophotometric techniques for quantification of phenolic compounds are the Folin-Ciocalteu method, which determines the ability of polyphenols to reduce Mo(VI) to Mo(V), by using the Folin-Ciocalteu reagent (mixed of phosphotungstic acid and phosphomolybdic acid). As a result of such reaction, a color change from yellow to blue is observed [3, 44]. The results of quantification of polyphenols obtained by this methodology, can be expressed in equivalents of gallic acid, catechin, colorgenic, caffeic, protocatechuic, vanillic or ferulic acid, making it difficult to establish comparisons among different determinations [13]. This methodology can be applied on a routine basis, due to the speed with determinations, which can be made at relatively low costs of these in comparison with other techniques [43]. However, it has been observed that the amount of phenols can be overestimated, since other compounds than phenols (sugars, organic acids, iron (II), nitrogenous compounds and other inorganic substances such as sulfates, phosphates and chlorides) can present interferences due to their reactions with reagent or at the wavelength used (close to 730 nm) in the determination [8, 13]. An example of the methodology is that proposed by García, for the quantification of phenolic compounds in plant extracts [8].

Another spectrophotometric technique is the one used for the quantification of total flavonoids where the ortho-dihydroxylated, meta-hydroxylated and para-hydroxylated flavonoids are chelated by reaction with  $AlCl_3$  [45]. However, since plant extracts may vary in their turbidity, is possible that in some samples there are alterations in the results [46]. For anthocyanins measurement, a colorimetric method based on the color change obtained as a result of pH variation can also be used. Taking into account that anthocyanins have a red, violet or purple coloring at acidic pH, while at basic pH, they are green or blue [47]. An example of its application was carried out by Gajula et al., for flavonoids and anthocyanins quantification in a plant extract (*Ocimum basilicum* L.) [48].

Apart from the spectrophotometric techniques are the chromatographic techniques for phenolic compounds quantification and identification. From these, the simplest technique is the column chromatography based on sample separation as it passes through a column packed with silica that allows compounds separation and their subsequent quantification through the use of a detector used at different wavelengths. Each type of compound is quantified by the preparation of a calibration curve and the results are expressed in terms of concentration or total amount of specific compounds in the analyzed extracts. One of the advantages of this technique when is used for separation and determination of phenolic compounds, is that does not require the previous sample derivatization [49]. Some examples of the application of this type of techniques are reported by Stefenona et al., Sánchez et al., and Martínez [14, 45, 50].

As a complement to chromatographic techniques and to improve the compounds identification found in the extracts, mass spectrometry is currently used, in the analysis of flavonoids extracted from plants [51]. This has allowed to know significant information about the structure of the present compounds even when sample quantities analyzed are very small or correspond to mixtures. With respect to flavonoids characterization applying mass spectrometry, the information obtained can be as follows: (1) the remains of aglycones, (2) types of carbohydrates (mono, di, tri, or tetrasaccharides and hexoses or pentoses) present, (3) the stereochemical assignment of terminal carbohydrate units, (4) the sequence of the glycan part, (5) the inter-glycosidic linkages and (6) points where the substituents bind to the aglycone [52]. Given that there are cases in which mass spectrometry and analysis through high performance liquid chromatography (HPLC) in the UV-Vis spectrum are not sufficient to identify the compounds present in a sample, it is necessary to use gas chromatography (GC) coupled to mass spectrometry to achieve more accurate results. However, in the case of gas chromatography for the analysis of non-volatile and thermolabile compounds it is necessary to previously carry out their conversion into volatile and thermotolerant chemical derivatives. In this sense, silylation of these compounds has been proposed, prior to GC quantification [49].

### 2.3. Methodologies for the quantification of antioxidant activity

The measurements of the antioxidant activity can be carried out based on the information you want to obtain:

- **Direct determination:** a radical is used as a quantification factor (since it produces an analytical signal). In this sense, the addition of the antioxidant, before or after the generation of the radical, causes a decreasing in the signal (ABTS<sup>•+</sup> or DPPH methods), which is proportional to the antioxidant activity of the sample.
- **Indirect determination:** the presence of free radicals causes the loss or appearance of a reagent and therefore, in the presence of an antioxidant, an increasing or decreasing in the signal is caused (ORAC and FRAP methods) proportional to the antioxidant activity of the sample.

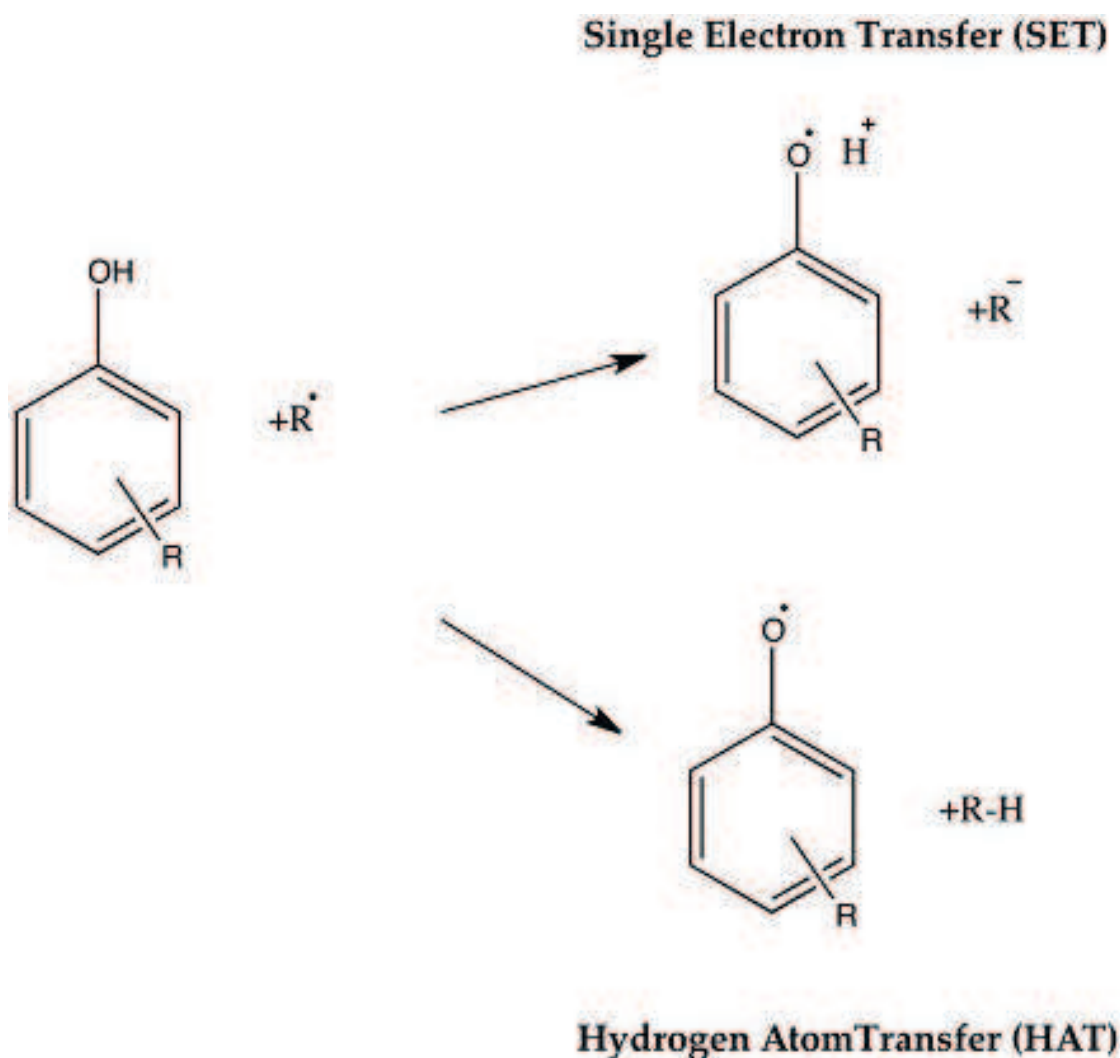
In that way, it is necessary to mention the differences between the free radical stabilizing activity or antiradicalaria (indirect methods) and the antioxidant activity (direct methods), the first being completely determined by the reactivity of an antioxidant against free radicals, characterized by reaction speed, while the second measures the ability to retard oxidative processes [53]. In this sense, the results of the antioxidant capacity measurement obtained by each of the methods do not always coincide, even among methods based on the same redox mechanism, there may be variations. Therefore, it is recommended that an assessment of the antioxidant capacity be carried out using more than one analytical technique and comparisons among results only be made when the same method has been used and samples have been obtained with the same solvents [8, 13].

In general, it has been suggested to combine FRAP and ABTS techniques [54]. This is because the use of the FRAP technique in combination with others such as ABTS and DPPH, allows

to evaluate different interactions of the antioxidant compounds, expanding the knowledge about them, which is relevant in the exploration of the antioxidant properties of nutraceutical products from natural sources or simply from some products included in the diet, such as fruits and vegetables [55]. When two techniques are used, as mentioned above, is generally sought that through one of them, is possible to determine the antioxidant activity based on transfer reactions of one electron (SET) and on the other, this same property is determined based on a transfer reaction of a hydrogen atom (HAT for its acronym in English) between an antioxidant and a free radical, allowing to evaluate the two mechanisms to extend the spectrum of the results obtained [8]. In **Figure 1**, the HAT and SET mechanisms are showed.

**Table 2** summarizes the principal methods to quantify the antioxidant activity [13].

Regarding the expression of results of antioxidant capacity, several methods (FRAP, ABTS and ORAC) express the results in  $\mu\text{mol Trolox/g}$  of sample on dry or wet basis (Trolox is a water-soluble analog of vitamin E). Likewise, these results can be expressed in terms of vitamin C and E. In summary, a suitable method for the quantification of antioxidant activity



**Figure 1.** Mechanisms of antioxidant reacting with free radical: single electron transfer (SET) and hydrogen atom transfer (HAT).

Principle	Method
Metal reduction	Ferric ion-reducing antioxidant power (FRAP)
Peroxy radical absorption capacity	Oxygen radical absorbance capacity (ORAC) Total radical-trapping antioxidant parameter (TRAP)
Hydroxyl radical absorption capacity	Deoxyribose assay
Capacity for radicals absorption generated from certain organic molecules	2,2'-azinobis acid (3-ethylbenzothiazolin)-6-sulfonic (ABTS), radical 2,2 diphenyl-1-picrilhydrazil (DPPH)
Quantification of products generated during the lipid peroxidation	Tiobarbituric acid reactive species (TBARs), oxidation of LDLs

**Table 2.** Principles of the most common methods used to quantify *in vitro* antioxidant activity.

should consider the electron transfer and hydrogen atoms reaction, establish the oxidation substrate, ensure that the substrate and how to induce oxidation, became relevant in terms of oxidative damage, be simple, have a mechanism and a defined endpoint, use available and affordable instrumentation, be reproducible, be adaptable to measure hydrophilic and lipophilic antioxidants, use different sources of free radicals with relevant biological characteristics and be adaptable for routine large-scale analyzes [13].

This is increasingly important, since is known that no single method reflects the total antioxidant capacity of a sample, that is, its ability to act as an antioxidant of lipophilic and hydrophilic compounds through specific mechanisms, in addition to its reactivity against different species [56]. In addition, is known that the antioxidant activity of a sample is not only given by the sum of the antioxidant capacities of the components present in it, but also depends on the synergistic and inhibitory effects that may exist among compounds and the microenvironment in which this one is [57].

### 2.3.1. Principal methods for *in vitro* antioxidant activity quantification

In **Table 3**, the advantage and disadvantages of *in vitro* antioxidant activity determination methods will be presented.

### 2.3.2. Principal methods for *in vivo* antioxidant activity quantification

There are different techniques to perform these measurements among which are as follows [68].

*Techniques based on lipid oxidation:*

- Malondialdehyde (MDA): a breakdown of lipid hydroperoxides generated from polyunsaturated fatty acids generates different aldehydes, such as MDA, which is also produced in food and can be absorbed in the gastrointestinal tract.
- Exhaled alkanes: in this technique the exhalation of volatile hydrocarbons, mainly ethane and pentane, derived from the oxidation of polyunsaturated fatty acids, is measured. The principal advantage of this method is that is a non-invasive procedure.

Method	Advantages	Disadvantages	Refs.
ABTS <i>(2,2'-Azinobis acid            (3-ethylbenzothiazolin)-6-sulfonic)</i>	<p>This method has a high sensitivity, is practical and fast. Is used to evaluate the antioxidant potential in plants, food and drink. Additionally, is a stable and soluble radical both in aqueous and organic media, allowing the evaluation of hydrophilic and lipophilic antioxidants; with the advantage that its spectrum has maximum absorbance at 414, 654, 754 and 815 nm in alcoholic medium, which represents a greater availability of wavelengths to carry out determinations, avoiding color interference.</p> <p>Correlations have been observed among ABTS values and the polyphenol content determined by the Folin-Ciocalteu method, especially when measurements are carried out at the beginning of the reaction (after 2 min) than when data came from measurements at longer times (15 min), which could be because in the first part of the reaction are the polyphenols that react, while in the second part are other metabolites derived.</p>	<p>In this case, the radical must be previously generated and reaction kinetics with some antioxidants can be slow, causing the determination of the endpoint to be carried out arbitrarily. This has led to differences in the literature regarding the time taken to carry out the determinations (between 1 and 7 min) affecting the antioxidant activity values, since the TEAC determination is dependent on the incubation time, as well as the relationship between the amount of sample and ABTS• concentration.</p> <p>It has also been found that the type of solvent used in antioxidants extraction from the sample influences the results obtained. In addition, as this technique is based on mechanisms of hydrogen atom transfer, the reactivity patterns and the reaction mechanism are difficult to interpret when the chemical structure of the evaluated antioxidant is unknown.</p>	[3, 13, 53, 55, 57–59]
FRAP <i>(Ferric ion-reducing antioxidant power)</i>	<p>Method based on the power that an antioxidant substance has to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>. In this sense, 2,4,6-tripyridyl-s-triazine (TPTZ) colorless ferric compound used in the test is reduced to a colored ferrous complex. Among its advantages is that the redox potential of Fe<sup>3+</sup>-TPTZ is comparable with that of the ABTS allowing to analyze similar compounds with both methods, although the reaction conditions are different. This technique, having as a base mechanism the metal reduction used as chain reaction propagator of lipid peroxidation through breakdown of hydroperoxides to alkoxyl radicals, allows its use to correlate the antioxidant activity with the ability of the compounds to act as prooxidants, in addition to its ferric ion-reducing capacity.</p>	<p>These types of techniques are quite sensitive to ascorbic acid and uric acid, compounds that could reduce the ferric ion to ferrous ion and react with the latter to generate new free radicals. Also, being based on an electron transfer mechanism, this technique does not allow detecting compounds that act by the hydrogen transfer mechanism. In addition, any compound with a redox potential lower than 0.7 V could reduce Fe(III), overestimating the value obtained for the activity.</p>	[3, 13, 53, 55, 58, 60]



Method	Advantages	Disadvantages	Refs.
DPPH (Radical 2,2 diphenyl-1-picrylhydrazil)	<p>In this method, DPPH* reacts with the sample in such a way that the determination is carried out indirectly by monitoring the decreasing in absorbance at 515 nm. A modification of this method, introduce kinetic parameters like EC<sub>50</sub> (effective concentration of the antioxidant necessary to reduce by 50% the initial amount of radical), tEC<sub>50</sub> (time necessary to reduce by 50% the initial amount of radical with the given antioxidant concentration) and the antiradical efficiency (AE) calculated based on the aforementioned parameters, which also have allowed to establish relationships between the concentration of phenolic compounds and their antioxidant activity. These kinetic parameters are determined as follows:</p> $AE = 1/(EC_{50} * t_{EC50})$ <p>At higher AE, better the performance of the antioxidant even at low concentrations and in short times. Among the advantages of this method are the stability of the organic radical, which does not have to be generated <i>in situ</i> and its simplicity by not requiring sophisticated equipment.</p>	<p>It is known that many substances have an absorption spectrum that overlaps with the only maximum absorption that the radical presents interfering with the measurement. Additionally, its application is restricted to the fact that only allows measuring the activity of lipophilic compound</p>	[13, 53, 55, 57, 58, 60]
ORAC (Oxygen radical absorbance capacity)	<p>It is based on the ability of antioxidant compounds to block free radicals by hydrogen atom donation. In this method, the AAPH (2,2'-Azobis-(2-aminopropane)-dihydrochloride) artificial radical, oxidizes fluorescein in such way that fluorescein loses its fluorescence. Among its advantages is that it is easy to follow when using a colored or fluorescent probe. In addition, the use of a protein as a substrate prevents the substrate itself from generation of free radicals due to its oxidation. Likewise, the method is used to determine the antioxidant capacity of aqueous and hydrophobic samples, varying the source of radicals and the solvent. On the other hand, based on a mechanism of hydrogen atoms transfer, in this method, the reactions are of approximately 30 min to guarantee the reaction stabilization, independent of pH and solvent.</p>	<p>It is found that the fluorescein solution must be prepared daily, the reaction kinetics may vary depending on the antioxidant concentration, the temperature and the presence of metals and reducing agents. In addition, the results may be affected by the solvent used in the extraction and by non-antioxidant compounds present in plant foods, such as some amino acids and uronic acids, which become interferences, providing overestimated results.</p>	[3, 13, 53, 58, 61]

Method	Advantages	Disadvantages	Refs.
CBA (Method of crocin decolorization or $\beta$ -carotene)	This method is based on crocin or $\beta$ -carotene oxidation, natural derivatives of the carotenoids, by peroxy radicals generated from AAP. For quantification, the crocin discoloration rate is measured in the presence and absence of antioxidants at 434 nm. In this sense, carotenoids can be decolorized by three main routes as follows: autoxidation, oxidation induced by heat or light and oxidation induced by peroxy radicals (generated by AAPH generator or by lipids oxidation). This bleaching can be prevented or diminished by the addition of some antioxidant compounds capable of donating hydrogen atoms to neutralize free radicals. Among the advantages of this method are its simplicity and speed, in addition to the fact that it does not require specialized equipment for the determination.	The wavelength at which is measured coincides with food pigments (carotenoids), which can lead into a variability in the measurements. In addition, the reaction mechanisms of different antioxidants with crocin may vary, affecting the interpretation of the results. Another disadvantage is the low crocin availability (mixture of natural pigments extracted from saffron) that induces variability in the measurements.	[13, 62]
TBARS (Tiobarbituric acid reactive species)	In this method, the absorbance at 532–535 nm of a chromogenic complex formed between tiobarbituric acid and malondialdehyde (MDA) is measured, which is a by-product of the lipid oxidation of polyunsaturated fatty acids of at least 3 double bonds. It is considered a rapid, sensitive and economic measurement technique.	It is lack of specificity, since tiobarbituric acid reacts with a variety of aldehydes in addition to those formed during lipid peroxidation, in such way, the analysis in biological fluids is limiting. Another disadvantage is that malondialdehyde and other short chain products are not stable for long periods of time.	[13, 53, 60]
TRAP (Total radical-trapping antioxidant parameter)	It is one of the most widely used technique to determine antioxidant activity in fluids. As an advantage, this methodology allows to determine the non-enzymatic antioxidant capacity of the tissue through a hydrogen atom transfer mechanism. In this assay, a hydrophilic radical generator and a substance that detects these radicals, such as phycoerythrin, are used. In this way, the oxidation is initially inhibited during a latency period by the antioxidant and what is done is to compare the duration of this period for the sample against Trolox.	Different endpoints for the reaction have been proposed in its application, preventing the results comparison. In addition, not all antioxidants have a fully established inhibition phase, making this method inaccurate when comparing compounds that exhibit different inhibition behaviors in fluorescence in a given oxidant system.	[63]
DMPD (N, N'-dimetil-p-phenylenediamine)	In DMPD method the free radical generated in the presence of an oxidizing solution of ferric chloride and acidic pH, becomes a colored and stable cationic radical, which has a maximum of absorbance at 505 nm. The experimental procedure is rapid, economical, sensitive and reproducible in the quantification of the antioxidant activity of hydrophilic compounds and in some cases lipophilic.	The technique is performed at a pH that is not physiological and reaction time that is required to perform the measurement (ranged from 18 to 21 h).	[13, 55]

Method	Advantages	Disadvantages	Refs.
TOSC (Total oxyradical scavenging capacity)	It is based on the oxidation of alpha-keto-gamma-methylbutyric acid to ethylene by the action of hydroxyl, peroxy and peroxy nitrite radicals generated from 2,2-azobis amidinopropane (ABAP). The TOSC value is found by results comparison obtained for the sample with those of a pattern. As advantages of this methodology application is its availability for the total capacity measurement of oxiradical elimination in biological tissues and the possibility of used it to discriminate different oxyradicals indicating the functions of these species or their metabolic pathways.	In reaction kinetics, there is no dose-response relationship between the amount of antioxidant and the percentage of inhibition, preventing comparisons among results.	[13]
CUPRAC (Cupric ion-reducing antioxidant capacity)	It is a method to assess the concentration and antioxidant capacity in biological samples, in samples from the food industry and in cosmetics. In addition, this method measures the total antioxidant capacity of a sample. This design is based on Cu(II) to Cu(I) reduction by the combined action of all antioxidants (reducing agents) in a sample. The CUPRAC assay uses a related compound neocuproin (2,9-dimethyl-1,10-phenanthroline), the Cu(I) complex which absorbs at 450 nm.	Slowly reacting antioxidants required an incubation at 50 °C for 20 min for color development. Certain compounds also needed incubation after acid hydrolysis for color development.	[64]
Oxidation method of LDLs (Low density lipoproteins)	In this method, the oxidation of LDLs isolated from different individuals is induced by different elements and compounds such as Cu <sup>2+</sup> or AAPH and subsequently, the absorbance at 234 nm is measured, which is absorbed by the conjugated dienes generated during the oxidation process of LDLs.	In some cases the duration of the determination is too long.	[13, 53]
Deoxyribose assay	This method measures the ability of an antioxidant or mixture of antioxidants to capture the hydroxyl radical. Is based on reaction of 2-deoxyribose (DR) when is oxidized in the presence of hydroxyl radical generated by the Fenton reagent in such way that malondialdehyde (MDA) is produced. MDA is mixed with 2-thiobarbituric acid (TBA) in acidic medium which allows the development of a pink color chromogen that can be measured at an absorbance of 532 nm. Higher absorbance values would indicate higher levels of OH <sup>•</sup> radicals [13, 65]. As an advantage, this assay has been widely used to determine the antioxidant activity of foods and medicines.	There may be a strong alteration of the result when working with ethanolic extracts due to the interference caused by alcohol on the measurement.	[65, 66]

Method	Advantages	Disadvantages	Refs.
XO (Xanthine oxidase assay)	It is an enzyme dehydrogenase that catalyzes the oxidation of hypoxanthine or xanthine to uric acid, by transferring an electron to the nicotinamide-adenine-dinucleotide (NAD). During the XO reoxidation, molecular oxygen acts as an electron acceptor producing radical superoxide and hydrogen peroxide.	Modifications to this method have also been developed in which the reaction is not monitored through determining changes in absorbance at 295 nm, but through HPLC or where what is determined is the evolution in uric acid formation and it can increase the cost and complexity of the method.	[13, 67]

**Table 3.** Principal methods for in vitro antioxidant activity quantification.

- *Ex vitro* oxidation of LDLs: this method consists of subjecting the LDLs isolated from a particular subject to the same oxidation process, in order to observe their basal level of antioxidants or the effects of an antioxidant supplementation.

*Techniques based on protein oxidation:*

Within these techniques, three biomarkers have been used: 2-amino adipic semialdehyde (AAS), gamma-glutamic semialdehyde (2-GGS) product of the lysine and proline oxidation, respectively (supposed as the major products of protein oxidation), and nitrosine, oxidized derivative of proteins that can be generated by action of peroxy nitrite or peroxidase.

Matrix	Folin ciocalteu	DPPH	ABTS	FRAP	DMPD	ORAC	VCEAC	CUPRAC	HPLC	Refs.
Pineapple, soursop, sweetsop, Artocarpus jackfruit, murici, papaya, mangaba, sapodilla, ciruela, umbu and tamarind	x	x	x				x			[58]
Broccoli, kale, cabbage and carrot.	x	x								[69]
Carrot, green pepper and lettuce.	x	x								[70]
<i>Ulva</i> species ( <i>Ulva clathrata</i> (Roth), <i>Ulva linza</i> Linnaeus, <i>Ulva flexuosa</i> Wulfen and <i>Ulva intestinalis</i> Linnaeus)	x	x								[71]
Kale, carrot, cabbage and barcoli	x	x								[72]
Edible fungi, <i>Marasmius oreades</i> , <i>Lactarius deliciosus</i> and <i>Macrolepiota procera</i>	x	x						x		[64]

Matrix	Folin ciocalteu	DPPH	ABTS	FRAP	DMPD	ORAC	VCEAC	CUPRAC	HPLC	Refs.
Marine algae, <i>Halimeda opuntia</i> and <i>halimeda</i> <i>monile</i>	x	x							x	[73]
Medicinal plants	x		x							[74]
Honey	x		x							[75]
<i>Rosa damascena</i> Mill.	x	x								[76]
Fruits such as: acai, banana, star fruit, purple prune, curuba, peach, strawberry, passion fruit, guava, apple guava, kiwi, lulo, tangerine, tommy mango, red apple and Colombian vegetables such as: garlic, white bulb onion, red bulb onion, cauliflower, spinach, capira potato, etc.	x					x				[77]
Fruits such as: blackberry, passion fruit, anana, granadilla, guava and feijoa	x	x				x			x	[78]
Guava		x	x							[79]
Lulo		x	x							[80]
Opuntia fruit				x						[81]
Gulupa				x	x				x	[82]
<i>Campomanesia</i> <i>lineatifolia</i> Ruiz & Pav.	x	x		x					x	[83, 84]

**Table 4.** Analytical methods for the quantification of antioxidant capacity and total polyphenols.

### 3. Quantification of antioxidant activity and phenolic compounds in plant extracts

In **Table 4**, a compilation of information associated with the analytical methods and the conditions used for the quantification of antioxidant capacity and total polyphenols in plant extracts, is presented.

## 4. Application of plant extracts with antioxidant activity in food processing

In **Table 5**, a list of some applications of plant extracts in food processing will be presented, highlighting its use in conservation processes improvement and shelf life extension.

Other applications can be found in the works developed in food matrices that include meats, oils, fruits, vegetables and cereal products [106].

Antioxidant	Application	Concentration	Results	Refs.
Achiote	Biodegradable container for palm oil	Proportion of the natural additive (0.25, 0.5 and 1%, respectively)	During the storage period (45 days), a decreasing in phenolic content (17.8–36.2%) was observed in the additive used to make the packaging and a decreasing in the peroxide value of the three evaluated formulations in comparison with controls. The protective effect of natural additive was established, since the one that underwent the oxidation processes was the additive and not the palm oil. The results were directly proportional to the used concentration. The incorporation of this natural additive did not change the mechanical and barrier properties of the containers.	[85]
Yerba Mate	Biodegradable container for palm oil	Proportion of the natural additive 20%	During the storage period (45 days) under accelerated oxidation conditions (63% UR/30°C), the total polyphenol losses in the films (40% total loss) correlated with a lower increase in the peroxide value of the packaged product, demonstrating, in such way, instead of the product, the packaging compounds were those that underwent the oxidation processes. The yerba mate extract did not alter the mechanical and barrier properties of the films.	[86]
Prune	Conservation of meat products		Prune extract showed antioxidant properties in products such as irradiated turkey, precooked pork sausage and roasted meat. However, in slices of ham the results were not adequate due to an increasing in cooking loss, values of cutting force and redness. The sensory quality of products derived from meat and poultry treated with prune products presented minimal differences with respect to untreated products.	[87]
Grape	Meat conservation	0.05 and 0.1%	The extract of grape seed has been shown to have an antioxidant potential 20 and 50 times higher than vitamin E and vitamin C, respectively. Numerous studies concluded that is an effective antioxidant for the preservation of raw and cooked pork. Likewise, it has been determined that the use of ActiVin (extract of grape seed) at 1.0% in minced meat, inhibits TBARS values by 92%. Likewise, it was established that low concentrations of grape seed extract, $\leq 0.2\%$ , do not present adverse effects on sensory characteristics such as color, odor and taste, while concentrations higher than 1% affected the color of the finished products.	[88]

Antioxidant	Application	Concentration	Results	Refs.
Bearberry and grape seeds	Additive in raw and cooked pork meat patties	(80–1000 $\mu\text{g/g}$ of meat).	The antioxidant activity of bearberry have allowed a significant decreasing in lipid oxidation compared to control under refrigeration conditions, the effect being greater in raw meats than in cooked ones. Likewise, it has been reported that bearberry did not generate differences in color, taste, texture and juiciness.	[89]
Pomegranate	Additive in cooked chicken burgers	10 mg of phenolic equivalents of tannic acid/100 g in fresh chicken	By using pomegranate powder in hamburgers preparation and refrigerating them for a period of 15 days, a greater reduction of TBARS values was observed in comparison with BHT control (68%). Likewise, it was determined that pomegranate powder and pomegranate juice powder have little effect on sensory or quality attributes when used in concentrations of 5–20 mg equivalents of phenolic tannic acid/100 g of meat. Similar results have been obtained in raw goat meat, where the reduction was 67%.	[90]
Blueberry	Turkey conservation and cooked pork	Blueberry powder juice at 0.32%	It has also been reported that the use of blueberry powder juice at 0.32% in turkey meat and cooked pork meat, have allowed to inhibit the lipid oxidation almost 10 times compared to control. This is attributed to the fact that this fruit has a high concentration of phenolic compounds (158.8 $\mu\text{mol}$ of total phenols/g of dry weight) and especially of anthocyanins, which can inhibit the oxidation of lipids.	[91, 92]
Lotus flower	Conservation of Cantonese Chinese sausage	Extract of lotus seed epicarp 0.1 and 0.2%	The extract delayed the initial oxidation of lipids and the effect was dose dependent, likewise it was determined that inhibited the generation of molecules responsible for unwanted odors. The extract did not have significant inhibitory effects on the enzymatic hydrolysis of lipids.	[93]
Potato peel and sugar beet pulp	Oxidation control of sunflower oil and soybean oil	5, 10, 50, 100 and 200 ppm	The oil conservation tests were carried out under accelerated conditions (72 h at 70°C) evaluating the action of natural extracts and synthetic antioxidants on the change in the peroxide value of the oils. It was observed that the action associated with an increasing in the peroxide index varied as follows: TBHQ > potato peel extract > BHT = sugar beet pulp > BHA.	[94]
Grape	Osmo-dehydrated foods		The study confirmed the potential of osmotic treatments for food development that incorporate functional ingredients such as antioxidants in a successful manner. It was observed that molecular weight of the phenolic compounds limits their penetration during treatment.	[95]
Wastes from wine	Oxidation control of frozen raw chicken meat	60 mg of total phenolic compounds/ (PC)/kg of meat	The protective effect of the extract against lipid oxidation of frozen raw chicken meat stored at freezing conditions ( $-18^{\circ}\text{C}$ ) was demonstrated. There were some differences in the color of the cooked product and its aroma, however these results were not very different from those obtained using synthetic antioxidants.	[96]

Antioxidant	Application	Concentration	Results	Refs.
Grape extracts	Bread enrichment in antioxidant compounds	Addition of 300 mg, 600 mg and 1 g of extract in 500 g bread	A decreasing in the antioxidant activity of the extract added to the bread was observed due to the thermal process involved in its elaboration. However, the use of the extracts has allowed to enrich the bread in compounds with antioxidant activity. The results showed that the addition of appropriate amounts of the extract contributed to the development of favorable changes in the bread color without altering the sensory properties thereof.	[97]
Moringa.	Extension of the shelf life of cookies	Extract in cookie mass 0.5, 1, 2 and 3%, respectively	The extract was used to control the oxidation of fats and oils present in cookies in such way that their shelf life could be prolonged. The extracts showed better results in comparison with those obtained using BHA, this perhaps due to its greater stability during the production process of the product.	[98]
Rosemary and Oreganum	Substitution of sodium erythorbate by natural extracts in lamb burgers	Concentration equivalent to 500 ppm of sodium erythorbate	The substitution did not affect the sensory quality of the product and managed to reduce the changes caused by meat deterioration, converting these plant extracts in a healthy alternative for the formulation of meat derivatives.	[99]
Natural antioxidants exploited commercially	Oxidation control in meat and meat products	Varies depending on the product and application	The natural antioxidants used in the elaboration of meat products (pork, chicken, goat, and cow) manage to reduce lipids and proteins oxidation in different meat matrices.	[100]
Rosemary and clove	Raw chicken conservation stored in refrigeration	Concentrations: 1% rosemary extract, 1% clove extract and mix 0.5% from each extracts.	The chicken was stored for 15 days at 4°C. As a control, the synthetic antioxidant BHT was used. It was demonstrated that the extracts of the studied spices showed a high effectiveness against microbial growth and lipid oxidation, demonstrating their potential as natural antioxidants for raw chicken meat.	[101]
Rosemary	Control of thermoxidation of soybean oil	3000 mg of rosemary extract/kg, a positive control of 50 mg of TBHQ/kg and extract mixture with TBHQ, were evaluated.	The assay included the addition of the antioxidant to the oil and its heating at 180°C for 20 h. Time during which determinations of oxidative stability, total polar compounds, tocopherol content and fatty acid profile, were carried out. The addition of the extract increased the oxidative stability and resulted in a low formation of polar compounds and a high retention of tocopherols. In the treatment with rosemary extract, a high amount of polyunsaturated fatty acids was observed after 20 h. No synergies were observed between the rosemary extract and TBHQ in terms of preventing the oil oxidation.	[102]
<i>Betula pendula</i>	Oxidative stability of meat empanadas.	0.1 and 0.3% w/w.	A reduction of lipid oxidation was observed in the studied samples, which was followed by color changes and metmyoglobin concentration. The extract demonstrated ability to delay the lipids degradation present in the muscle of the meat.	[103]



Antioxidant	Application	Concentration	Results	Refs.
Mango shell extract	Stability of pork meatballs stored at 4°C.	0.05, 0.10, 0.15 and 0.20% (v/w)	During storage, the amount of phenolic compounds added to the product decreased slightly, while the lipid oxidation increased slightly. Mango shell extracts showed greater efficiency in the control of lipid oxidation against other extracts such as santol and rambutan. It was also determined that the treatments that included mango shell extract presented lower rancidity than control (without addition of antioxidants) after 10 days of storage. Mango extract applied at 0.2% (v/w) in pork meatballs showed the greatest effectiveness against lipid oxidation.	[104]
<i>Campomanesia lineatifolia</i>	Enzymatic browning control of potato	Potato immersion in a extract solution of 0.5, 1 and 1.5%, respectively	A decreasing in the speed change of the color parameter "L", corresponding to the brightness, had achieved by extract addition. The potatoes treated with the extract, presented a lower darkening than potatoes that were not treated during storage at 5°C and 55% of relative humidity.	[105]

**Table 5.** Applications of plant extracts with antioxidant activity in food processing.

## 5. Conclusions

The compiled information in this work demonstrates the need to continue developing techniques for the extraction of phenolic compounds with antioxidant activity from plant species in order to improve the obtained yields, have greater control over the extracts composition and their mechanism of action, to facilitate their implementation in food industry, where they can find great acceptance due to their natural character and their properties to lessen the impact of diseases attributed to the oxidation processes proper to human organism.

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# **Biological Activities of the Doum Palm (*Hyphaene thebaica* L.) Extract and Its Bioactive Components**

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## **Abstract**

The doum palm (*Hyphaene thebaica*) is a type palm tree which has a wood texture and has edible oval fruits and the origin native to upper Egypt. The trunk of this small palm is dichotomous. It is one of the most important useful plants in the world. All parts of doum palm have a useful role such as fiber and leaflets which used to weave baskets and doum nuts which have antioxidants and secondary metabolites such as tannins, phenols, saponin, steroids, glycosides, flavonoid, terpenes and terpinoids. Also, roots, stems and leaves are used in medicine, ropes and baskets. Studies on anti-inflammatory, antioxidant, antimicrobial, anticancer and pharmacological potential of *Hyphaene thebaica* extracts and its major phytoconstituents like the phenolic, essential oil and flavonoid compounds are extensively discussed in this review.

**Keywords:** doum, antioxidant, antimicrobial, anticancer, phenolic compounds

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## **1. Introduction**

*Hyphaene thebaica* is commonly referred to as doum, and it is a type of palm tree with edible oval fruit which belongs to the mint family (Arecaceae). They have several vernacular names like doum palm, doom palm, gingerbread palm, zembaba, mkoma, arkobkobai and kam-bash [1, 2]. The doum palm is native to the northern half of Africa. It grows in the west from Mauritania and Senegal, and east to Egypt, Kenya and Tanzania. It tends to grow along the Nile River in Egypt and Sudan in the areas which contain groundwater. It is also native to the Levant and the Arabian Peninsula (Israel, Sinai, Yemen and Saudi Arabia). It grows in wadis and at oases, but it is considered as drought-tolerant and sometimes grows on rocky hillsides.

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Also, it is very resistant to destruction by fire in scrub or a forest [3]. This chapter focuses on the biological activities and beneficial effects of the Doum palm extract and its bioactive components in humans.

## 2. Botanical description

The doum palm is a dioecious palm and grows up to 17 m (56 ft) high. The trunk, which can have a girth of up to 90 cm (35 in), the trunk divided into two branches, each branch divided again into two branches, and the ends of the branches contain tufts of large leaves. The bark is smooth, dark gray and contains the scars of fallen leaves. The petioles are about 1 m long, sheathing the branch at the base and contain curved claws. The leaves are fan-shaped and measure about 120 by 180 cm (47 by 71 in) (**Figure 1**). Male and female flowers are produced on separate trees. The inflorescences are similar in general appearance, up to about 1.2 m (3 ft. 11 in) long, irregular in the branching and have two or three spikes in each branch. Male flowers have a short-stalk, solitary in pits of the spadix, spathe-bracts encircling the spadix, pointed. Branches of female spadices become thicker in the fruiting stage. Woody fruits are produced in the female palm that continues on the tree for a long time. They are 6–10 × 6–8 cm, smooth, rectangular to cubical with rounded edges, shiny brown when ripe. Its fresh weight is about 120 g and dry weight is about 60 g and each one containing a single seed. The size of seeds about 2–3.5 × 3 cm, the color is ivory, truncate at the base and the apex is obtuse [4].



**Figure 1.** *Hyphaene thebaica* L. (Doum).

## 3. Traditional uses

*H. thebaica* tree is one of the most useful plants in the world [5]. Along the Nile, people used its fiber and leaflets to spin baskets. The fruits of doum palm are contained antioxidants [6]. Palms are used for firewood and charcoal. Leaves are probably the most important part of

the palm, providing the raw material used in basketry, making mats, brooms, coarse textiles, ropes, thatching and string [7]. Leaves may also be used as fuel. The fibers of roots obtained after soaking in water for 2–3 days, and flogging of the roots are used for making fishing nets. Due to the high amounts of fibers in wood, it is difficult to cut them using an ax. Wood produced from the male palm is considered better than that of the female. It is often used for building, providing for support and rafters for houses, railway sleepers, planks, water ducts and wheels fence posts and raft construction. Dried bark is used to produce a black dye for leather wear [8]. Roots are used in the treatment of bilharzia, while fruit pulp is helped in the reduction control hypertension [9]. The hard seed inside the fruit, known as (vegetable ivory) is used to treat sore eyes in livestock using charcoal from the seed kernel as well as making buttons and small carvings, and artificial pearls [1]. In Turkey and Kenya, the powder made from the outer covering of the fruit is added to water and milk and left to stand to make a mild alcoholic drink; in other countries, the terminal meristem is tapped for making palm wine. The thin dried brown rind is used in the manufacture of sweetmeats, cakes, and molasses. In Egypt, the fruit is sold in herbalist shops and is popular among children. Apart from the use of the fruit as food, juice is extracted from the young fruit and palm wine is prepared from the sap [10]. Doum palm fruit in its powder form was applied in some food products as a source of fiber, stabilizer and minerals as well as for its potential healthy effect [11]. Research on the fruit pulp of *H. thebaica* showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid [12].

Also, aqueous doum palm extracts increased the viability and activity of some certain dairy starter cultures which used in the manufacture of some dairy products especially probiotics [13].

#### 4. Chemical composition of doum fruit

Doum fruit has a high-quality protein varied between 2.86 and 5.01%, high proportion of lysine and cysteine of crude protein varied between 4.09–4.16% and 0.2–1.62%, respectively, the limited amino acid threonine, crude fat varied between 1.2 and 8.4%, crude fiber varied between 52.26 and 66.5%, the most important carbohydrates component was mannose varied between 13 and 75.9%, also the presence of calcium, magnesium, potassium, iron sodium and negligible amount of nickel, cobalt and molybdenum. Phytochemical compounds of doum fruit such as tannins, saponin, steroids, glycosides, flavonoid, terpenes and terpinoids were found at low and moderate concentrations [14].

#### 5. Pharmacological activities of doum

Various extracts of *H. thebaica* (L) Mart are used in the treatment of hypertension, bilharzias and as a hematinic agent [15]. The water extract of doum fruits can reduce hyperlipidemia in nephrotic syndrome and leads to decrease the risk of glomerulosclerosis and atherosclerosis and consequently the natural, safe and nontoxic *H. thebaica* fruit could be of great merit for use as hypolipidemic drugs [16]. It is also good as hypocholesterolemic agent, hypolipidemic

and hematinic suspensions lipidemic, and hematinic suspensions [17]. The identification of compounds by thin-layer chromatography showed that the doum fruit contains significant amounts of saponins, coumarins, hydroxyl cinnamates, essential oils and flavonoids [18]. It was found that the administration of flavonoid extracts to diabetic rats significantly increased adiponectin levels that stimulate the hypoglycemic action of insulin without altering the concentration of insulin in blood and decrease the weight and volume of contents of granuloma in inflammation [19]. Therefore, this might be its probable mechanism of anti-inflammatory action. Furthermore, the hypoglycemic effect of these herbs may be due to the increased level of serum insulin by increasing the pancreatic secretion of insulin from cells of islets of Langerhans or its release of bound insulin and also may be due to the enhancement of peripheral metabolism of glucose [20]. The decoction of doum fruits is well tolerated and no mortality or morbidity until the dose of 5 g/kg b. wt. Repeated oral administration of doum fruits at 0.5 g/kg b. wt. or 2 g/kg b. wt. was ineffective on the normal reproductive parameters. While the red blood corpuscles, packed cell volume, hemoglobin concentration and percent of phagocytic activity were significantly increased [21]. A significant decrease in blood glucose, cholesterol, triglycerides and total lipid levels was observed after 1 and 2 months of administration of the decoction of doum fruits [21]. The obtained results confirm the value of doum fruits as hematinic potentials, hypolipidemic, improve the hepato-renal functions and without side effects on the studied reproductive parameters.

Also, triglycerides were independently related to coronary heart disease and most of the anti-hypercholesterolemic drugs did not decrease triglycerides levels, but the aqueous extracts of doum fruits lower it significantly [22]. This effect may be related to the increase in endothelium bound lipoprotein lipase which hydrolyzes the triglycerides into fatty acids. Previously, the authors reported that the hypolipidemic properties of the aqueous pulp suspension of doum could be partly due to the presence of glycosides [23]. Saponins have been reported to form complexes with cholesterol and bile in the intestine thereby indirectly reducing the cholesterol level in the blood [24]. In addition, administration of 200, 400 and 800 mg/kg body weight of aqueous extracts of both stem and bark of *H. thebaica* (L) Mart showed no significant ( $p > 0.05$ ) difference in feed intake, this may be due to the absence of tannin in both the stem and bark extracts. Decrease in feed intake was observed at the highest dose of 800 mg/kg of the methanolic fruit pulp extract of the same plant, this may be due to tannin content of the methanolic fruit pulp extract of the plant [25].

## 6. Bioactive compounds of doum palm

### 6.1. The chemical structure of volatile components

Doum fruit yielded a yellowish color volatile oil with a fragrant aromatic odor at a yield of 0.5% (fresh wt). Physical constants measured include: specific gravity (0.168) and refractive index (1.383). The result of analysis of essential oil of doum by GC and GC-MS techniques revealed the presence of a total 57 compounds (**Figure 2**). Monoterpenes represented 15.97% including compounds such as sabinene (0.82%),  $\beta$ -pinene (1.97%), limonene (2.42%), terpinen

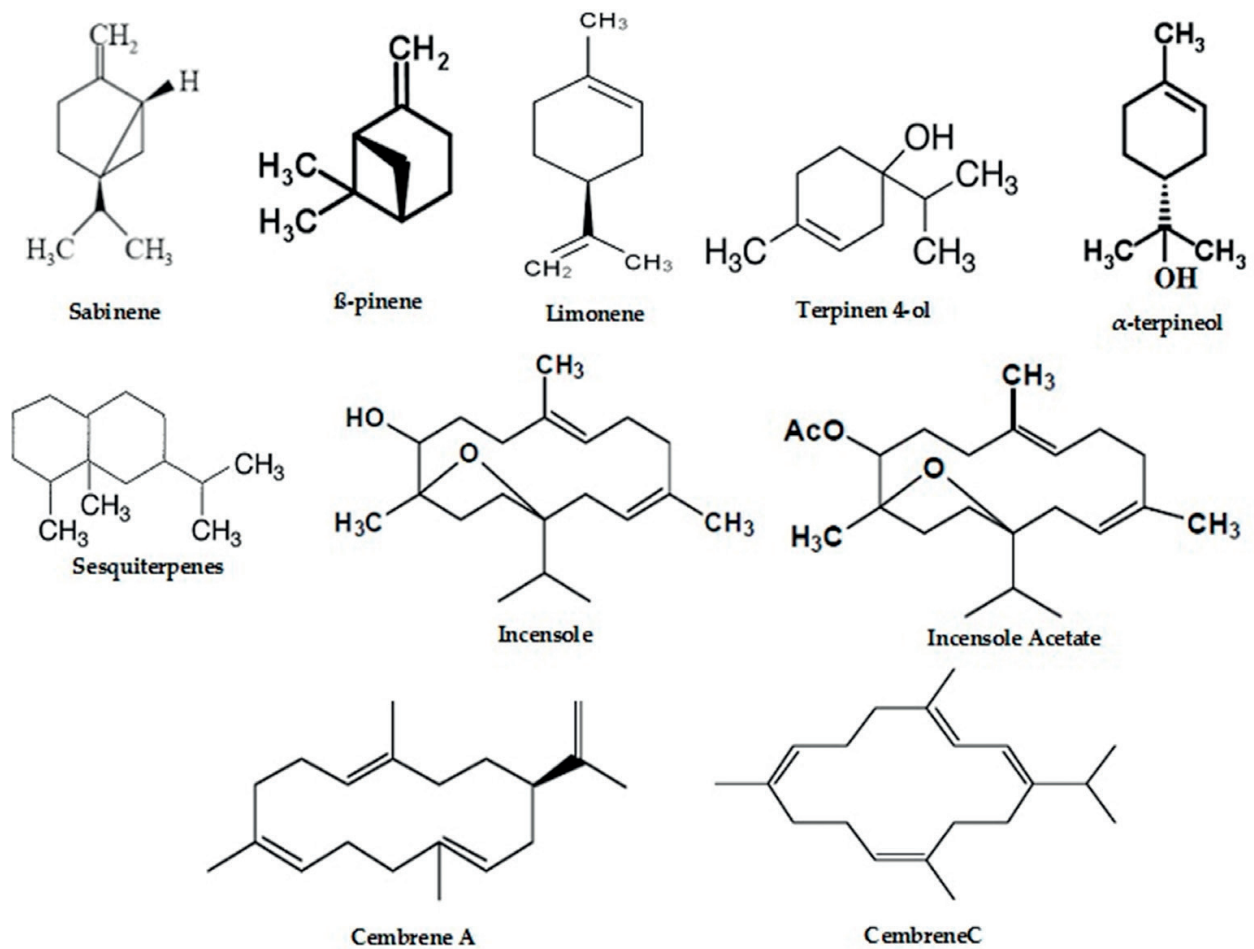


Figure 2. Chemical structures of doom fruit essential oil.

4-ol (1.77%), α-terpineol (0.95%), sesquiterpenes (3.2%), diterpenes represent 40.49%, of which incensole (19.81%) and incensole acetate (17.52%) were found to be the main components, non-terpenoidal components amount to 15.21% of which octylacetate (9.38%) was found to be the major and fatty acid (8.55%) with the main component palmitic acid (5.90%). Oxygenated compounds constituted 66.78% of the total compounds identified which indicated the economic value of this oil. Fruit of doom oil was found to contain volatile diterpenes especially cembrene A which showed cytotoxic activity, and this revealed the medical importance of the volatile oil of doom which could be utilized medicinally [26].

## 6.2. Chemical structure of doom fruit phenolic compounds

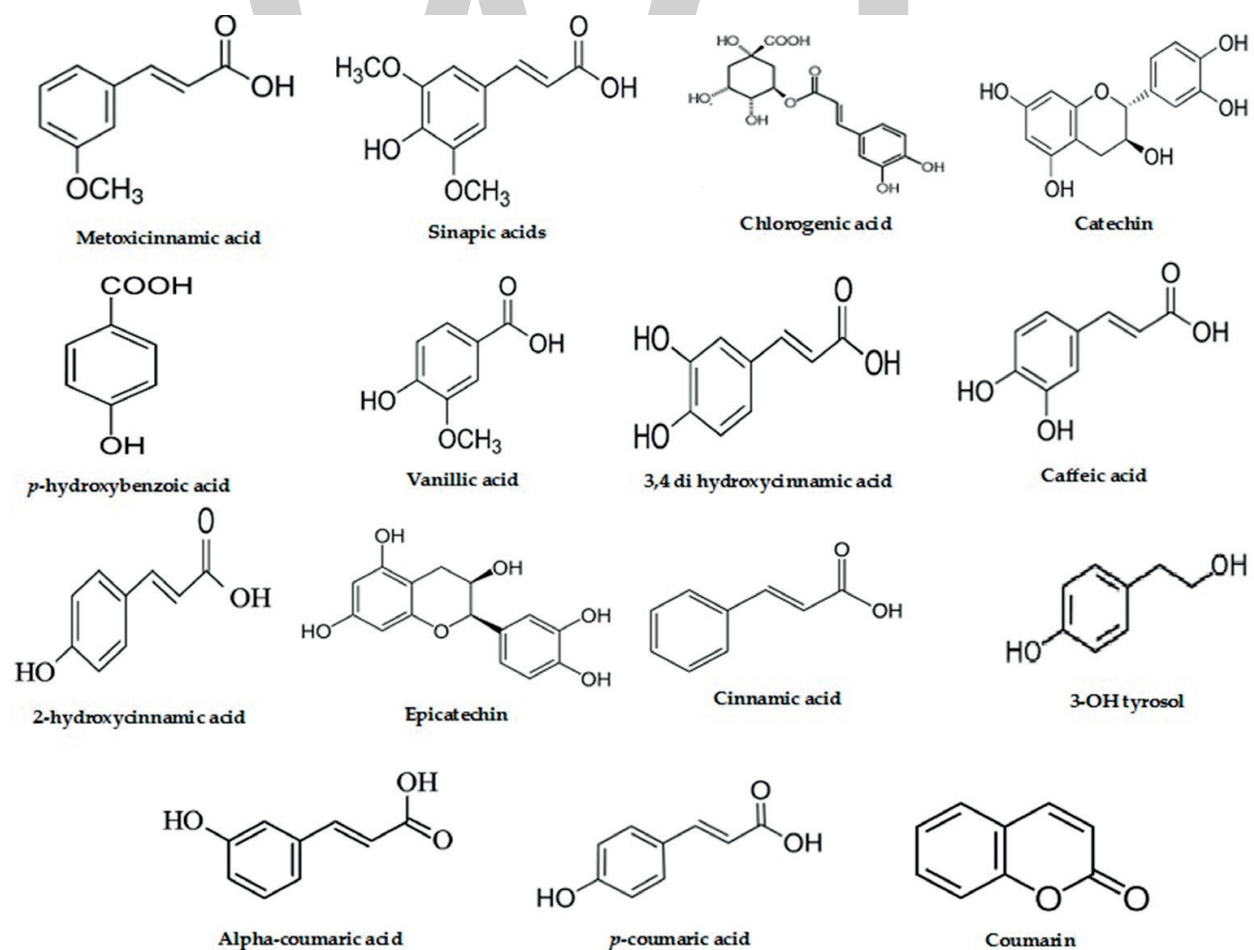
### 6.2.1. Total soluble phenols content and compounds

Different total soluble phenols values in doom were published in different studies; it ranged from 45.08 to 64.90 mg GAE/g DW [27]. While it recorded the highest values in pitted doom fruit extracts varied from 116.26 to 139.48 mg GAE/g DW [16]. The bioactive potential of fruits and vegetables attributed to their high content of polyphenols [28].

The most abundant phenolic compounds recorded in doum were metoxycinnamic acid, sinapic acids (hydroxycinnamic acids), chlorogenic acid, catechin, p-hydroxybenzoic acid, vanillic acids, 3,4 di hydroxycinnamic acid, caffeic acid, 2-hydroxycinnamic acid, Epicatechin and cinnamic acid, respectively (**Figure 3**) [29]. Doum pulps exhibited higher caffeic acid contents in comparison to the domestic fruits [30]. The highest four concentrations of phenolic compounds in doum fruit aqueous extracts were found to be 3-OH tyrosol, E-vanillic acid, catechin and chlorogenic acid, while the lowest were of alpha-coumaric acid, cinnamic acid, p-coumaric acid and coumarin [31].

### 6.2.2. Total flavonoids content and compounds

The total flavonoids content in different extracts of doum fruit extracts varied widely ranging from 24.04 to 47.17 mg rutin/g DW [16]. Similar results found that the content of flavonoids (mg/g) of fruits of *H. thebaica*, in the quercetin equivalent was 46.28 mg/g DW [27]. HPLC analysis of aqueous doum fruit extracts showed 11 flavonoid compounds (**Figure 4**). The highest concentrations were quercetin, hesperetin, naringin and rutin compounds [18]. Five flavone glycosides were isolated and identified from doum fruits namely, luteolin 7-O- $\beta$ -glucuronide, apigenin 7-O- $\beta$ -glucuronide, luteolin O- $\beta$ -glycoside, luteolin 7-O-rutinoside



**Figure 3.** Chemical structures of doum fruit phenolic compounds.

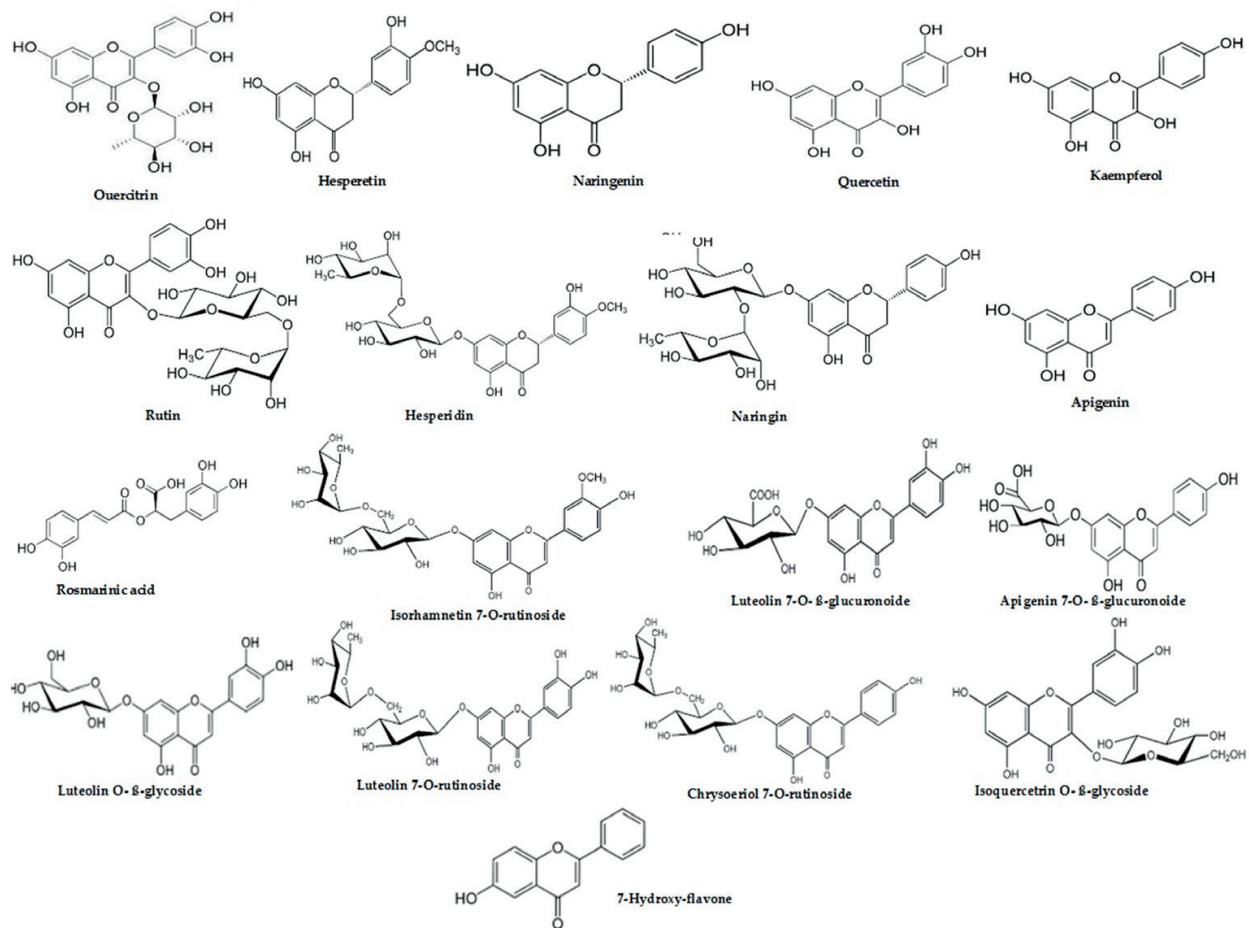


Figure 4. Chemical structures of doum fruit flavonoid compounds.

and chrysoeriol 7-*O*-rutinoside [32]. Glycosides of luteolin and chryseriol flavones previously isolated from doum fruit were identified. In addition, isoquercetrin and isorhamnetin rutinoside are reported for the first time in *H. thebaica* [33].

### 6.2.3. Chemical structure of doum palm leaves phenolic compounds

Doum leaves were extracted in 80% ethanol and filtrate. The aqueous ethanolic extract used to scavenge reactive oxygen species (ROS). The phenolic content of doum was determined by using HPLC and recorded the presence of four major compounds correspond to Gallic acid, Quercetin glucoside, Kaempferol rhamnoglucoside, Dimethoxy quercetin rhamnoglucoside, respectively. An in-depth phytochemical investigation showed the presence of 14 compounds (**Figure 5**): 8-*C*- $\beta$ -D-glucopyranosyl-5, 7, 4'-trihydroxyflavone (vitexin), 6-*C*- $\beta$ -D-glucopyranosyl-5, 7, 4'-trihydroxyflavone (iso-vitexin) [34, 35]. Quercetin 3-*O*- $\beta$ -4C1-D-glucopyranoside, gallic acid [36], quercetin 7-*O*- $\beta$ -4C1-D-glucoside [37], luteolin 7-*O*- $\beta$ -4C1-D-glucoside, tricetin 5-*O*- $\beta$ -4C1-D-glucoside [38], 7, 3' dimethoxy quercetin 3-*O*-[6''-*O*- $\alpha$ -L-rhamnopyranosyl]- $\beta$ -D-glucopyranoside (rhamnazin 3-*O*-rutinoside) [39] (kaempferol-3-*O*-[6''-*O*- $\alpha$ -L-rhamnopyranosyl]- $\beta$ -D-glucopyranoside (nicotiflorin) [40], apigenin, luteolin, tricetin, quercetin and kaempferol. All these compounds were isolated and identified for the first time in doum leaves [41].



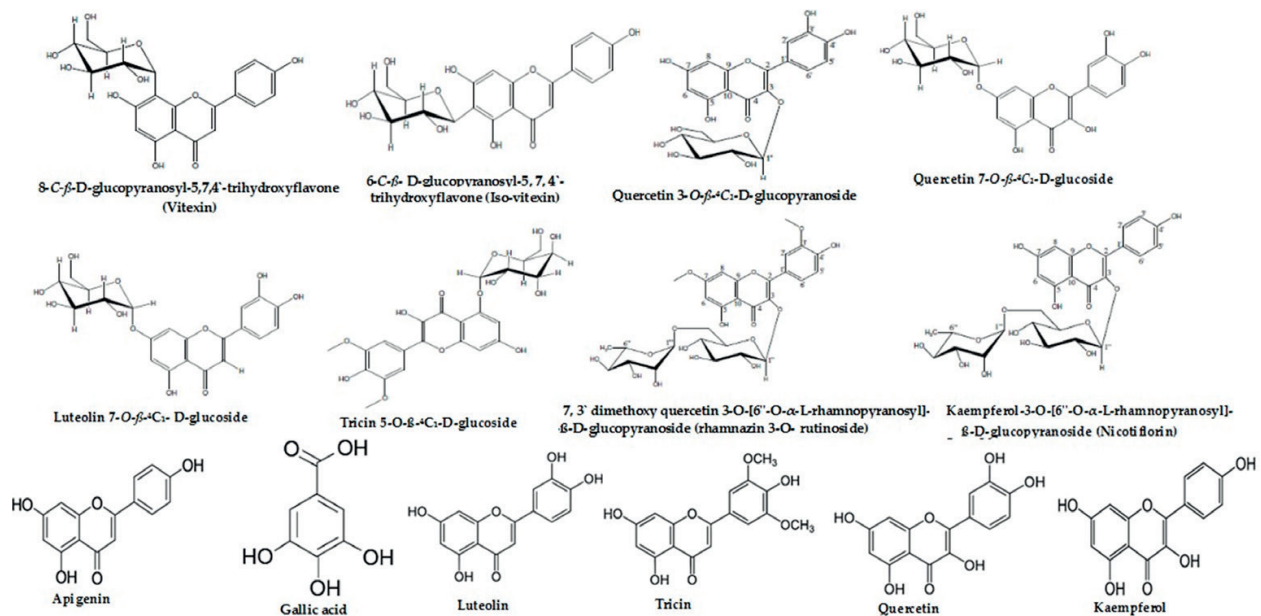


Figure 5. Chemical structures of doum leaf phenolic compound.

## 7. Biological activities of doum fruit extracts

### 7.1. Antioxidant activity of doum fruit extracts

Doum is one of the commonly consumed traditional beverages in Egypt and is rich in polyphenolic compounds. Several studies have recorded that doum fruit extracts contain high amount of flavonoids, phenols and used as antioxidant and antibacterial activities (Table 1), which can alleviate the adverse effects of oxidative stress and prevent diseases caused by pathogenic bacteria [16]. It is well-known that plant phenolic compounds are highly effective free radical scavengers (Figure 6). Phenolic compounds antioxidant activity is associated with the presence of functional groups in the ring and the annular structure of the molecule, conjugated double bonds [42]. The antioxidant activity increased with the increase in concentration and the consumption of doum plant which would exert several beneficial effects by the value of its antioxidant and antimicrobial activities [27].

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) is a free radical that received an electron or hydrogen radical and become a stable diamagnetic molecule [43]. DPPH was determined by the decrease in its absorbance at 517 nm, which was induced by antioxidants. The ability of hydrogen donating in the polyphenolic compounds in the doum fruit extracts helps in the scavenging ability of DPPH. The antioxidant activity measured from doum extracts was 343.4  $\mu$  moles trolox equivalents/g DW in hydrophilic extract and 42.67  $\mu$  moles trolox equivalents/g DW in lipophilic extract when using DPPH [29]. In addition, the  $IC_{50}$  values of doum extracts varied from 107.6 to 172.7  $\mu$ g/ml [16]. These results are lower than the results reported by Abou-Elalla [44] who found that the aqueous doum extract exhibited 50% antioxidant activity ( $IC_{50}$ ) at the concentration of 1000  $\mu$ g/ml, also 1500  $\mu$ g/ml extract exhibited 80% antioxidant activity. Also, the aqueous ethanolic extract of doum leaves appeared to be a potent scavenger of reactive oxygen species [41]. Strong correlation between total phenolics content and total

Doum part	Method used	Extract type	Antioxidant activity/ IC <sub>50</sub> Inhibition values	References
Fruit	DPPH	Dichloromethane	343.4 μ moles trolox /g	Salih and Yahia [29]
Fruit	DPPH	Hexane	42.67 μ moles trolox /g	Salih and Yahia [29]
Fruit	DPPH	Ethanol	IC <sub>50</sub> = 172.7 μg/ml	Aboshora et al. [16]
Fruit	DPPH	Methanol	IC <sub>50</sub> = 107.6 μg/ml	Aboshora et al. [16]
Fruit	DPPH	Water 12 h	40.77%	Aamer [18]
Fruit	DPPH	Methanol	64.55%	Mohamed et al. [27]
Bark	DPPH	Methanol	90.7%	Fayad et al. [52]
Fruit	FRAP	Methanol	28.93%	Sani et al. [47]
Fruit	FRAP	Distilled water	31.91%	Sani et al. [47]
Fruit	FRAP	Methanol	24.3%	Mohamed et al. [27]
Fruit	FRAP	Dichloromethane	13.57 μmoles trolox /g	Salih and Yahia [29]
Fruit	FRAP	Hexane	7.69 μmoles trolox /g	Salih and Yahia [29]
Leaves	Superoxide anion radical	Ethanol	IC <sub>50</sub> = 1602 μg/ml	Eldahshan et al. [41]

Table 1. Antioxidant activity/ IC<sub>50</sub> Inhibition values of doum part by different methods.

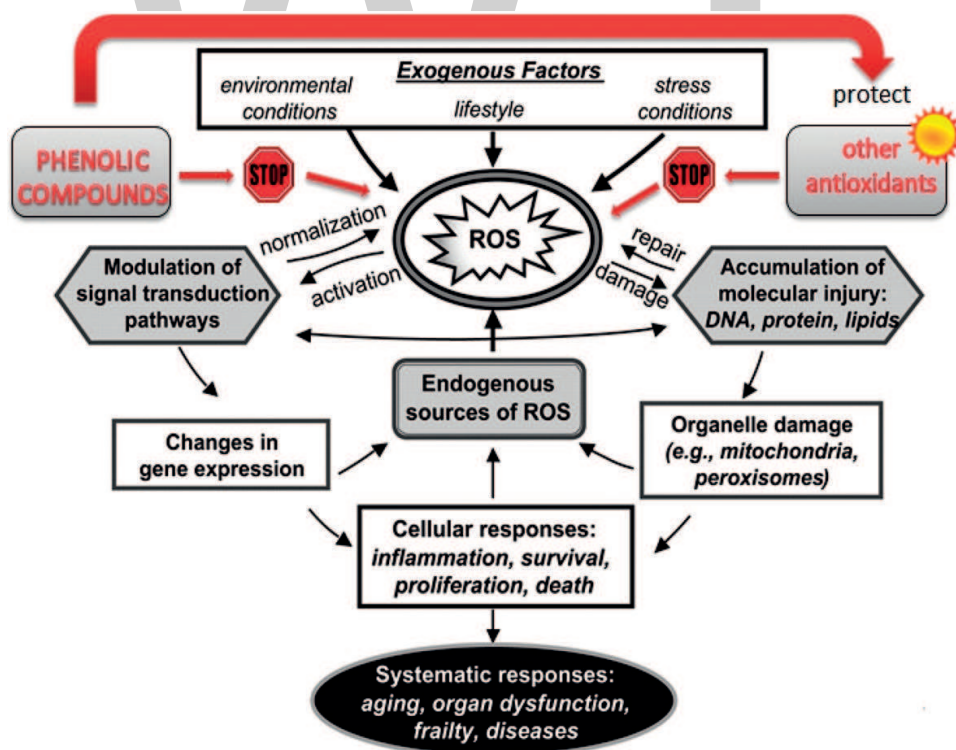


Figure 6. The scheme of factors involved in the formation of free radicals and a cellular response to reactive oxygen species (ROS). The red arrow and the text in red emphasize the importance of phenolic compounds, other reactive oxygen species (ROS). The red arrow and the text in red emphasize the importance of phenolic compounds, other antioxidants and the relationship between them. The sun signifies protection of other antioxidants by phenolic compounds.

antioxidant activity reported in many studies concluded the significant role that total phenols can play in antioxidant activity [29].

Iron is known as an essential transition metal element in the human body for the activity of many enzymes and for some important proteins participated in cellular respiration, O<sub>2</sub> transport, and redox reactions. However, because its transition metal, it contains one or more unpaired electrons that enable them to share in one-electron transfer reactions. Hence, its potent catalysts of autoxidation reactions, such as involvement in the production of OH<sup>-</sup> from H<sub>2</sub>O<sub>2</sub> in the Fenton reaction and in the decay of alkyl peroxides to alkoxy and hydroxyl radicals [45]. Because of this property, transition metal chelation to shape low redox potential complexes is significant antioxidant property and measuring chelation of iron (II) is one method for estimating this property [46]. The reaction is based on the relationship of an antioxidant toward iron (II) in relation to ferrozine, the assay is affected by both concentration of antioxidant and binding constant and thus only powerful iron antioxidant chelator is detected. With this assay, many plant phenolic compounds have been described as antioxidants due to their chelating ability to iron ions. *H. thebaica* fruit has antioxidant activity of 28.93 ± 0.23% and 31.91 ± 0.14% for methanol and aqueous extract respectively [47]. The extracts of doum fruits showed an antioxidant potential, and this is due to the substantial amount of their water-soluble phenolic content [6]. The percentage of metal scavenging capacity at 200 µg/ml of tested methanol extracts *H. thebaica* and was found to be 24.3%. The antioxidant activity increased when extract concentration increased [27].

It displayed the Fe<sup>2+</sup> chelating effect in a concentration-dependent manner. The antioxidant activity measured from doum extract was 13.57 µmoles trolox equivalents/g DW in hydrophilic extract and 7.69 µmoles trolox equivalents/g DW in lipophilic extract when using FRAP assay [29]. These results are in agreement with previous study, who found that doum fruit contained contain iron (II) chelating activity [6].

Superoxide radical is harmful to the body because it is a portent of the hydroxyl radical in the Fenton reaction and is the part of lipid peroxidation as an allylic hydrogen abstractor. Phenazine methosulphate/β-NADH/nitro blue tetrazolium (PMS/NADH/NTB) system is used to determine superoxide radical [6]. Again, an antioxidant activity can come about by antioxidant donation of hydrogen or electron to superoxide or by direct reaction with it. Using this assay, doum fruit was found a very poor activity toward superoxide; it contains 0.02 mmol gallic acid equivalents/g extract. In addition, the significant inhibition percentage of superoxide generation was shown at 300 µg/ml concentration of *H. thebaica* as 63.22% [27]. The Doum extract is found to be an efficient scavenger of superoxide radicals generated in a PMS-NADH system in vitro and its activity is comparable to that of quercetin.

## 7.2. Anticancer activity of doum fruit extracts

Free radicals can react with biomolecules, causing extensive damage to DNA, protein, and lipid, which are considered to be related to aging, degenerative diseases of aging, cancer [48, 49]. Antioxidants play an important role in the later stages of cancer development. The oxidative stress is defined as the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage in human cells. Physiologically, antioxidants play a

major role in preventing the formation of free radicals, which are responsible for many harmful oxidative processes [50]. Antioxidants may be synthetic or of natural source [51]. Antioxidants play an important role in the later stages of cancer development. The methanol extract of *H. thebaica* bark showed high cytotoxicity against human cancer cells and free radical scavenging activities, but showed no cytotoxic effect on human normal immortalized fibroblast cells (BJ- 1) [52]. *H. thebaica* extract showed cytotoxicity against A549 (lung carcinoma) and MCF-7 (breast adenocarcinoma) (87% and 89% respectively). Others reported that the fruit extract of *H. thebaica* had antioxidant and anticancer activities against acute myeloid leukemia [43]. It found that the incubation of tumor cells with doum extract significantly reduced the viability of these cells and the dead cells were significantly increased with high extract concentration. At concentration of 2 µg/ml, the extract reduced the viability from 98 to 83% (17% death). The dead cells produced by extract reached to 50% by 3 µg/ml when compared to control (2% death). Also, doum extract reduced the viability from 98 to 60% (61% death at 4 µg/ml and the dead cells reached to 92% dead by 8 µg/ml). This anticancer activity may be due to the antioxidant activity of doum extract. This is due to the substantial amount of their water-soluble phenolic compounds [6]. Plants extract which combines antioxidant and anticancer activities and at the same time safe to healthy cells is a promising cancer chemopreventive candidate.

The logic behind this is that the antioxidant will reduce, if not prevent, the DNA mutations and adducts caused by cytosolic free radicals and consequently prevent the initiation of cancer through induction of mutations. The anticancer activity will be useful in early eliminating any newly formed neoplastic cells that are not clinically detectable. However, these cancer cytotoxic agents should be with minor or no side effects as they are planned to be used for prolonged time preventing cancer formation [52].

### 7.3. Anti-inflammatory activity of doum fruit extracts

Ulcerative colitis (UC) and Crohn's disease (CD) are two major categories of inflammatory bowel diseases (IBDs). Familiar drugs that are administrated for the management of IBD include glucocorticoids and sulfasalazine. Antibiotics, monoclonal antibodies (Infliximab) and immunosuppressants are also sometimes used for difficult disease conditions [53]. These medicinal agents have side effects, and they could not suitably cure IBD patients [54]. In many studies, it has been reported that antioxidants show useful effects in experimental colitis [55]. The effect of various herbal drugs on experimental models of inflammatory bowel diseases (IBD) has been reported earlier with the antioxidant potential as the main mechanism of action against IBD [56]. As the plant *H. thebaica* is thought to possess anti-inflammatory and antioxidant properties, proving its role in the management of experimentally induced IBD [27]. It showed significant amelioration of experimentally induced IBD, which may be attributed to its antioxidant and anti-inflammatory properties [57]. The previous studies found that the weight and volume of contents of granuloma in inflammation were decreased after treatment with doum extract. This may be due to the presence of flavonoids, coumarins and saponins in doum extract which has anti-proliferative activity [58]. Therefore, this might be its probable mechanism of anti-inflammatory action. Also, flavonoids and coumarin derivatives have been reported as protective products to prevent and treat intestinal inflammatory processes induced by different chemical indicators of experimental colitis [59]. In addition, diet supplementation

with doum has a promising anti-inflammatory influence on attenuating the complications associated with the renal dysfunction. Moreover, anti-inflammatory status of animals injected with cyclosporine and supplemented with doum showed a significant amelioration in the kidney functions as compared to animals injected with the cyclosporine only [60]. The anti-inflammatory activity of doum was possibly due to its saponin content which acts against the oxidative damage and suppresses the serum transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) expression [61]. Therefore, doum administration declines the oxidative damage and the renal interstitial fibrosis in rats [60]. The significant increase in white blood cells caused by crude mesocarp extract of *H. thebaica* could be due to stimulation of bone marrow stem cells to produce these cells, which is an indication of immune—modulatory effect as was observed by other researchers exhibited by some plants [62]. The presence of phytochemicals such as glycosides and reducing sugars could be the reason for the leukocytosis [63]. Flavonoids protect both the hematopoietic committed stem cells and the formed blood cells from the attack of the reactive free radicals hence improving leucocytic production [64]. In *H. thebaica*, flavonoid conjugates, oxygenated fatty acids, and sphingolipids enriched in fruits are likely to mediate for its anti-inflammatory effect [65, 66].

Doum extracts treatments inhibited the activity of cyclooxygenase (COX-1), an enzyme known to be involved in inflammation [33].

#### 7.4. Antimicrobial activity of doum fruit extracts

Methanol and aqueous extracts of doum fruit showed higher antibacterial activity against Gram-positive bacteria and Gram-negative bacteria except for *Listeria monocytogenes*, where only a slight inhibition was observed [27]. Moreover, the ethyl acetate extract of doum fruit was active against five pathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* while methanol extract was active against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. *Penicillium* sp. growth was slightly affected by high concentration of methanolic extract [67]. *H. thebaica* fruit extracts reduced the growth of *Erwinia carotovora* and produced inhibition zones up to 38 mm in diameter [68]. All doum extracts showed strong antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi*, while methanol/ultrasonic (MU) extract inhibited the growth of all pathogenic bacteria used in the study. However, all doum fruit extracts demonstrated no antibacterial activity against *E. coli* colonies except the methanol/ultrasonic (MU) extract, which had slight activity [16]. The antibacterial activities against both Gram-positive and Gram-negative bacteria may indicate the presence of broad spectra antibiotic compounds or simply metabolic toxins in plant extracts [69]. Doum fruits showed antimicrobial and antihypertensive activities, and these activities were attributed to the presence of flavonoids [70]. The mechanism of polyphenol toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesins, cell envelope transport proteins, and non-specific interactions with carbohydrates [71]. Also, the methanolic extract of *H. thebaica* showed stronger antifungal and anti-yeast activities than aqueous extracts [27]. Similar results were observed in previous studies, which showed that polar solvent extract of *H. Tobacco* has high antifungal activity against a wide range of fungal isolates, including *Aspergillus niger*, *Microsporium gypseum*, *Trichlorophyton rubrum*, *Mucor* sp., *Fusarium solani* and *Candida albicans* [72]. Antimicrobial activity may involve complex mechanisms, like the

inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nucleic acids [73].

## 8. Conclusion

*Hyphaene thebaica*, a well-known plant for its antioxidant, anticancer and anti-inflammatory potential because of its phenolic and flavonoid content was explored for its antimicrobial potential against various Gram-positive and Gram-negative bacteria and fungal pathogens. This chapter evidently reveals that the doum (leaf and fruit) extracts are effective antimicrobial and pharmacological agents. Further detailed study on its mechanism and safety profile may develop them as good candidates for food preservation or functional foods, as well as for pharmaceutical and natural plant-based products.

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## **Biological Activities of Plants from Genus *Annona***

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### **Abstract**

Species of the genus *Annona* (Annonaceae) are distributed in the tropical and subtropical regions of the world and are characterized by their highly valued exotic fruit. The commercial species are *A. muricata*, *A. crassiflora*, *A. squamosa*, *A. cherimola*, and *A. reticulata*. In addition, different parts of the tree, including leaf, bark, and roots, are used in traditional medicine to treat conditions such as diabetes, hypercholesterolemia, hypertension, cancer, and gastrointestinal diseases. Phytochemical studies are helping to determine the biological properties of extracts and characterize bioactive principles from extracts of genus *Annona*. The main chemical compounds isolated from genus *Annona* are phenols, acetogenins, alkaloids, and cyclopeptides. All these compounds have antioxidant properties and generally are associated with other biological properties. The aim of this chapter is to carry out an analysis of the properties related to combating oxidative stress of the five most important species of the genus *Annona*, as well as the relationship these properties have with the bioactive principles present in these plants.

**Keywords:** genus *Annona*, phenols, acetogenins, antioxidant, antitumor, antidiabetic

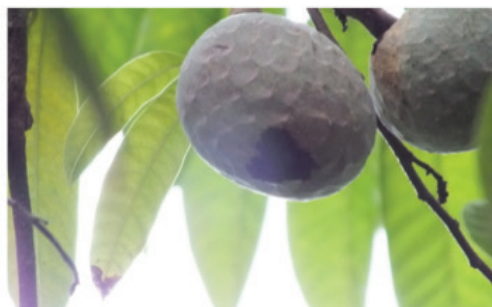
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### **1. Introduction**

Bioactive compounds are extra nutritional constituents present in small amounts in higher plants. They reinforce the immune system, combat oxidative stress, and reduce the risk of a number of diseases, especially cancer, diabetes, and cardiovascular diseases [1]. Traditional medicine has developed diverse treatments based on the use of plant extracts with well-known results worldwide. Based on these results, *in vitro* and *in vivo* studies, mainly with mice and rats, have

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been carried out to validate the effects described. Studies have been done to identify and classify the chemical compounds responsible for the biological properties. *Annona* have been used traditionally for medicinal and nutritional purposes. Pinto et al. published a monograph that addresses the main medicinal uses of plants of the genus *Annona* in detail in one of its chapters [2]. Additionally, Moghadamtousi et al. conducted a review of the traditional uses of *A. muricata* [3].

*Annona muricata**Annona crassiflora**Annona squamosa**Annona cherimola**Annona reticulata*

**Figure 1.** Photographs of the five *Annona* species reviewed in this chapter. The images of *A. muricata*, *A. squamosa*, *A. cherimola* and *A. reticulata* were obtained from <https://www.inaturalist.org/taxa>, while the image of *A. crassiflora* was obtained from <http://tropical.theferns.info>.

*A. muricata* is one of the best known and most studied species of the genus *Annona*. However, there are other species of this genus that produce edible fruit, such as *A. squamosa*, *A. crassiflora*, *A. cherimola*, *A. reticulata*, etc., which are also highly valued and used in traditional medicine. *Annona* fruit contains a considerable quantity of phenolic compounds and other bioactive compounds [4, 5]. Many of these compounds are antioxidants that can help with the prevention and treatment of diseases like cancer, atherosclerosis, diabetes mellitus 2, etc. [6–8].

Another group of compounds are the acetogenins, which are a chemical group representative of the genus *Annona*. These compounds are polyketides that are characterized by linear chains of 32–34 carbons with hydroxyl, ketones, epoxides, tetrahydrofurans, and tetrahydropyrans groups. Acetogenins comprise more than 450 compounds isolated mainly from species of the genus *Annona*. The properties of acetogenins are closely associated with their antiproliferative activity on cancer cell lines. This activity is related to the reduction of ATP levels and the induction of apoptosis. In addition, other biological properties have been demonstrated, including antineoplastic, antiparasitic, cytotoxic, immunosuppressive, neurotoxic, etc. [9]. Alkaloids and cyclopeptides with potent antiulcer and anticancer activity are also present in *Annona* extracts.

The objective of this chapter is to carry out a critical review of the biological properties of extracts of the five most important *Annona* species (*A. muricata*, *A. crassiflora*, *A. squamosa*, *A. cherimola*, and *A. reticulata*) **Figure 1**, as well as associating the biological properties with the bioactive principles present in the extracts.

## 2. Genus *Annona*

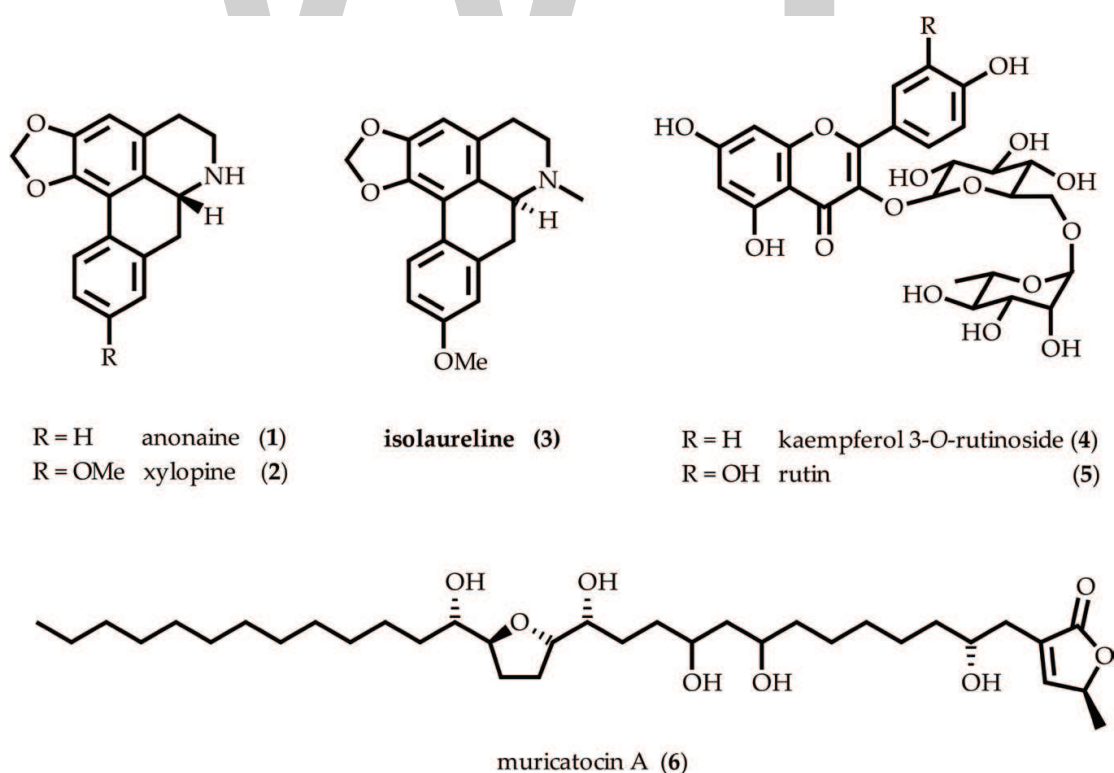
The review is organized by species, and the principal studies for every species discussed are described. The methods of evaluation of antioxidant and other activities are briefly mentioned. The reports demonstrate the diversity of the chemical structures present in *Annona* species; among them, phenols, acetogenins, and alkaloids are found. This chapter describes some of these compounds and their relation to corresponding biological properties.

### 2.1. *Annona muricata*

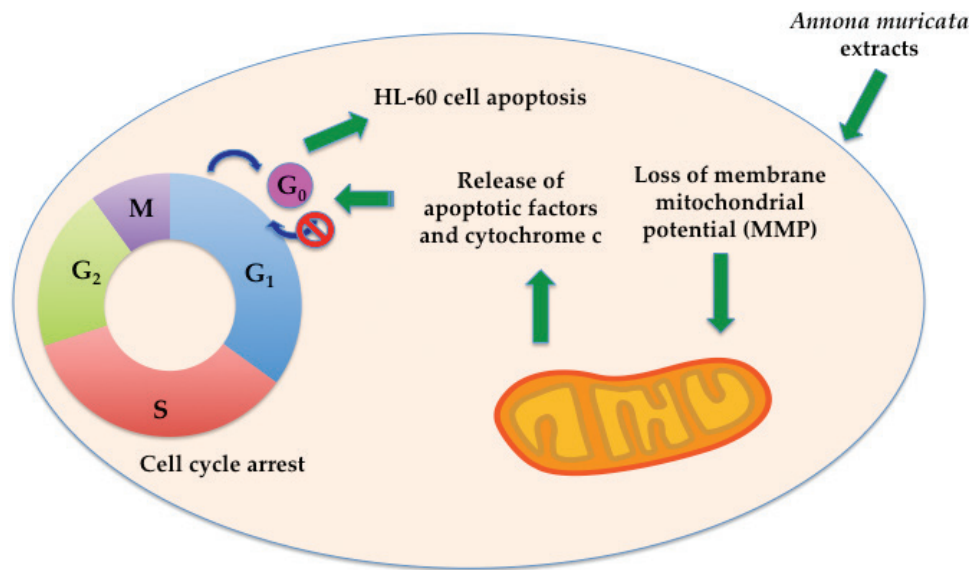
*A. muricata* L. is known as soursop, graviola, paw-paw, and sirsak and is native to the warmer tropical areas of America. It has also been found in some tropical and subtropical regions, including India, Malaysia, and Nigeria. *A. muricata* is a perennial, terrestrial, upright tree that reaches 8 m in height and has an open canopy, with roundish, large, bright, dark green leaves. The edible fruit of the tree is large, heart-shaped, and green, with a diameter between 15 and 20 cm [10]. *A. muricata* has antihypertensive [11], antidiabetic [12–14], antimalarial [15], antiviral [16], anthelmintic [17], anticonvulsant [18], antibacterial [19], antioxidant [20], and anticancer [21–25] properties. Florence et al. evaluated the antidiabetic activity of aqueous extracts at concentrations of 100 and 200 mg of extract·(bw)<sup>-1</sup> and found that the polar extract of *A. muricata* reduced blood glucose level, body weight, food and water intake, lipid profile, and oxidative stress to near normal. These results can be attributed to antioxidant and protective effects of pancreatic  $\beta$ -cells of *A. muricata* extracts [13]. In the same study, Florence et al.

administered, for 28 consecutive days, an aqueous extract from *A. muricata* at doses of 100 and 200 mg·kg<sup>-1</sup> to rats with diabetes induced by streptozotocin (STZ) [13]. Streptozotocin, in addition to inducing diabetes, promotes an increase in triglycerides, total cholesterol, low-density lipoprotein, atherogenic index, and a decrease in high-density lipoprotein. After 4 weeks of treatment with the aqueous extract, a reduction in triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein concentrations was observed. In addition, a decrease in the hepatic levels of aspartate aminotransferase and alanine transferase, as well as malonaldehyde in liver and kidney, and an increase in the levels of superoxide dismutase and catalase in liver, kidney, and aorta were seen. *A. muricata* contains 212 different bioactive compounds including phenolic compounds [26], which have been shown to have an antidiabetic and anti-inflammatory effect. Damayanti et al. evaluated, through a computational study, the potency of *A. muricata* as FOXO1 inhibitor for diabetes mellitus treatment [14]. The results of this study showed that the main components responsible for FOXO1 inhibition were anonaine (1), xylopine (2), isolaureline (3), kaempferol 3-*O*-rutinoside (4), rutin (5), and muricatocin A (6), **Figure 2**. This inhibition ability is possible because the compounds are capable of strongly and spontaneously binding with the active site of FOXO1.

Pieme et al. evaluated *in vitro* the antiproliferative activity and apoptosis induction of extracts coming from three different parts of *A. muricata* at concentrations in the range of 1–100 µg·mL<sup>-1</sup> against human promyelocytic leukemia (HL-60) cells [22]. The authors concluded that *A. muricata* has the potential as a chemotherapeutic and cytostatic agent against HL-60 cells because it induced loss of cell viability, morphology changes, loss of membrane mitochondrial potential, and G0/G1 phase cell arrest **Figure 3**. Yang et al. carried out a study that demonstrated synergistic



**Figure 2.** Molecules with FOXO1 inhibition activity isolated from *Ammonia muricata* [14].



**Figure 3.** Schematic representation of mechanism for *Annona muricata* extracts-induced HL-60 cell apoptosis. G<sub>0</sub> = quiescence, G<sub>1</sub> = Gap 1, S = DNA replication/synthesis, G<sub>2</sub> = Gap 2, M = mitosis.

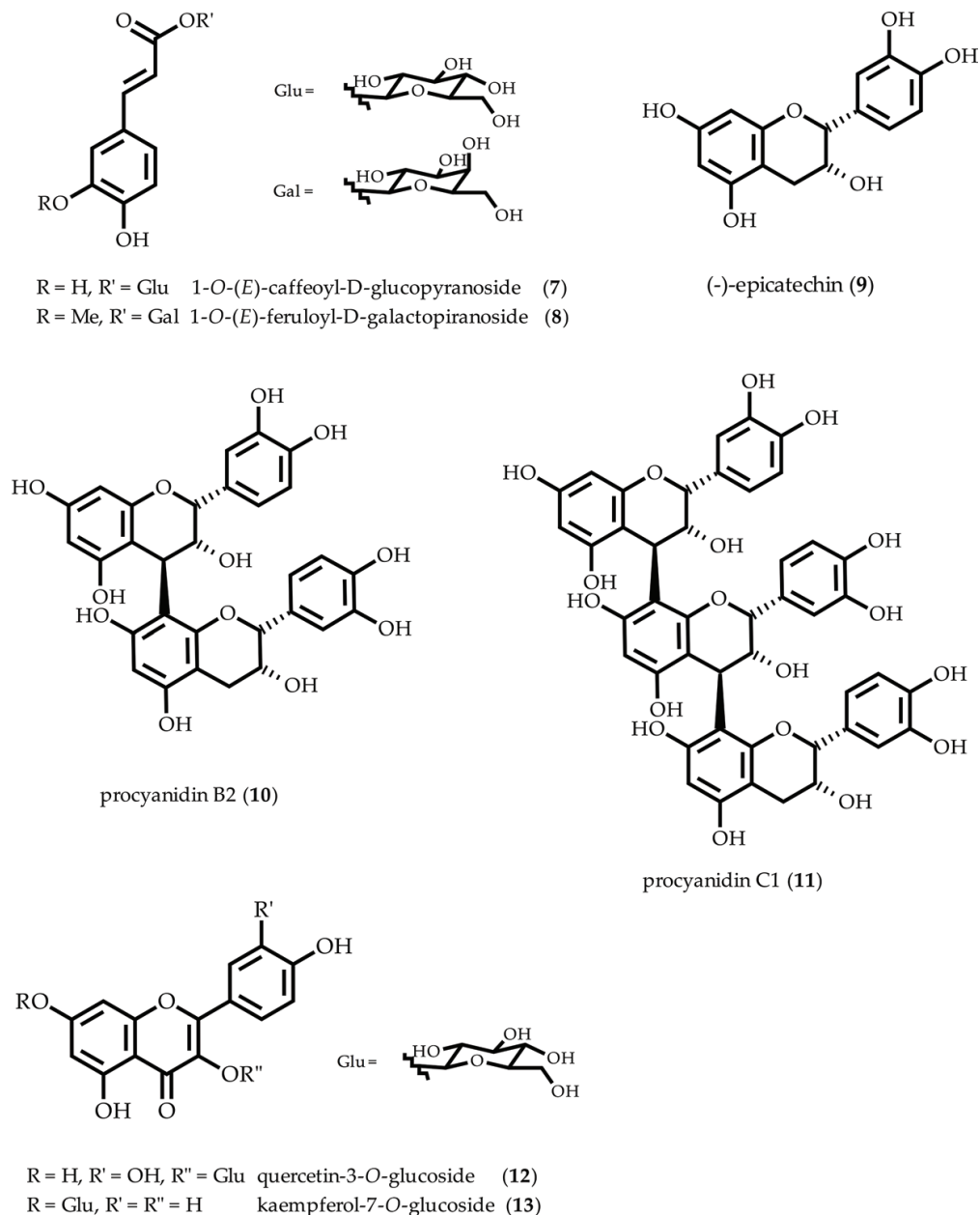
interactions between flavonoids and acetogenins of leaf extracts from *A. muricata* against prostate cancer [27]. This study was conducted with mice exposed to androgen-independent prostate cancer (PC-3) cells that were fed with dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), acetic acid (AcOH), ethyl acetate (EtOAc), ethanol (EtOH), or methanol (MeOH) extracts from leaves of *A. muricata* at doses of 100 mg of extract·(kg of body weight (bw))<sup>-1</sup>. The acetogenins that were supplied as part of an extract from *A. muricata* leaves were more effective than acetogenin-enriched fractions, which were toxic and in some cases led to the death of the mice. Generally, the extracts used are polar, containing significant quantities of antioxidant compounds [19].

## 2.2. *Annona crassiflora*

*Annona crassiflora* is known as araticum, araticum-do-cerrado, ariticum, articum, marolo, bruto, cabeça-de-negro, pinha-do-cerrado, and pasmada [29]. *A. crassiflora* is a perennial, terrestrial, upright tree that reaches 6–8 m in height and has oval, coriaceous leaves. *Annona crassiflora* has antioxidant [28], antimicrobial [29, 30], antidiabetic [31], hepatoprotective [32], antiobesity [33], and anticancer [34] activities. Débora and Neuza carried out a study of the lipid extract of *A. crassiflora* Mart. seeds, identifying and quantifying fatty acids, phytosterols and tocopherols, as well as evaluating the antioxidant activity expressed as radical DPPH• scavenging. The results of this study showed that the lipid fraction of *A. crassiflora* Mart. seed contains a relevant quantity of tocopherols (expressed as α-, β-, γ-, and δ-tocopherol) and phytosterols (campesterol, stigmasterol, and β-sitosterol) with a content of 683.59 and 138.90 mg·kg<sup>-1</sup>, respectively. They also showed that the antioxidant activity was significantly influenced by the phytosterols and the fatty acids composition of the sample [28]. Cavalcante et al. evaluated the antimicrobial activity of EtOH extracts from *A. crassiflora* root wood and root bark against *Candida albicans* [29]. The minimum inhibitory concentration (MIC) of extract used in this study was 2000 μg·mL<sup>-1</sup>. The authors suggest that the responsible compound for antimicrobial activity is goniodonin, an acetogenin [29]. Another study related to the antimicrobial activity of *A. crassiflora* was made



by Silva et al. [30]. They evaluated the antibacterial activity of ethanol aqueous extracts of fruit rind, stem, seed, pulp, and leaf from *A. crassiflora* against oxacillin-resistant *Staphylococcus aureus*. The stem extract showed a better selectivity index against oxacillin-resistant *Staphylococcus aureus*. The authors reported that the EtOH aqueous extract from *A. crassiflora* contains tannins, a group of phenolic compounds. Justino et al. evaluated the antidiabetic activity expressed as  $\alpha$ -amylase,  $\alpha$ -glucosidase, and glycation inhibitory activity of extracts rich in antioxidant compounds from *A. crassiflora* fruit peel [31]. The results of this study showed that fractions of EtOAc and *n*-butanol from 98% EtOH aqueous extract have a high antioxidant activity and  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities. The spectrometric analysis revealed the presence of caffeoyl-glucopyranoside

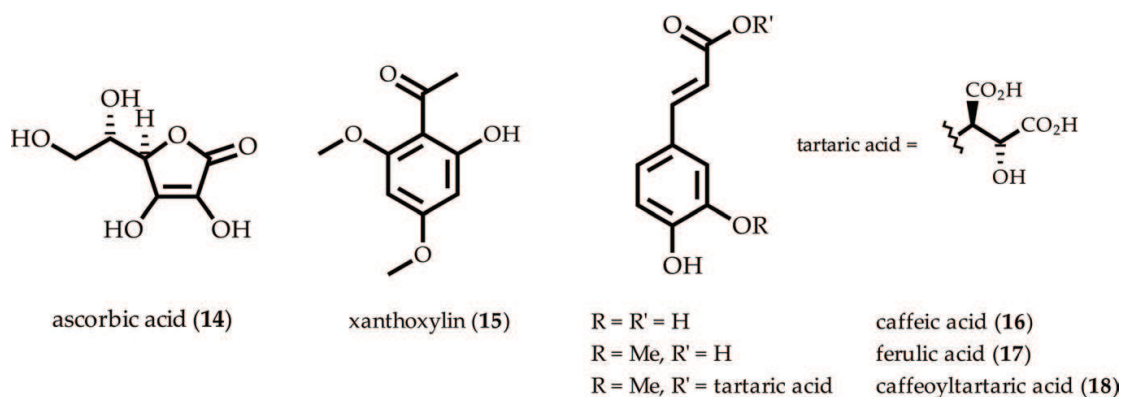


**Figure 4.** Main bioactive compounds of *Annona crassiflora* fruit peel with antioxidant capacity and  $\alpha$ -amylase,  $\alpha$ -glucosidase, and glycation inhibitory activities [31].

(7), feruloyl-galactopyranoside (8), (-)-epicatechin (9), and procyanidin B2 (10) in both fractions. In addition, in the EtOAc fraction, the study identified procyanidin C1 (11) and the flavonoids quercetin-3-*O*-glucoside (12), kaempferol-7-*O*-glucoside (13), **Figure 4**, and rutin (5), **Figure 2**. Roesler evaluated the effect of EtOH extracts from *A. crassiflora* fruit on the hepatic antioxidant enzymes using Wistar rats with CCl<sub>4</sub>-induced liver damage [32]. The results showed that ethanol extracts of *A. crassiflora* fruit could enhance or maintain the hepatic activity of antioxidant enzymes, such as catalase, glutathione peroxidase, and glutathione reductase. This effect can be attributed to ascorbic acid (14), xanthoxylin (15), caffeic acid (16), ferulic acid (17), caffeoyltartaric acid (18), **Figure 5**, and flavonoid 5, **Figure 2**. All these compounds are potent antioxidants present in *A. crassiflora* fruit.

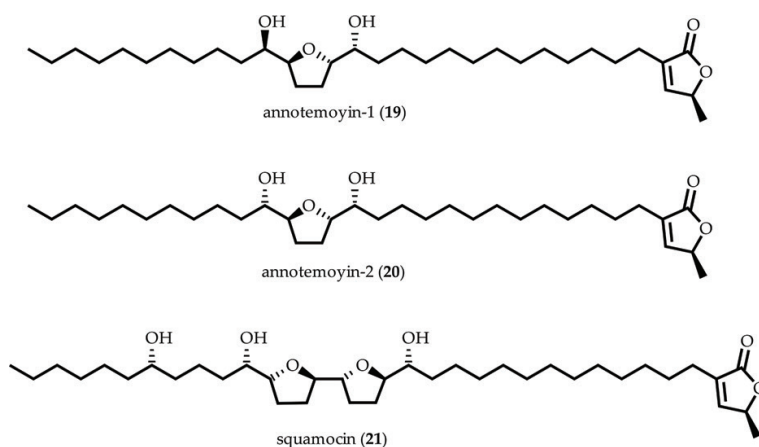
### 2.3. *Annona squamosa*

*Annona squamosa* is known as sweetsop, sugar apple, custard apple, ata, saramuya, and Aztec. The *A. squamosa* tree is deciduous and much smaller than the *A. muricata*, reaching a maximum height of 6.0 m, with abundant lateral branches. Shirwaikar et al. evaluated the effects of the consumption of aqueous extracts of *A. squamosa* leaves in diabetic rats for 12 days [35]. The doses used were 250 and 500 mg·kg<sup>-1</sup> (both doses without toxicity), and plasma glucose levels, serum insulin levels, liver glycogen levels, level of reactive substances to thiobarbituric acid (TBARS), and pancreatic and blood lipid levels (cholesterol and triglycerides) were measured with fasting. A significant reduction in plasma glucose was found 30 min after the oral glucose tolerance test. In addition, plasma glucose levels and serum insulin levels decreased significantly with the two doses administered. The aqueous extracts of *A. squamosa* contain a large quantity of mucilages, which are analogous to gums (soluble fraction of the fiber), which have been shown to have a powerful role in the treatment of hyperglycemia and hyperlipidemia [36]. Insulin is an important inhibitor of lipolysis in adipose tissue and the release of fatty acids into the bloodstream; when there is a deficiency of this hormone, dyslipidemia can be induced [37, 38]. In this same study, rats that did not receive *A. squamosa* extract had twice as high triglyceride and cholesterol levels. In addition, a significant increase in hepatic glycogen levels was observed in diabetic rats treated with the aqueous extract, probably due to a reactivation of the glycogen synthase system. The effects of diabetes include the affecta-



**Figure 5.** Antioxidants from *Annona crassiflora* [32].

tion of glycogen synthesis in the liver and skeletal muscle [39]. The diabetic rats treated with the aqueous extract of *A. squamosa* increased in weight, probably due to a protective effect in the control of energy expenditure of muscle. Shirwaikar et al. [35] also found that *A. squamosa* extract showed antioxidant activity by decreasing TBARS levels in the pancreas. Numerous studies indicate that oxidative stress plays an important role in the pathogenesis of diabetes and its complications. Both insulin resistance and beta cell dysfunction, two central events in the pathogenesis of diabetes mellitus 2, have been linked to a redox imbalance [40]. Thus, it has been found that *A. squamosa* extracts have significant amounts of phenols and flavonoids, which may be involved in the reduction of oxidative stress associated with diabetes. Finally, the antidiabetic activity was independent of doses administered at concentrations of 250 and 500 mg·kg<sup>-1</sup> of extract. Gupta et al. [41] evaluated the effect of ethanolic extracts of *A. squamosa* leaves in different doses (200, 300, 350, and 400 mg·kg<sup>-1</sup>) on glucose tolerance in diabetic rats (induced with streptozotocin). It was found that the hypoglycemic effect occurred 1 h after glucose loading and was maintained up to 3 h. The EtOH extracts of *A. squamosa* were administered to diabetic rats in a single dose of 350 mg·kg<sup>-1</sup> for 15 days. The levels of total cholesterol, low-density lipoproteins, very low-density lipoproteins, and triglycerides, which after treatment with the extract were high, decreased significantly 15 days after treatment. In addition, an increase of 30.3% in high-density lipoproteins was found. El-Chaghaby et al. evaluated the effect of different polar solvent extracts on the antioxidant and antibacterial activities of *A. squamosa* leaves [42]. The antioxidant activity was evaluated using the phosphomolybdenum method, reducing power assay and hydrogen peroxide-scavenging assay. Antibacterial activity was evaluated using six test bacterial species (*Bacillus subtilis*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis*). The results showed moderate antibacterial activity with respect to a standard antibacterial agent and this activity correlates positively with the phenolic contents. These compounds were found to be the major contributor to the antioxidant and antibacterial activity of polar extracts from *A. squamosa* leaves. In a different study, Rahman et al. evaluated extracts and four compounds from *A. squamosa* seeds for antimicrobial activity [43]. Three of these compounds were acetogenins [annotemoyin-1 (19), annotemoyin-2 (20), and squamocin (21)], **Figure 6**. The antimicrobial activity was evaluated against 10 bacteria species [five gram (+) and five gram (-)]



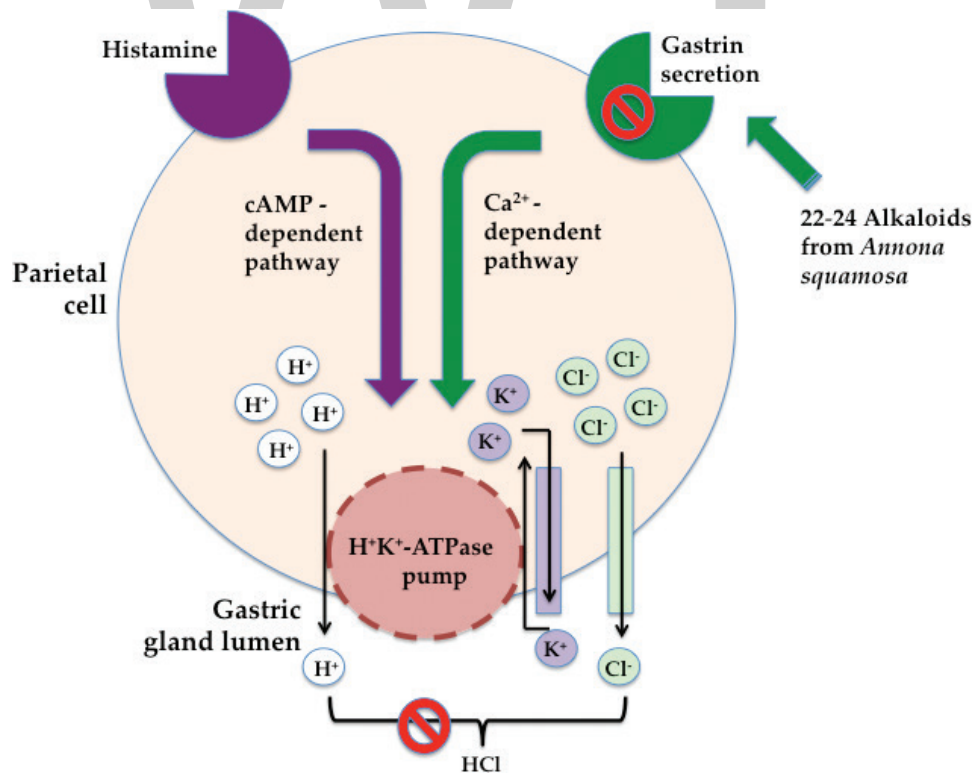
**Figure 6.** Acetogenins isolated from the seeds of *Annona squamosa* with antimicrobial and cytotoxic activities [43].

and four fungi. The results showed that acetogenins have antibacterial activities against all test microorganisms at MIC in the range of 60–130  $\mu\text{g}\cdot\text{mL}^{-1}$ .

Yadav et al. evaluated antiulcer activity in rats of extracts and 11 compounds isolated from *A. squamosa* twigs [44]. The models used in this study were cold-restraint-induced gastric ulcer, aspirin-induced gastric ulcer, and pyloric ligation-induced ulcer; they also used histamine-induced duodenal ulcer in guinea pigs and *in vitro* assay of  $\text{H}^+$ ,  $\text{K}^+$ -ATPase activity. Extracts of *A. squamosa* twigs at concentrations of 25, 50, and 100  $\text{mg}\cdot\text{kg}^{-1}$  were used. The results showed that ethanol extracts of *A. squamosa* twigs inhibit *in vitro*  $\text{H}^+$ ,  $\text{K}^+$ -ATPase (proton pump) activity and simultaneously strengthen the mucosal defense mechanism **Figure 7**. The compounds (+)-*O*-methylnorpavine (**22**), *N*-methylcorydaldine (**23**), and isocorydine (**24**) were the active components of the extract, **Figure 8**. Zahari et al. found that alkaloid **24** has antioxidant activity with DPPH assay, metal-chelating activity assay, and ferric-reducing antioxidant power assay (FRAP) [45].

#### 2.4. *Annona cherimola*

*Annona cherimola* is a tropical tree native to Peru and Ecuador. The word cherimoya comes from the Quechua name “chirimuya,” which means “cold seeds” [46]. The tree is small, upright, and/or somewhat spreading, deciduous with a maximum height of 7.5 m. Its trunk frequently divides at the ground level into several trunks [47]. *A. cherimola* has been cultivated since the Incan Empire, dating back to 1200 BC. Albuquerque et al. evaluated the antioxidant activity of extracts from pulp, peel, and seeds of *A. cherimola* fruit [48]. The results showed a significant



**Figure 7.** Inhibition of  $\text{H}^+\text{K}^+$ -ATPase activity by alkaloids 22-24 from EtOH extracts of *A. squamosa* twigs.

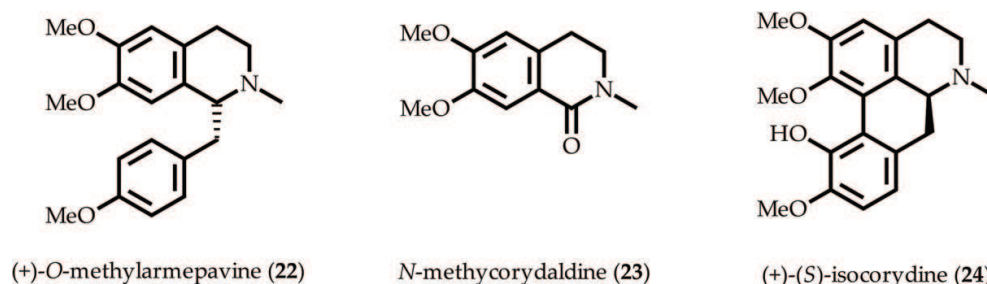


Figure 8. Alkaloids from *Annona squamosa* with antiulcer effect *in vivo* [44].

antioxidant activity of *A. cherimola* fruit, mainly in the peel. These results are similar to those reported by Loizzo et al. who examined the antioxidant properties of *A. cherimola* pulp and peel and found that the peel extract showed the greater capacity for free radical-scavenging DPPH $\cdot$  and ABTS $\cdot^+$  and good antioxidant activity through FRAP assay,  $\beta$ -carotene bleaching assay, and Fe $^{2+}$  chelating assay [49]. The peel extract showed the highest content of phenols and flavonoids. Gupta-Elera et al. evaluated antioxidant properties of *A. cherimola* fruit *in vitro* using the oxygen radical absorbance capacity (ORAC) assay and simulating conditions of cells under oxidative stress. The results showed that *A. cherimola* peel, pulp, and juice have antioxidant activity [50]. The juice showed the highest antioxidant activity, while the pulp exhibited the lowest. The results also indicate that pre-exposure to oxidative stress may contribute to an increased antioxidant uptake in both Raji (Burkitt's lymphoma) and HT-29 (colon cancer) cell lines. In the two cell lines, cell lysate antioxidant capacity was significantly higher when cells were exposed to oxidative stress. This is an indication that oxidative stress contributed to the uptake of antioxidants as a response mechanism. Barreca et al. evaluated antioxidant and cytoprotective properties of extracts from the pulp of *A. cherimola* fruit [51]. The extracts from *A. cherimola* pulp had powerful antioxidant activity expressed as scavenging activity toward DPPH $\cdot$ , ABTS $\cdot^-$ , O $_2^{\cdot-}$  radical, and ferric-reducing antioxidant power (FRAP) assay, while the ethanol extract showed the highest activity against lipid peroxidation induced by *tert*-butyl hydroperoxide. Garca-Salas et al. carried out an exhaustive study of identification and quantification of phenolic compounds in *A. cherimola* fruit [52]. The method used for identification and quantification was HPLC-DAD-ESI-QTOF-MS. The main results indicate the presence of 21 phenolic and organic acid compounds in the edible portion of *A. cherimola* fruit, 37 in the peel and 22 in the seeds. The *A. cherimola* seeds contain acetogenins, *cis*-annonacin (25), and (2,4)-*cis*- and *trans*-isoannonacins (26 and 27, respectively), Figure 9. These acetogenins showed cytotoxicity against the human tumor lines A-549 (lung carcinoma), MCF-7 (breast carcinoma), HT-29 (colon adenocarcinoma), A498 (renal carcinoma), PC-3 (prostate adenocarcinoma), and MIA PaCa-2 (pancreas carcinoma), with a remarkable selectivity to this last line with a power of 1000 times higher than adriamycin [53]. Adriamycin is a commercial anticancer (antineoplastic or cytotoxic) chemotherapy drug. This compound is classified as an anthracycline antibiotic. Earlier, Kim et al. isolated and evaluated the anticancer activity of anomolin and annocherimolin. Anomolin has anticancer activity against the PC-3 line and annocherimolin against the MCF-7 and HT-29 lines [54]. Cyclopeptides with anticancer activity have also been isolated from the seeds of *A. cherimola*. For example, cherimolacyclopeptide C (28), cherimolacyclopeptide E (29), and cherimolacyclopeptide F (30) showed significant cytotoxicity against tumor cells KB (nasopharyngeal carcinoma), Figure 10 [55, 56].

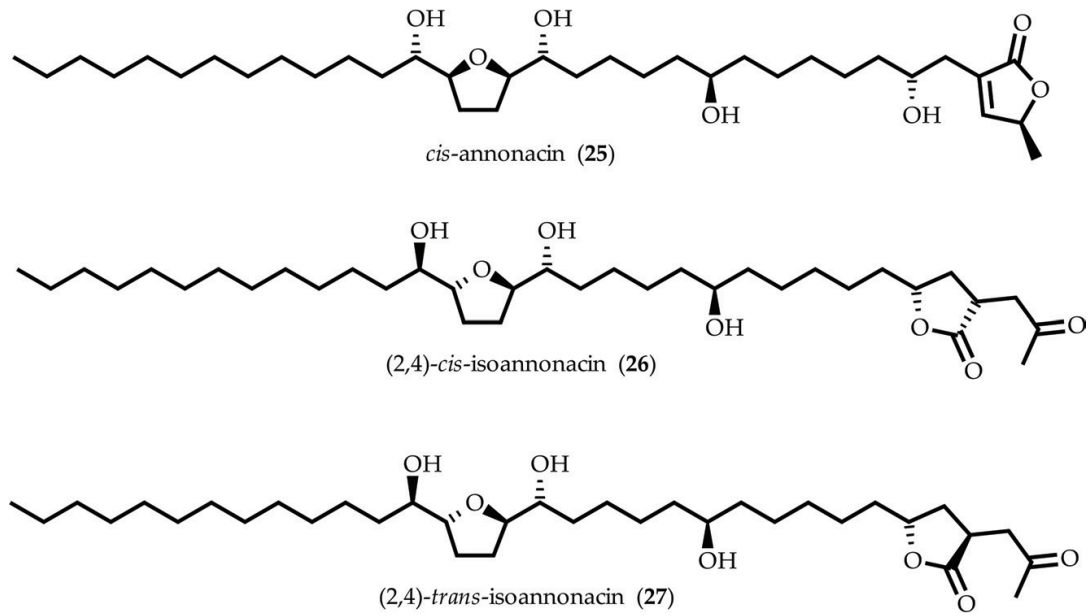


Figure 9. Acetogenins with cytotoxicity activity from seeds of *Annona cherimola* [52].

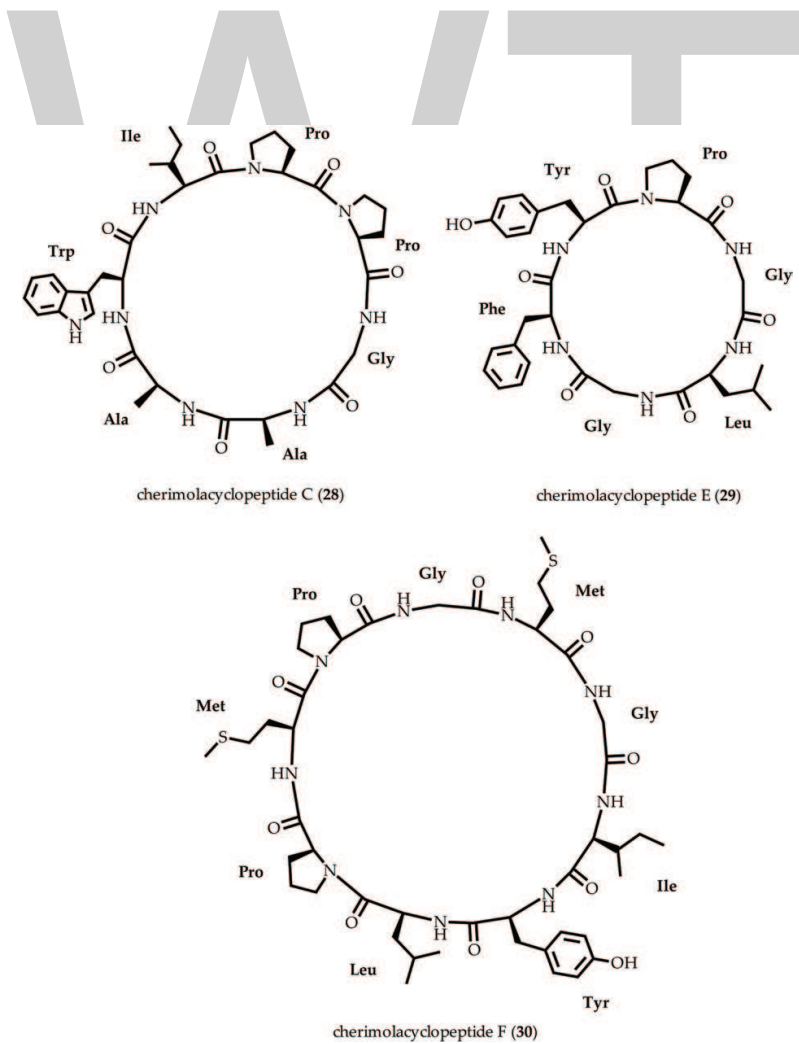


Figure 10. Cyclopeptides with anticancer activity isolated from the seeds of *A. cherimola* [54].

Arun et al. carried out a review related to the pharmacological potential of *A. cherimola* and found antidiabetic activity of leaf extract in streptozotocin (STZ)-induced hyperglycemia in rats [57]. The extract effect was evaluated by measuring fasting plasma glucose levels, serum insulin levels, serum lipid profiles, and body weight in normal rats. In addition, the measurement of liver glycogen levels and pancreatic lipid peroxidation levels was considered for diabetic rats [58]. A significant reduction in blood glucose level and a loss of body weight in diabetic rats were observed. Calzada et al. recently evaluated antihyperglycemic activity of *A. cherimola* leaves on alloxan-induced diabetic rats. The effect of ethanol extract at concentrations of  $300 \text{ mg}\cdot\text{kg}^{-1}$  was measured through the blood glucose level [59]. A computational molecular docking was also done to show the interaction of flavonoid **5**, **Figure 2**, with enzyme  $\alpha$ -glucosidase. Calzada et al. confirm that rutin (**5**) is the main compound responsible for antihyperglycemic activity of *A. cherimola* leaves. Before this study, Fale et al. found that **5** is the main compound in decoctions of *A. cherimola* leaves, responsible for inhibiting the HMG-CoA reductase activity and decreasing the cholesterol uptake in intestinal cells [60]. HMG-CoA reductase is the dependent enzyme of NADH, which controls the mevalonate pathway rate, which produces cholesterol.

## 2.5. *Annona reticulata*

*Annona reticulata* is a small deciduous tree that is grown in diverse parts of the world, including southern and eastern Asia, central and southern America, Australia, and western Africa. It grows up to 10 m in height. The leaves are narrow, lanceolate, alternating, and oblong, measuring approximately 10–20 cm long and 2–5 cm wide with conspicuous veins and a bad odor [61]. Extracts from different parts of *A. reticulata* have shown antioxidant and antimicrobial [62], antihyperglycemic [63, 64], and anticancer [65, 66] activities. Jamkhande et al. (2014) evaluated antioxidant and antimicrobial activity of root extract from *Annona reticulata*. The antioxidant activity was evaluated by DPPH free radical-scavenging assay and antibacterial and antifungal activities by agar cup method and poison plate method, respectively. The results of this study showed that methanol extracts of *A. reticulata* roots have significant antioxidant activity and a wide spectrum antibacterial and antifungal efficacy [61]. Ginguine et al. (2016) evaluated the anticancer activity of methanol extract from *Annona reticulata* leaves [64]. This activity was investigated for anticancer potential using sulforhodamine B (SRB) cytotoxicity assay against colon cancer (HCT15), human lung cancer (Hop65), and human hepatoma (HEPG2) cell lines. The extract exhibited a moderate anticancer effect against all the cell lines. Suresh et al. (2011) evaluated the anticancer activity of ethanol extract from *Annona reticulata* roots against melanoma cells in mice and *in vitro* activity on MDA-MB-435 human melanoma cells by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [65]. The ethanol extract exhibited significant *in vitro* and *in vivo* inhibitory activities against melanoma tumor cells. In both studies, the anticancer activity is attributed to the presence of acetogenins in the extracts used to perform the evaluations. Rahman et al. evaluated antihyperglycemic activity of methanol extract from *A. reticulata* leaves in Swiss albino mice [63]. The extracts were administered at doses of 50, 100, 200, and  $400 \text{ mg}\cdot\text{kg}^{-1}$ . The results showed lowered blood sugar in mice, and the authors suggest that the responsible compounds are acetogenins. Acetogenins have been reported from the plant seeds [66]. These compounds, including squamone (**31**), solamin (**32**), annononicin (**33**), and rolliniastatin 2 (**34**), have also been isolated from the leaves (**Figure 11**) [67]. Santos Lima et al.

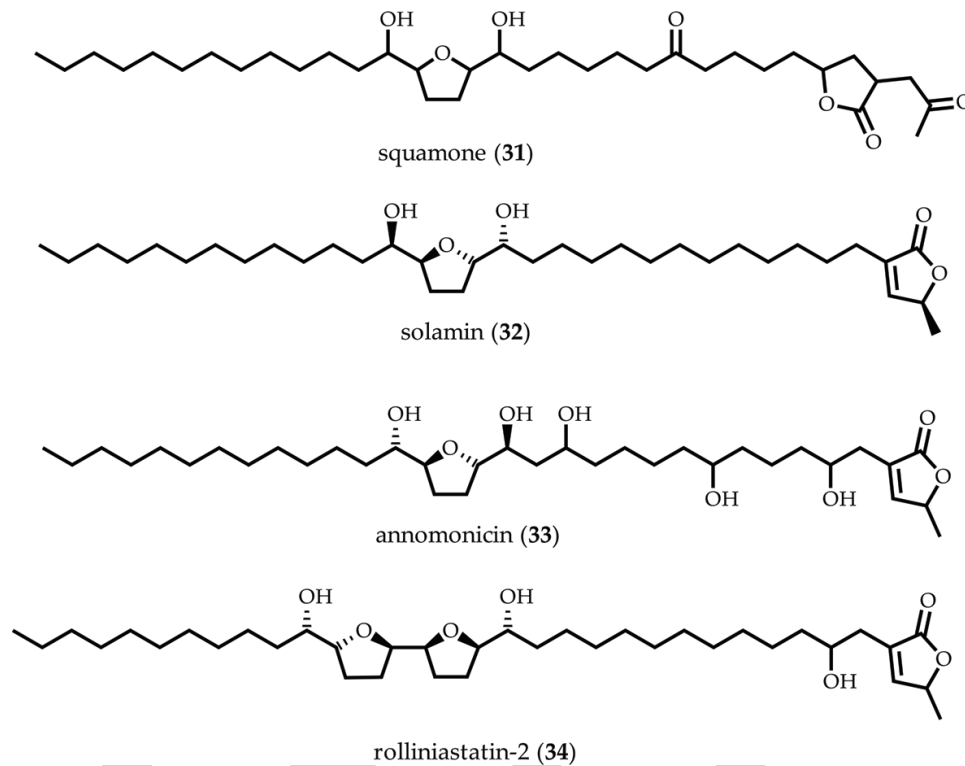


Figure 11. Acetogenins isolated from *A. reticulata* leaves [67].

evaluated the antioxidant activity of acetogenins and found that they have strong DPPH radical-scavenging activity, like that of ascorbic acid [68].

### 3. Conclusion

After analyzing the five most studied species of genus *Annona*, it can be concluded that they have great potential for the treatment of diseases associated with oxidative stress, including diabetes, hyperglycemia, cancer, and gastric ulcers, among others, because they are rich in antioxidant compounds. The most studied species of this genus are *A. muricata* and *A. cherimola*. The stems, trunks, and leaves of the trees are the most frequently studied and used in traditional medicine. Undoubtedly, the most representative bioactive compounds of the genus *Annona* are the acetogenins because they are abundant, mainly in the seeds of the fruit. There are several studies that show the anticancer properties of the genus. In addition, the phenolic compounds found in this genus are capable of inducing antioxidant properties in extracts. It is also possible to find alkaloids and cyclopeptides with properties similar to acetogenins in the species of this genus.

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## Conflict of interest

The authors have no conflict of interest to declare and are responsible for the content and writing of the manuscript.

## Ethical approval

This chapter does not contain any studies with human participants or animals performed by any of the authors.

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