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Antioxidant Activity of Plant Extracts Containing Phenolic Compounds

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Asian Journal of Organic & Medicinal Chemistry

Volume: 8 Year: 2023
Issue: 3–4 Month: July–December
pp: 29–34
DOI: https://doi.org/10.14233/ajomc.2023.AJOMC-P28926

Received: 3 November 2023
Accepted: 26 December 2023
Published: 9 January 2024

ABSTRACT

Polyphenols present in various plant and vegetable extracts possess enormous antioxidant, anti-inflammatory, anti-carcinogenic and anti-atherogenic qualities providing medical benefits. Antioxidants scavenge free radicals and other reactive oxygen species (ROS) by interrupting their mechanism of oxidation. Since free radicals cause neurological illnesses, cancer, heart disease, diabetes, atherosclerosis, and lipid and DNA damage, inhibiting their formation has recently been the subject of several medical investigations. Present studies described the determination of total phenolic content and DPPH activity of a large number of commonly used plant and vegetable extracts utilizing Folin-Ciocalte and Soler-Rivas procedures. This study aims to conduct a biochemical analysis of the therapeutic effects of okra, tamarind, and other phenolic compounds. The comparative data provided in this work for antioxidant and polyphenolic substances is expected to be highly valuable for readers who are interested in this subject.

KEYWORDS

Polyphenols, Reactive oxygen species, Diabetes, Antioxidants, Advanced glycation end products (AGEs).

INTRODUCTION

The presence of phenolic substances in many common fruits and vegetables has been at the forefront of the recent scientific studies because of their antioxidant abilities. Antioxidants are important as they scavenge free radicals and other reactive oxygen species (ROS) to prevent an excess, known as oxidative stress. The inhibition of free radicals and ROS production has been the focus of many medical studies as they precipitate many diseases such as cancer, heart disease, diabetes, atherosclerosis and neurodegenerative diseases by causing the direct damage of proteins, lipids and DNA [1-4]. ROS particularly damage proteins by driving the reaction forming advanced glycation end products (AGEs). AGEs are the end products of non-enzymatic glycation reaction of proteins, known as the Maillard reaction, wherein a sugar aldehyde reacts with the N-terminal of free amino groups in proteins, thus distorting the protein. Advanced glycation end products (AGEs) and oxidative stress are major components of the pathogenesis of diabetes related bodily degeneration, atherosclerosis and cardiovascular disease [3-5]. As antioxidants, polyphenols scavenge free radicals by interrupting the mechanism of their oxidation. They also

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prevent the formation of other ROS by inhibiting the enzymes that drive the process or chelating the metal traces used in their production [6]. Flavonoids, phenolic acids and tannins, as well as other phenolic compounds, are extremely important in plant development as well, providing defense against plant injury or infection. In addition, anthocyanins are commonly found to provide pigmentation in fruits, vegetables, flowers and leaves. Foods attributed to being abundant in polyphenols include blackberries, plums, pomegranate, apples, tea and wine. According to a study done by the University of Barcelona [7], a daily intake of 650 mg or more of polyphenols proved to reduce mortality by 30% compared to a daily intake of 500 mg or less. However, an average American diet a person only consumes 213 mg of phenols per day [8].

Previous studies have quantified the phenolic content and antioxidant ability of different okra tissues (Table-1) and identified okra seeds to be the most potent free radical scavenger [8-11]. In 2012, our group [12] conducted research that defined and established the capacity of okra (*Abelmoschus esculentus*) seeds (Fig. 1a) to effectively hinder protein glycation. Additionally, we discovered the primary flavonoids found in the extracts of okra seeds that are responsible for their ability to scavenge free radicals.

TABLE-1
DETERMINATION OF PHENOLIC AND
ANTIOXIDANT ACTIVITY IN OKRA VEGETABLE

Okra parts	Phenolic (mg/g)	Antioxidant activity
Skin	0.20	0.22
Skeleton	0.09	0.23
Seed	2.85	7.55
Stem	0.13	0.59

Inhibition of AGE formation was determined by comparing the effect of okra seed extract on the glycation of bovine serum albumin (BSA). BSA was incubated both with glucose and okra seed extract and with just glucose. The anti-glycation effects of the okra extracts on BSA were also measured by SDS-PAGE analysis and nano-drop spectrophotometer. Okra seed extracts showed a significant inhibitory potential (45-50%) at 0.1 mg/mL concentration in a dose dependent manner. To extract the biologically active compounds present in the okra seeds, the seeds were treated with methanol and heated in a microwave. The major flavonoids (Fig. 2) present in the okra seed extract were identified by LC-MS [11].

In view of the substantial anti-glycation/antioxidant capacity of okra seeds and paralleling high concentration of bioactive phenolic compounds. Dayal *et al.* [12] acknowledged the necessity of quantifying these two variables across a range of natural foods and food components in order to identify prospective therapeutic options for diabetes, atherosclerosis, neurodegenerative illnesses and cardiovascular diseases. By measuring the free radical scavenging activity of present phenolic compounds through various assays, the antioxidant capabilities of natural foods and food ingredients can be determined. The present study conducts several DPPH assays and total phenolic content assays to investigate the antioxidant properties of okra (*Abelmoschus esculentus*), taro (*Colocasia esculenta*), red onion (*Allium cepa*), loofah (*Luffa acutangula*), cauliflower (*Brassica oleracea botrytis*), papaya (*Carica papaya*), spinach (*Spinacia oleracea*), celeste figs (*Ficus carica*), kalamata figs (*Ficus carica*), turmeric (*Curcuma longa*), banana (*Musa cavendishii*), apple banana (*Musa sapientum*), watermelon (*Citrullus lanatus*), beet (*Beta vulgaris*), asparagus (*Asparagus officinalis*), dates (*Phoenix dactylifera*), tamarind (*Tamarindus indica*) and plumcot (Hybrid: *Prunus cerasifera* and *P. armeniaca*).

EXPERIMENTAL

The chemicals *viz*. methanol, Folin-Ciocalteu's phenol reagent, sodium carbonate and diphenyl picrylhydrazyl, Trolox were purchased from Aldrich Chemical Co, USA.

Plant materials: The different plant materials were purchased from the local market in New Jersey, USA. All plants were dissected into parts such as skin, seeds, stem, tissue and skeleton among others. Individual plant material was weighed. Methanol was added to plant material on a 1 mL into 1 g plant material basis. Extractions were left overnight for at least one night and then further microwaved (without methanolic extract evaporation). Extracts were centrifuged for at least 10 min at 5000 rpm (further centrifugation was conducted if pellet was not dissolved) in Sorvall RB-5C. Extracts were stored in freezer at -18 ºC and used and analyzed by different methods within 3 months of collection and preparation.

Determination of total phenolics: Total phenolic content is a good indicator of the potential level of antioxidant ability of food. The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure [13] and chlorogenic acid (1 mg/mL) was used to standardize the procedure. Samples (1-200 µL) were introduced into test tubes and

(a) (b) (c)

Fig. 1. Okra (*Abelmoschus esculentus*) seeds (a), vegetable (b) and plant (c)

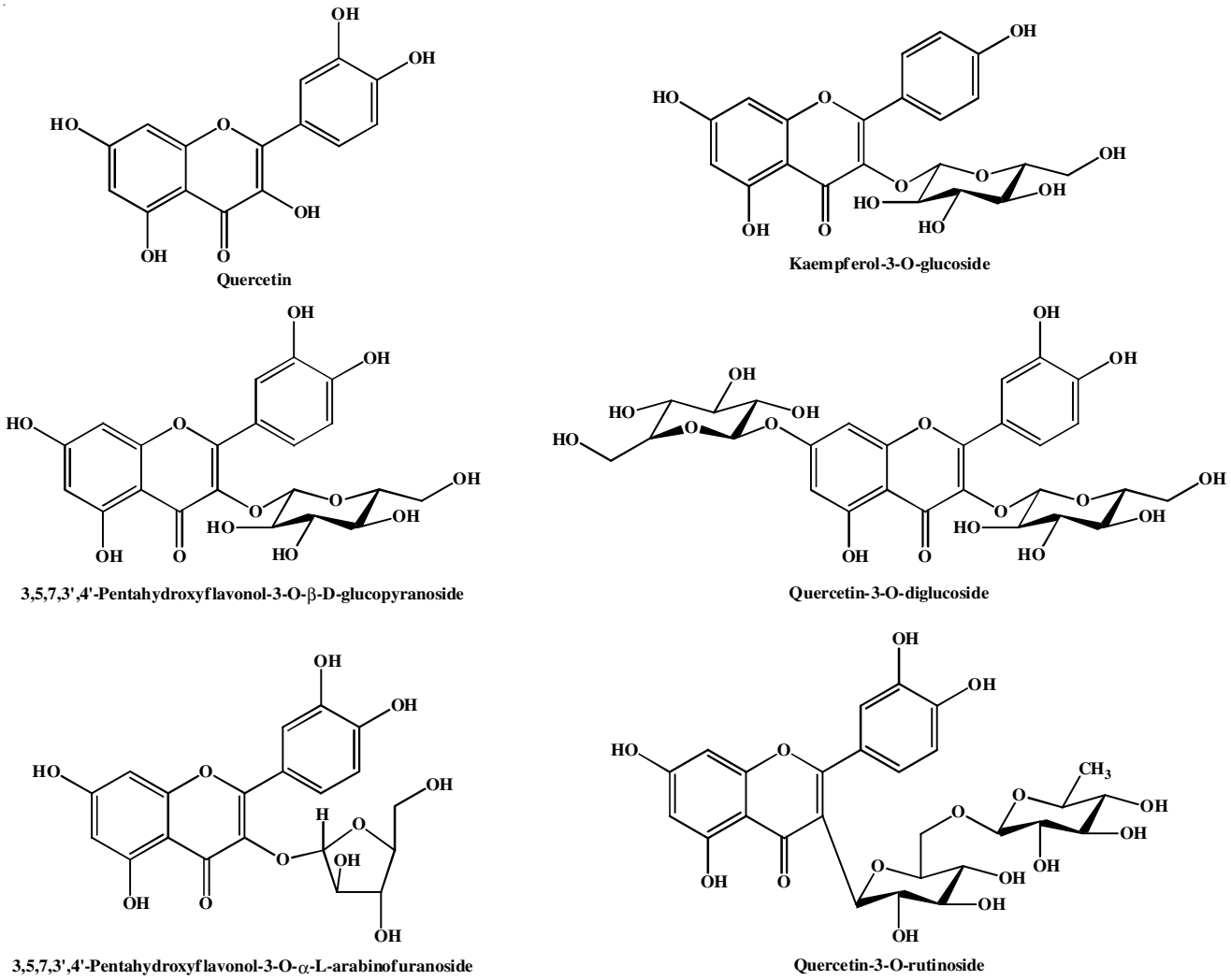

Fig. 2. Chemical structures of flavonoids identified from okra seed extract

then 100 µL Folin-Ciocalteu's reagent and 200 µL of sodium carbonate were added. Absorption at 725 nm was measured by UV-Vis spectroscopy. The total phenolic content was expressed as chlorogenic acid equivalents (CGAE) in milligrams per gram plant material.

The CGAE in mg/g plant material was calculated by the using the following equation:

$$\frac{\text{Sum of O.D. of standards}}{\text{Total volume of chlorogenic acid used}} = \text{Absorbance for 1 mL}$$

$$\frac{\text{O.D. of the singular unknown}}{\text{Volume of unknown} \times \text{Absorbance for 1 mL}} = \text{mg/mL}$$

DPPH radical scavenging activity: In this study, the DPPH scavenging activity was determined according to Soler-Rivas method [14] by adding 0.04 mm DPPH 0.5 mL to each test tube and Trolox (1 mg/mL) was used to standardize the procedure. Samples (1-200 µL) were introduced into test tubes and then methanol was added to give each test tube a total volume of 2 mL. The control sample contained only methanol and DPPH. The reaction was left overnight and absorption at 515 nm was measured by UV-Vis spectrophotometer. The antioxidant activity was expressed as µg Trolox per gram plant material.

Trolox per gram plant material was calculated by the following equations:

$$\frac{\text{O.D. Set 1} + \text{O.D. Set 2}}{2} = \text{Average O.D.}$$

Average O.D. of RB – Average O.D. of unknown = ΔO.D.

$$\frac{\Delta\text{O.D. Trolox}}{\mu\text{g Trolox}} = \frac{\text{Absorbancy}}{\mu\text{g Trolox}}$$

$$\frac{\mu\text{g Trolox}}{\text{g Wet wt.}} = \Delta\text{O.D. of unknown} \times \frac{1}{\text{mL Volume known}} \times \text{mL } CH_3OH \text{ used to extract} \times \frac{1}{\frac{\Delta\text{O.D. Trolox}}{\mu\text{g Trolox}}} \times \frac{1}{\text{g Wet wt.}}$$

UV/Vis spectroscopic analysis: To quantitatively determine the different analytes, the NanoDrop 1000 was used to conduct UV/Vis spectroscopy. The machine was initialized using a 1.5 µL of distilled water and λ_1 was set to 220 nm and λ_2 was set to 750 nm. The maximum absorbance was set to 5.0 blanked against 1.5 µL of methanol. A 1.5 µL of unknown extract was placed on the sampling pedestal and the wavelength/absorbance spectrum of the sample was measured.

RESULTS AND DISCUSSION

Food extracts rich in phenolic content are available commercially as dietary supplements. The supplements, however, only contain the polyphenols present in one specific part of the food it comes from, neglecting the abundant polyphenols in its other parts and the other essential nutritional compounds present in the food. Specifically, it does not provide the consumer with the dietary fibers present in fruits and vegetables. Dietary fibers help reduce the risk of heart disease, diabetes and colon cancer by increasing glucose tolerance and insulin sensitivity [15,16].

Total phenolic content: A total phenolic content analysis was performed on the extracts in order to determine the plants and their components that exhibited the highest levels of antioxidant activity. The amount of total phenolics varied greatly between the species. Loofah tissue displayed the least amount of phenolic content at 0.044 CGAE/g of plant weight (Table-2). Tamarind seeds had the most amount of phenolic content at 62.220 CGAE/g of plant weight (Table-2). Okra, red onion, apple banana, plumcot and tamarind were the foods that showed significantly high levels of phenolic content. The rest of the studied foods had less than 2 CGAE/g of plant weight (Fig. 3).

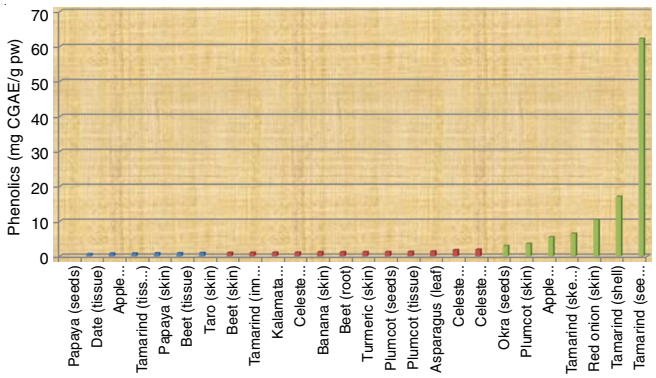

Fig. 3. 26 Plant extracts with highest total phenolic content (CGAE in mg/g plant material). Data values for plant extracts with higher phenolic content than okra seeds are indicated on the graph

Antioxidant activity of extracts: Antioxidant activity can be found in a wide variety of actions, such as chelation of transition metals, transfer of hydrogen or single electron to radicals (ROS), inhibition of oxidation enzymes, singlet oxygen deactivation or enzymatic detoxification of ROS [17]. The extracts were examined with regard to scavenging capacity toward DPPH. The white tissue of the watermelon reduced the least amount of DPPH, alluding to a low antioxidant capability (4.516 µg Trolox/g of plant weight) (Table-2). Tamarind seeds, however, had the highest antioxidant capability (9562.7 µg Trolox/g of plant weight). Similar to the total phenolics assay, the DPPH assay results (Fig. 4) also suggest that okra, red onion, plumcot and tamarind have the highest antioxidant abilities (< 1500 µg Trolox/g of plant weight). The efficacy of the functionality of phenolic compounds and dietary compounds in human health has been heavily interconnected. Thus, foods such as tamarind, okra and plumcot, which are high in both, should be added to regular diet.

Similarly, it was also observed that leafy greens (*e.g.* spinach, asparagus, *etc.*) have higher phenolic content and antioxidant activity in their leaves compared to stem (Table-2). Also, data analysis showed that plants and fruits with vivid skins (plumcot, red onions) have higher phenolic content and antioxidant activity in the skin due to flavonoids than their respective tissue (Table-2). Adding these plants and fruits to a regular diet may further bolster the antioxidant intake in the body.

Other than polyphenols, other common antioxidants require for human body are vitamin E, vitamin C, β-carotene

TABLE-2
TOTAL PHENOLICS ASSAY AND DPPH ASSAY RESULTS

Plant extract		Phenolics (mg CGAE/g pw)	DPPH (µg Trolox/g pw)
Okra (*Abelmoschus esculentus*)	Skin	0.421	530.123
	Skeleton	0.133	355.041
	Seeds	2.939	1576.301
	Stem	0.155	270.081
Taro (*Colocasia esculenta*)	Skin	0.955	983.409
	Tissue	0.157	930.011
Loofah (*Luffa acutangula*)	Skin	0.239	353.781
	Tissue	0.044	292.715
	Stem	0.208	237.218
Red onion (*Allium cepa*)	Stem	0.064	123.307
	Skin	10.429	2285.199
	Tissue	0.068	86.228
Cauliflower (*Brassica oleracea botrytis*)	Stem	0.295	362.965
	Leaf	0.115	81.235
	Tissue	0.129	114.129
Papaya (*Carica papaya*)	Skin	0.871	825.091
	Tissue	0.068	73.033
	Seeds	0.590	966.819
Spinach (*Spinacia oleracea*)	Leaf	0.530	418.943
	Stem	0.195	296.171
Celeste fig (*Ficus carica*)	Skin	1.916	1118.056
	Seeds	1.086	998.256
	Tissue	1.771	1114.649
Kalamata fig (*Ficus carica*)	Skin	0.266	576.734
	Seeds	0.143	144.102
	Tissue	1.069	847.370
Turmeric (*Curcuma longa*)	Skin	1.221	1145.408
	Tissue	0.448	666.848
Banana (*Musa cavendishii*)	Skin	1.187	815.821
	Tissue	0.271	521.199
Apple banana (*Musa acuminata*)	Stem	0.529	983.426
	Skin	5.465	1318.694
	Tissue	0.791	1315.640
Watermelon (*Citrullus lanatus*)	Skin	0.430	327.445
	Seeds	0.071	100.463
	Red tissue	0.050	15.741
	White tissue	0.209	4.516
Beet (*Beta vulgaris*)	Root	1.200	1257.712
	Skin	1.008	920.319
	Tissue	0.883	854.722
Asparagus (*Asparagus officinalis*)	Leaf	1.383	1196.754
	Stem	0.576	468.823
Date (*Phoenix dactylifera*)	Tissue	0.631	799.099
	Seeds	0.484	734.556
Plumcot (*Hybrid: Prunus cerasifera & P. armeniaca*)	Skin	3.589	3382.200
	Seeds	1.234	2762.304
	Tissue	1.291	2377.807
Tamarind (*Tamarindus indica*)	Shell	17.066	9492.856
	Skeleton	6.482	6715.869
	Tissue	0.809	808.346
	Inner skin	1.030	1935.177
	Seeds	62.220	9562.700

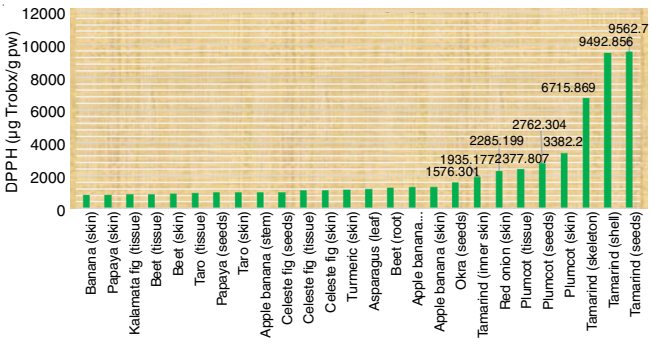

Fig. 4. 26 Plant extracts with greatest amount of scavenging by DPPH (µg Trolox/g plant material). Data values for plant extracts scavenged by DPPH more than okra seeds are indicated on the graph

and lycopene (Fig. 5). All four of these antioxidants are implicated in reducing the progression of cardiovascular disease by inhibiting the glycoxidation of LDL, which delays or prevents atherogenesis [18-20]. Polyphenols, if widely available in regular diet, have been found to be a viable replacement and can possibly be a more effective antioxidant than vitamin E. β-Carotene is abundantly found in orange, root vegetables (carrots, sweet potatoes) and leafy green vegetables (spinach) [21].

A strong but not absolute correlation between total phenolic content and antioxidant activity was observed upon data analysis ($R^2 = 0.7407$) (Fig. 6). Thus, it can be assumed that other natural products present in the plant extracts, besides phenolic compounds, may also be involved in the scavenging of free radicals. The antioxidant properties of polyphenols and similar other nutritional compounds present in the food work synergistically to enhance the overall effectiveness in promoting health. This result corroborates the idea that adding certain foods to regular diet will be more helpful in reducing the risk of certain diseases than taking extract supplements instead. It should be emphasized that in the present study, the comparative chemical and biological examinations of the various plant species were undertaken for the first time. The preliminary results of their antioxidant activity certainly suggest that certain fruits and vegetables can be used as potent natural antioxidants. Tamarind, okra and plumcot, in particular, have been found to contain a high concentration of biologically active phenolic compounds that exhibit significant scavenging capabilities.

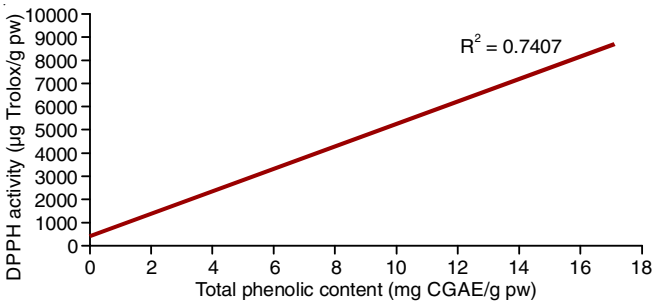

Fig. 6. Correlation between total phenolic content and antioxidant activity, without (tamarind seeds)

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Jasmine Desai and Leka Racharla in the preparation of this manuscript. The authors also appreciate the support provided by the Rising Star program.

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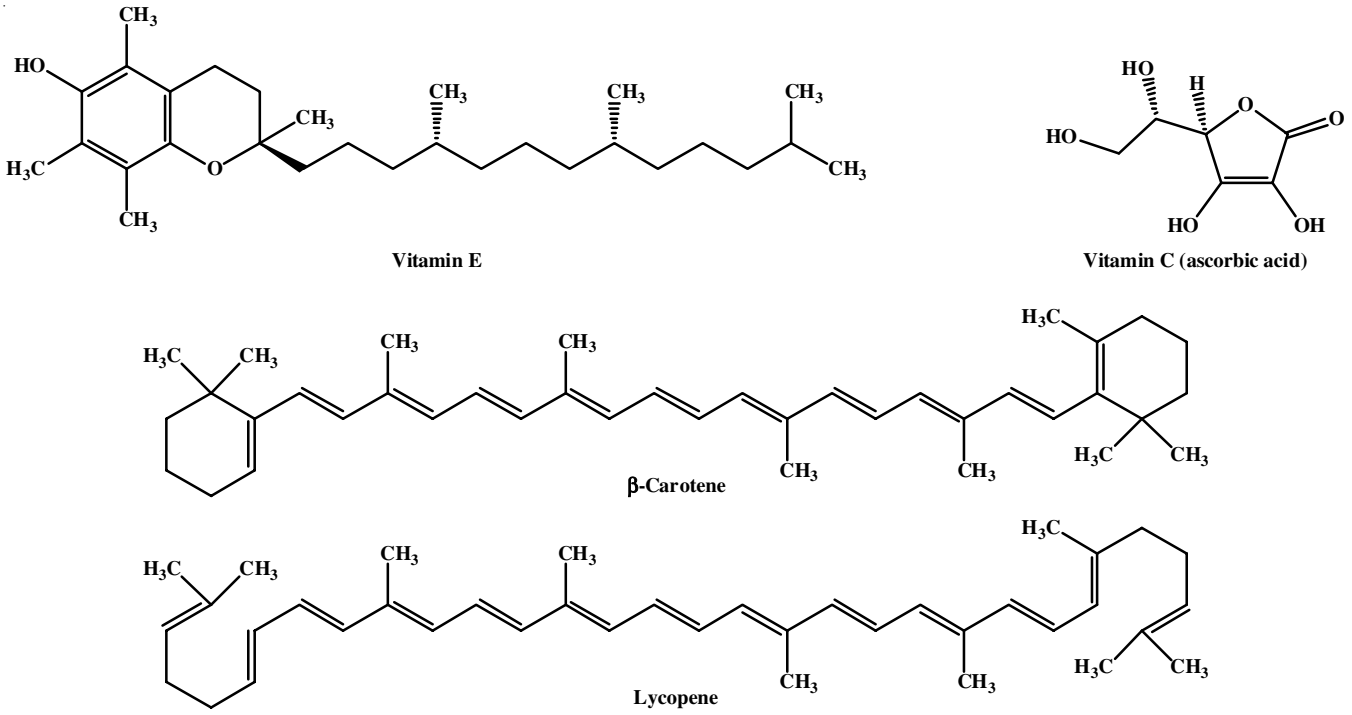

Fig. 5. Common dietary antioxidants other than polyphenols

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