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REVIEW ARTICLE

Review On-Natural Compounds Used For Antioxidant Activity

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ABSTRACT

Natural antioxidants compounds are present in leaf, rhizome, stem, fruits, vegetables, tea etc. They are recognized as important compounds in conferring stability against oxidation in vitro. Natural antioxidant compounds can be classified into Exogenous antioxidants and Endogenous antioxidants, Enzymatic and Non-Enzymatic antioxidants. Naturally occurring antioxidants compounds are carotenoids, tocopherols, a hydrophilic group, phenolic acids, anthocyanins, flavonoids and tannins. In this we reviewed the most important groups of natural antioxidants, and methods for in vitro assessment of the antioxidant activity of natural compounds.

Keywords: Natural antioxidants, Plants, Extracts, Polyphenols, Antioxidant activity, Oxygen

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INTRODUCTION

Oxygen is most prevalent element in the earth's crust .It exist in air as a diatomic molecule. Reactive oxygen species are involved in several disorders. The harmful action of the free radicals can, however, be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism. Oxidative stress is considered to play a pivotal role in the pathogenesis of antiaging & several degenerative diseases , such as atherosclerosis, cardiovascular disease, cancer[1,2]. Therefore, plant derived antioxidants are now receiving a special attention. A large number of phenolic compounds present in vegetable, foods, such as fruits and nuts, peanuts, essential oils, aromatic plants have been reported to possess good antioxidant properties [3,4].

Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells. Although almost all organisms possess antioxidant defense and repair systems to protect against oxidative damage, they cannot prevent the damage entirely. Interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, replacing synthetic antioxidants which are often restricted due to carcinogenic effects [5]. Aromatic and medicinal plants source natural antioxidants like polyphenols and essential oils which are secondary metabolites [6].

Natural compounds and antioxidant activity

The main classes of natural antioxidant compounds (Figure 1), as well as methods for determination of *in vitro* antioxidant activity of natural products and plant extracts are discussed. Antioxidant activity is an important and fundamental function in life systems. Many other biological functions such as the anti-mutagenic, anticarcinogenic, and anti-aging responses, originate from this property [5, 7, 8].

The main classes of antioxidant compounds [9]

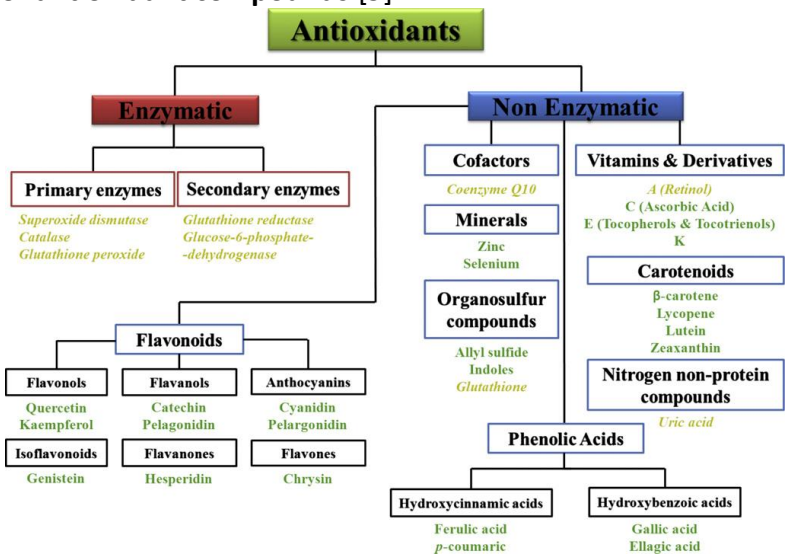

Figure1. Natural antioxidants separated in classes

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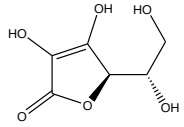
Green words represent exogenous antioxidants, while yellow ones represent endogenous antioxidants.

Natural antioxidants [10, 11]

Ascorbic acid

As an oxygen scavenger, ascorbic acid serves as a reducing agent. It transfers its hydrogen atoms to oxygen, making the oxygen unavailable for further reaction. In the process, the ascorbic acid is oxidized to dehydroascorbic acid, which in turn can function as an oxidizing agent by removing hydrogen from reducing agents such as sulfahydryl groups. Ascorbic acid and dehydroascorbic acid are thus reversible forms of vitamin C and both have physiological activity. Further, ascorbic acid may exert a chelating action, binding heavy chemical, if present will promote oxidation. When chelated with heavy metals, ascorbic acid loses its physiological vitamin activity. Ascorbic acid (Figure 2.) functions as antioxidant.

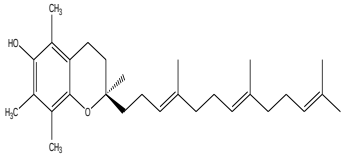


ascorbic acid

Figure 2

Tocopherols

Vitamin E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols. There are many different forms of vitamin E, of which γ-tocopherol is the most common in the North American diet. γ-Tocopherol can be found in corn oil, soybean oil, margarine and dressings. In the North American diet, α-Tocopherol(Figure3.), the most biologically active form of vitamin E, is the second most common form of vitamin E. This variant of vitamin E can be found most abundantly in wheat germ oil, sunflower, and safflower oils. It is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation.



Alpha tocopherol

Figure 3.

Carotenoids

Carotenoids are powerful anti-aging antioxidants, protecting the cells of the body from damage caused by free radicals. They work in combination with each other and other antioxidants, including the network antioxidants vitamin C, vitamin E, lipoic acid, and the

antioxidant enzymes produced in our body: superoxide dismutase, catalase and glutathione peroxidase.

Carotenoids protect lipids against peroxidative damage by inactivating singlet oxygen (without degradation) reacting with hydroxyl, superoxide, and peroxyl radicals. The β-carotene is the most abundant of the carotenoids and widely used in therapies. It is almost completely insoluble in water but readily soluble in hydrophobic environments, and slightly polar solvents. β-carotene (Figure 4.) is highly reactive with electrophiles and oxidants. While many studies have shown β-carotene inhibition of lipid auto-oxidation in biological tissues and food, few details of the kinetics or mechanism of these reactions have been revealed. Lycopene (Figure 4.) is also well known for its antioxidant activity.

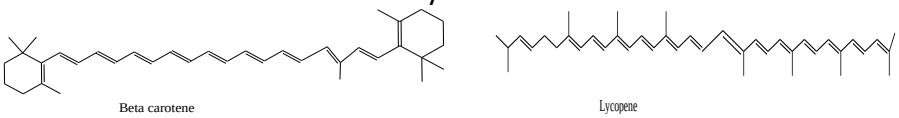


Figure 4. Carotenoids

Phenolic compounds [12]

Phenolic compounds (Figure 5.) are an important group of secondary metabolites, which are synthesized by plants as a result of plant adaptation to biotic and abiotic stress conditions (infection, wounding, water stress, cold stress, high visible light). Protective phenyl propanoid metabolism in plants has been well documented. In recent years phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenolics is principally based on the redox properties of their hydroxy groups and the structural relationships between different parts of their chemical structure. Epidemiological data have indicated beneficial effects of antioxidant compounds in the prevention of a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders.

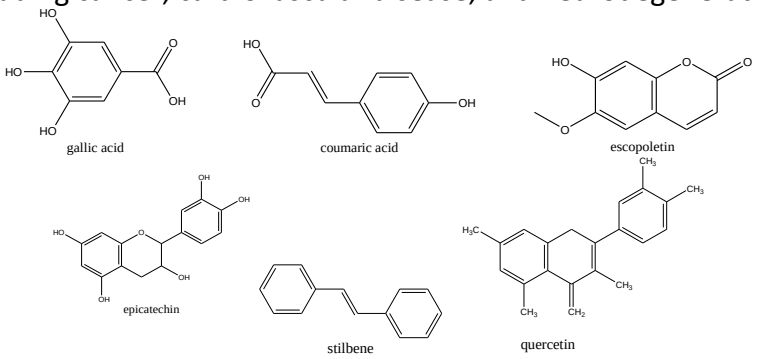


Figure 5. Chemical structures of some phenolic compounds.

Flavonoids

Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial,

antiviral, anti-inflammatory, anticancer, and anti-allergic activities. With their biological activity, flavonoids are important components of the human diet, although they are generally considered as non-nutrients. Sources of flavonoids are foods, beverages, different herbal drugs, and related phytomedicines. Flavonoids are an important class of phenolic compounds, and have potent antioxidant activity. The antioxidant property of flavonoids was the first mechanism of action studied with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases.

The antioxidant mechanism involves suppressing reactive oxygen formation, by inhibiting enzymes, chelating trace elements involved in free-radical production, scavenging reactive species, and up-regulating and protecting antioxidant defences .More than 4000 flavonoids have been identified, and the number is still growing. Flavonoid scan be further divided into chalcones, anthocyanins, flavones, isoflavones, flavanones, flavononols and flavanols. The chemical structures of the main classes of flavonoids are shown in (Figure 6.)
Anthocyanins are probably the largest group of phenolic compounds in the human diet, and their strong antioxidant activities suggest their importance in maintaining health.

When consumed regularly, by humans, these flavonoids have been associated with a reduction in the incidence of diseases, such as cancer and heart disease.

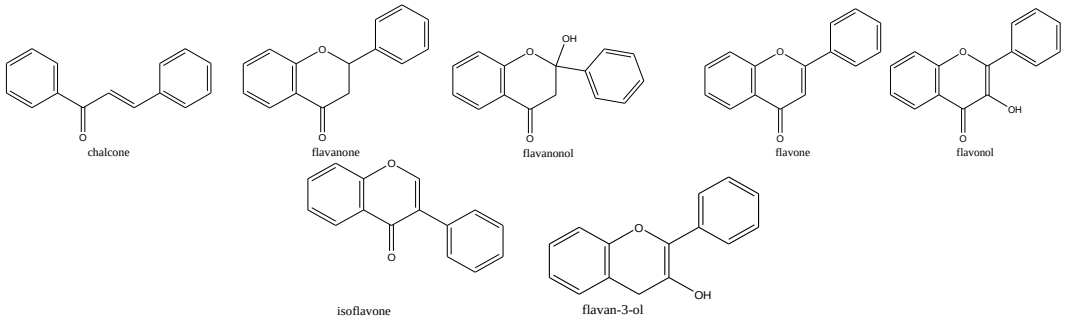


Figure 6. Basic structures of the main classes of flavonoids.

Essential oils

Essential oils also called volatile or ethereal oils are aromatic compounds, oily liquids obtained from different plant parts, and widely used as food flavors. Essential oils are complex mixtures comprised of many single compounds. Chemically they are derived from terpenes, and their oxygenated compounds. Essential oils have been useful in food preservation, aromatherapy and the fragrance industry.

In nature, essential oils have an important role in protecting plants. They serve as antibacterial agents, antivirals, antifungals, and insecticides, and also against the action of herbivores. They sometimes attract insects to help the spread of pollen or repel other unwanted insects. They are liquid, volatile, transparent, rarely coloured, soluble in organic solvents, and have lower densities than that of water. Synthesized by all organs of the plant, Such as buds, flowers, leaves, stems, seeds, fruits, roots and bark, they are stored in secretor cells, cavities, channels, epidermal cells, and glandular trichomes.

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Terpenoids form a large and structurally diverse family of natural products derived from isoprenoid units C5. These compounds have carbon skeletons being multiples of n (C5), and are classified as hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes(C20), sesterpenes (C25), triterpenes (C30) and tetraterpenes (C40) .

Monoterpenes are primary components of essential oils, and the effects of many medicinal herbs have been attributed to them. Among various monoterpenes that have antioxidant activity are carvacrol, thymol, γ-terpinene and terpinolene, linalool, and isopulegol, among others (Figure 7.).

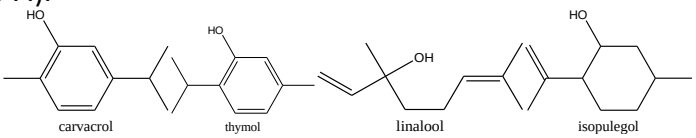

Figure 7. Chemical structures of monoterpenes with antioxidant activity.

Antioxidant activity of aromatic plants [13]

Consumer interest in natural food additives, have reinforced the interest in natural antioxidants. Herbs and spices are harmless sources for obtaining natural antioxidants.

Table1. Aromatic plants which shows antioxidant activity

Aromatic Plants	Plant Parts	Antioxidant Sources
Salvia officinals	Leaves, stem	Methanol Extract, Acetone Extract
Calendula officinals	perianthus	Methanol Extract
Melilotus officinals	Leaves, stem	Chloroform Extract
Lavandulaangus lifolia	Leaves,stem, blossoms	Methanol Extract
Geranium macrorrhizum	Leaves(growing stage)	Methanol Extract
P.guajava L.	Leaf	Methano Extract
Curcuma amada	Rhizome	Ethyl Acetate Extract , Methanol Extract
Zingiber officinals	Stem, Rhizome	Methanol Extract, Chloroform Extract
Curcuma longa	Rhizome	Methanol Extract
Curcuma caesia	Rhizome	Methanol Extract
Curcuma indica	Rhizome	Chloroform Extract, Methanol Extract

A very important compound in herbs of *Lamiaceae* family is rosmarinic acid, showing high scavenging DPPH potential, this being related to the presence of four hydroxyl groups in its molecule . Oregano is particularly rich, 55000 ppm, in this compound and peppermint and lemon balm also contain high amounts, about 30000 and 37000 ppm, respectively .*Curcuma zedoaria* (Berg.) Rosc. (*Zingiberaceae*) has long been used as achinese folk medicine. The essential oil of its dried rhizome was moderate to Good in antioxidant activities by three different methods, good in reducing power and excellent in scavenging effect on 1, 1-diphenyl-2-picrylhydrazylradical but low in chelating effect on ferrous ion. Although epicurzerenone and Curzerene were found with moderate to good antioxidant activity, the compounds 5-isopropylidene-3,8-dimethyl-1(rH)-azulenone was responsible for better antioxidant properties. However, natural curcuminoids were also isolated from *Curcuma longa* and showed

reducing antioxidant activities. Many aromatic plants have antioxidant activity [14, 15] (Table 1.)

Methods of antioxidant activity assessment for natural products [16]

Total antioxidant activities of the Plant extracts can not be evaluated by any single method, due to the complex nature of phytochemicals. Thus, two or more methods should always be employed in order to evaluate the total antioxidative effects of natural products.

DPPH assay [17, 18]

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was carried out according to the methods described earlier [14, 15]. Briefly, 1 mL of DPPH· (500 mM in ethanol) was added to a mixture of test samples (10-100 mL) and 0.8 mL of Tris-HCl buffer (pH=7.4). After vigorous shaking, the mixture was allowed to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (UV- 160A, Shimadzu Co, Japan). BHA was used as positive control. Radical scavenging potential was expressed as EC50 value, which represents the sample concentration at which 50 % of the DPPH radicals were scavenged. The method is influenced by the solvent and the pH of the reactions. The antioxidants BHA, BHT and Trolox® can be used as references in the experiments.

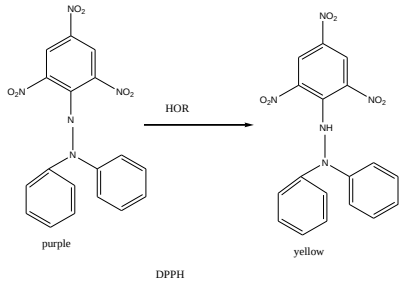

Figure 8. Radical and non-radical forms of DPPH.

The electron donation ability of natural products can be measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured (Figure 8.) solution bleaching. The anti-radical activity (three replicates per treatment) is expressed as IC50 (µg/ml), the concentration required to cause a 50% DPPH inhibition. The presence of the phenolic hydroxyls appears essential for scavenger properties.

Ferric reducing antioxidant power (FRAP) assay [19]

The method for determining the ferric reducing ability of the essential oil has been taken in modified form from the method used the method used by Chaiyasut *et. al.*[20] and Jukic *et. al.*[21]. Briefly FRAP reagent has been prepared by mixing 0.1 M . Acetate buffer (pH 3.6) with 10mM 2, 4, 6-tripyridil-s-triazine(TPTZ) and 20 mM ferric chloride in the ratio of 10:1:1 (v:v:v), then 3 mL of the Frap Reagent has been added

to 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM of samples of essential oil (taken in triplicate), and left at room temperature for 5 min. until the reaction is complete. The reacted sample were read at 593 nM using ferrous sulphate (with same concentration) as standard. An increase in the absorbance of the reaction mixture indicate an increase in the reducing power of sample.

Determination of lipid peroxidation inhibitory activity [22]

Lipid peroxidation inhibitory activity was carried out according to the method of Duh and Yen as described earlier [23]. In brief, lecithin (in 3 mg/mL of phosphate buffer, pH=7.4) was sonicated in a UP 50H ultrasonic processor (Hielscher Ultrasonics GmbH, Teltow, Germany).The test samples (100 mL) were added to 1 mL of sonicated lecithin, and then 10 mL of $FeCl_3$ (400 mM) and 10 mL of L-ascorbic acid (400 mM) were added to induce the lipid peroxidation. The reaction was stopped by adding 2 mL of 0.25 M HCl containing 15 % TCA and 0.375% TBA, after incubation for 1 h at 37 °C. The absorbance of the supernatant was measured at 532 nm. BHA was used as positive control. Inhibitory activity was expressed as EC50 value, which is sample concentration at which 50 % of lipid peroxidation was inhibited.

ABTS method

The 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), commonly called ABTS. It is based on the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce a radical cation. The improved method generates a blue/green ABTS**.** Chromophore via the reaction of ABTS and potassiumpersulfate. It is now widely used. Along with the DPPH method, the ABTS radical scavenging method is one of the most extensively used antioxidant assays for plant samples.

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm. Decolourisation assays measure the total antioxidant capacity in both lipophilic and hydrophilic substances. The effects of oxidant concentration and inhibition duration, of the radical cation's absorption are taken into account when the antioxidant activity is determined. Trolox is used as a positive control. The activity is expressed in terms of Trolox-equivalent antioxidant capacity for the extract or substance (TEAC/mg).

β-carotene bleaching test [19]

The β-carotene/linoleic acid oxidation method evaluates the inhibitory activity of free radicals generated during the peroxidation of linoleic acid. The method is based on Spectrophotometric discoloration measurements or (oxidation) of β -carotene-induced oxidative degradation products of linoleic acid. This method is suitable for plant samples. Theβ-carotene bleaching method is based on the loss of β-carotene's yellow colour due to its reaction with radicals formed by linoleic acid oxidation when in an emulsion. The rate of the β-carotene bleaching can be slowed in the presence of antioxidants. There action can be

monitored by spectrophotometer, β-carotene loss of staining at 470 nm, with intervals of 15 min for a total time of 2h. The results are expressed as IC50 (µg/ml), the concentration required to cause a 50%β-carotene bleaching inhibition. Tests are realized in triplicate. The results can be compared with synthetic standards such as BHA, BHT and Trolox®, or natural, such as gallic acid and quercetin.

ORAC assay [6]

Oxygen Radical Absorbance Capacity method**.** When a free-radical generator such as an azo-initiator compound is added to a fluorescent molecule such as beta-phicoerythrin or fluorescein and heated, the azo-initiator produces peroxyl free radicals, which damage the fluorescent molecule, resulting in the loss of fluorescence. Curves of fluorescence intensity vs time are recorded, and the area under the curves with and without addition of an antioxidant is calculated and compared to a standard curve generated using the antioxidant (±)-6-hydroxy-2,5,7,8- tetramethylchromane-2-carboxylic acid, a water-soluble vitamin E analog trademarked by Hoffman- LaRoche as *Trolox*™.

Reducing power assay [24]

In this assay, the yellow colour of test solution changes into green depending on the reducing power of the test specimen.The reductants (of the solution) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Fe^{2+} can be monitored by absorbance measurement at 700 nm. In this method, the sample is mixed in 1 ml of methanol with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%). The mixture is incubated at 50 ^{o}C for 20 min. 5 ml of trichloroacetic acid (10%) are added to the reaction mixture, which is then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5 ml) is mixed with distilled water (5 ml), and ferric chloride (1 ml, 1%), and the absorbance is measured at 700 nm. A stronger absorbance indicates increased reducing power.

Superoxide anion scavenging activity assay [6, 25]

Xanthine convert into uric acid, Xanthine oxidase (XO) enzyme is responsible for this conversion; hydrogen peroxide and superoxide are produced. (Figure 9). It is considered a major biological source of reactive oxygen species. It is possible that inhibition of this enzymatic process by compounds that exhibit antioxidant properties may have therapeutic use.

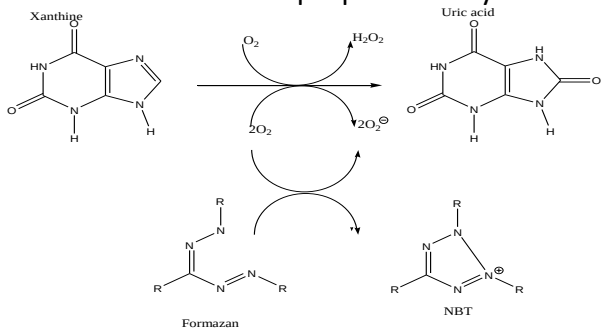

Figure 9. Formation of formazan from NBT

The scavenging potential for superoxide radicals is analysed with a hypoxanthine/xanthineoxidase-generating system coupled with nitrobluetetrazolium (NBT) reduction (measured spectrophotometrically). Reagents in this is prepared in 50 mM KH_2PO_4-KOH buffer (pH 7.4). The reaction mixture contained 20 µl of 15mM Na_2 EDTA (pH 7.4), 50 µl of 0.6 mM NBT in buffer, 50 µl of xanthine oxidase solution (1 unit in 10 ml of buffer), 30 µl of 3 mM hypoxanthine in 50 mM KOH, 145 µl of buffer, and 5 µl of various concentrations (1, 5, 10, and 50 µg/ml) of plant extracts or 5 µl of DMSO (as control). The reaction mixture was incubated at ambient temperature and the absorbance at 570 nm was determined every 30 sec up to 8 min using the Microplate reader. Quercetin was also used as a positive control. Three replicates were made for each test sample. The inhibition ratio (%) was calculated as follows: % inhibition = [(rate of control - rate of test sample)/rate of control] ×100.]

Superoxide scavenger activity is expressed as percent inhibition compared to the blank, in which buffer is used in place of the extract. When using this system, any inhibition by tannins in the plant extracts must be due to their antioxidant activity and any action upon the enzyme must be excluded as a possibility.

Chelating effect on ferrous ions [6, 26]

Chelating activity of samples can be determined by the ferrozine assay. Ferrozine quantitatively forms complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with a resulting decrease in the red colour of the complex. Measurement of the rate of colour reduction allows estimation of the chelating activity of the coexistent chelator.

Peroxynitrite scavenging [27]

The scavenging activity was measured by using an Evans Bmlue bleaching assay. 1 mL reaction mixture contained 50 M phosphate buffer (pH 7.4), 0.1 mM DTPA, 90 mMNaCl,5 mM KCL, 12.5 µM evans blue different concentration prepared peroxynitrite. 1 mM freshly. The absorbance was measured after 30 minute of incubation at 25**°C.** The concentration of ONOO- was measured spectrophotometrically at 302 nm (€ = 1 670 $M^{-1}cm^{-1}$).The percentage scavenging of ONOO- was calculated using this formula·

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Hypochlorous acid scavenging [28]

Hypochlorous acid (HOCL) was freshly prepared adjusting the pH of a 10% (v/v) solution of NaOCl to 6.2 with 0.6 M H_2SO_4 and the concentration of HOCl was determined by measuring the absorbance at 235nm using the molar mixture contained, 100 $M^{-1}cm^{-1}$. The reaction mixture contained, 1.5mM of HOCl and different concentration of the extract or standard ascorbic acid and incubated for 1h at 37°C. After the taurine (30mM) was added and

incubated again 30 min at 37°Cfollowed by the addition of thionitro benzoic acid (TNB). Absorbance was measured at 412 nm against blank and % scavenging was calculated according to the standard formula.

Total Oxyradical Scavenging Capacity method.

This method is based on the reaction between peroxyl radicals and α-keto-γ-methiolbutyric acid (KMBA), which is oxidized to ethylene. Added antioxidant competes with KMBA for the peroxyl radicals, reducing the production of ethylene, which is generally measured by gas chromatography. Syft Technologies Ltd. has developed a Selected Ion Flow Tube Mass Spectrometric (SIFT-MS) test that is based on TOSC.

Total Radical-Trapping Antioxidant Parameter method.

This method uses a luminescence spectrometer to measure the fluorescence decay of R-phycoerythrin during a controlled peroxidation reaction. TRAP values are calculated from the length of the lag-phase caused by the antioxidant compared to that of *Trolox.*

Ferric Thiocyanate method [12]

Various extracts (4.0mL) would be dissolved in ethanol (4.0mL) and mixed with 4.1 mL 2.5% linolic acid (in ethanol), 8.0 mL phosphate buffer (0.02M, pH 7.0) and 3.9 mL distilled water will be added to make up a volume to 20 mL. BHT will be taken as positive control and a solution without extracts will be taken as negative control. The mixture will be incubated at 40-45ºC for 30º min. Ethyl alcohol (75%) 9.7 mL and 0.1 mL ammonium thiocyanate (30%) will be added to 0.1 mL of the mixture. The readings are taken at an interval of 24 h. for one week.

TBA(Thiobarbituric acid) Method [12]

To 1.0 ml of the FTC sample used above will be added 2.0 mL of TCA and 2.0 mL of TBA 0.8%(w/v) TBA in 1.1%(w/v) sodium do decyl sulphate solution and this mixture will be placed in a boiling water bath at 100ºC for 10 min. After cooling it will be centrifuged at 3000rpm f or 20 min. and absorbance of the supernatant would be measured at 532 nm using UV -visible spectrophotometer.

Determination of phenol content by Folin-Ciocalteu method [29]

In Folin-Ciocalteu phenol reagent, a mixture of the hetero-polyphosphormolybdic and phoshotungstic acids is present in which the molybdenum and tungsten are in the 6^+ oxidation state. When these are reduced with certain reducing agents, molybdenum blue and tungsten blue are formed, in which the mean oxidation state of the metals is between 5 and 6. It is known that Folin-Ciocalteu reagent reacts not only with phenols but also with a variety of other compounds. Total phenolic contents determined by this method, using gallic acid as a standard.

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The test samples were dissolved in 5ml of methanol/water (50/50). The sample solution (500 µl) was mixed with 500 µl of 1N Folin-Ciocalteu reagent. The mixture was allowed to stand for 5min, which was followed by the addition of 1ml of 20% Na_2CO_3. After10 min of incubation at ambient temperature, the mixture was centrifuged for 8 min (12,000*g*), and the absorbance of the supernatant was measured at 730 nm. The total phenolic contents were expressed as gallic acid equivalents (GAE) in milligrams per gram sample.

Gallic acid is used as a standard for the calibration curve. The total phenolic content is expressed as mg of gallic acid equivalent (GAE). (Figure10.) show the reaction of gallic acid with molybdenum, a component of the Folin-Ciocalteu reagent.

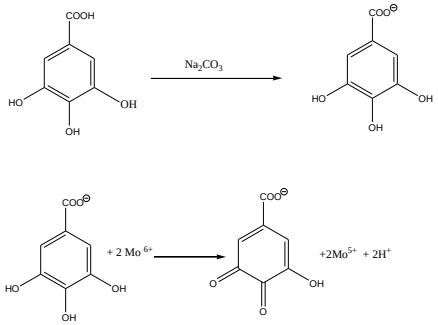

Figure 10. Reaction of gallic acid with molybdenum, a component of the Folin-Ciocalteu reagent.

Total flavonoid content [30, 31]

Total flavonoid content is determined by using a colorimetric method. Briefly, 0.30 mL of the EtOH and AcOEt extracts or (+)-catechin standard solution is mixed with 1.50 mL of distilled water in a test tube followed by addition of 90µL of a 5% $NaNO_2$ solution. After 6 min, 180 µL of a 10% $AlCl_3.6H_2O$ solutionis added and allowed to stand for another 5 min before 0.6 mL of 1 M NaOH is added. The mixture is brought to 330 µL with distilled water and mixed well. The absorbance is measured immediately against the blank at 510 nm using a spectrophotometer incomparison with the standards prepared similarly with known (+)-catechin concentrations.The results are expressed as mg of catechin equivalents per gram of extract (mg CE/g)through a calibration curve with catechin.

Free radicals and oxidative stress mechanisms [32]

$ONOO^-$.RSS are easily formed by the reaction of ROS with thiols. Regarding ROS, the reactions leading to the production of reactive species are displayed in (Figure 11). The hydroperoxyl radical(HO_2°) disassociates at pH 7 to form the superoxide anion (O_2).This anion is extremely reactive and can interact with a number of molecules to generate ROS either directly or through enzyme or metal-catalyzed processes. Superoxide ion can also be detoxified to hydrogen peroxide through a dismutation reaction with the enzyme superoxide dismutase (SOD) (through the Haber-Weiss Reaction) and finally to water by the enzyme catalase (CAT). If hydrogen peroxide reacts with an iron catalyst like Fe_2^+, the Fenton reaction can take place ($Fe_2^+ + H_2O_2Fe3^+ + OH° + OH^-$) forming the hydroxyl radical HO°. With regard to RNS, the mechanism forming $ONOO^-$ is: $NO^\circ + O2^-$. Finally, RSS derive, under oxidative conditions, from

thiols to form a disulfide that with further oxidation can result in either disulfide-S-monoxide or disulfide-S-dioxide as an intermediate molecule. Finally, a reaction with a reduced thiol may result in the formation of sulfenic or sulfinic acid.

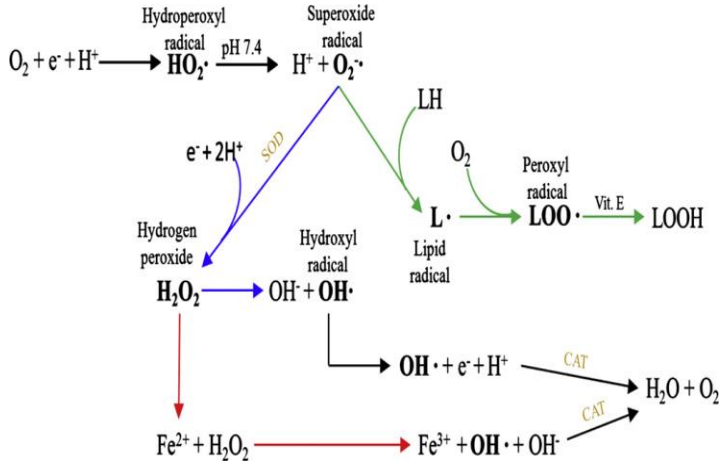

Fig. 11.Overview of the reactions leading to the formation of ROS. Green arrows represent lipid peroxidation. Blue arrows represent the Haber-Weiss reactions and the red arrows represent the Fenton reactions. The bold letters represent radicals or molecules with the same behavior (H_2O_2). SOD refers to the enzyme superoxide dismutase and CAT refers to the enzyme catalase.

CONCLUSION

The antioxidant activity of natural compounds is determined by various methods *in vitro* which are discussed above. These tests have demonstrated the importance of diets rich in fruits and vegetables by confirming the presence of antioxidants that help fight free radicals, and which in moderate consumption are beneficial to human health.

Aromatic plants have great antioxidant potential which is due to their contents of variable phytochemical compounds. The most important free radical in biological systems is radical derivatives of oxygen that is why phenolic compounds posses the largest group among other phytochemical groups in such activity. All compounds discussed in this review exhibit antioxidant activities.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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