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A REVIEW ON- ANTIOXIDANTS

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ABSTRACT

Free radicals and related species have attracted a great deal of attention in recent years. Oxidative stress has been considered a major contributory factor to the diseases. They are mainly derived from oxygen (Reactive Oxygen Species/ROS) and nitrogen (Reactive Nitrogen Species/RNS), and are generated in our body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radical damage to protein can result in loss of enzyme activity. There are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. The sources and origin of antioxidants which include fruits and vegetables, meats, poultry and fish were treated in this study. The classification and characteristics of antioxidant; its measurements and level in food and free radicals were also documented. The Chemistry of antioxidants which include chain reactions, molecular structures, food antioxidants and reaction mechanisms, biochemical activity, therapeutic properties and future choice of antioxidants were reported in this review.

KEY WORDS: Antioxidants, Free radicals, Oxidative stress.

INTRODUCTION

The use of plants whether herbs, shrubs or trees in parts or in whole in the treatment and management of diseases and disorders date back to pre historic days. Plant extract have been used in folk medicine practices for the treatment of various ailments since antiquity. Natural phytochemicals present at low levels in fruits, vegetables, herbs and spices offer many health benefits, but these compounds may not be effective or safe when consumed at higher dose [1].

The presence of free radicals in biological materials was discovered less than 50 years ago [2]. Exposure of biological systems to xenobiotics, pollutants, ionizing radiation or U.V. light, smoking, and development of certain pathological conditions lead to oxidative stress, consequently increase production of oxy radicals [3]. Cell damage caused by free radicals appears to be a major contributor in aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, compromised immune system, rheumatoid arthritis and brain dysfunction. The cellular injury caused by oxidative stress has been linked to over 200 clinical disorders, many of which are seen in ICU patients units [4]. Free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Antioxidants are capable of stabilize, deactivate or scavenge free radicals before they attack cells.

Antioxidants can be defined as substances whose presence in relatively low concentrations significantly inhibits the role of oxidation of the targets. Due to continuous generation of partially reduced forms of oxygen by constitutive metabolic pathways, a number of protective antioxidant enzyme, such as Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Peroidase (GSHPx), Glutathione Reductase (GSHRx), Glutathione-S-Transferase (GST) and non- enzymatic antioxidants have involved to deal with toxic species.

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Table 1. Reactive species

Reactive species	Symbol	Half life(in sec)	Reactivity / remarks
Reactive oxygen species			
Superoxide	O_2^*	10^{-6} s	Generated in mitochondria, in cardiovascular system and others
Hydroxyl radicle	$^*OH$	10^{-9} s	Very highly reactive, generated during iron overload and such conditions in our body
Hydrogen peroxide	H_2O_2	Stable	Formed in our body by large no of reactions and yields potent species like $^{\cdot}OH$
Peroxyl radicle	ROO*	s	Reactive and formed from lipids, proteins, DNA, sugars etc during oxidative damage
Organic hydroxide	ROOH	Stable	Reactive with transient metal ions to yield reactive species
Sinlet oxygen	$^1O_2$	10^{-6} s	Highly reactive, formed during photosensitization and chemical reactions
Ozone	O_3	s	Present as an atmospheric pollutant can react with various molecules.
Reactive nitrogen species			
Nitric oxide	NO*	s	Neurotransmitter and blood pressure regulator, can yield potent oxidants during pathological status
Peroxy nitrile	$ONOO^-$	10^{-3} s	Formed from nitric oxide and superoxide highly reactive
Peroxy nitrous acid	ONOOH	Fairly stable	Protonated from of $ONOO^-$
Nitrogen dioxide	NO_2	s	Formed during atmospheric pollution

Table 2. Different types of plants having antioxidant activity

S.No	Plant name	Family	Part used	Chemical constituents responsible for antioxidant activity	References
1	*Amaranthus paniculatus*	Amaranthaceae	Leaf	Carotenoids, ascorbic acid, flavonoids and phenolic acids	[88]
2	*Amaranthus gangeticus*	Amaranthaceae	Leaf	Carotenoids, ascorbic acid, flavonoids and phenolic acids	[88]
3	*Amaranthus blitum*	Amaranthaceae	Leaf	Carotenoids, ascorbic acid, flavonoids and phenolic acids	88
4	*Amaranthus spinosus*	Amaranthaceae	Leaf	Carotenoids, ascorbic acid, flavonoids and phenolic acids	[88]
5	*Amaranthus viridis).*	Amaranthaceae	Leaf	Carotenoids, ascorbic acid, flavonoids and phenolic acids	[88]
6	*Coriandrum sativum*	Umbelliferae	Leaf, Fruit	S-(+)-linalool, monoterpenes, hydrocarbons viz. α-pinene,limpnene, γ- terpinene, p-cymene, borneol, citronellol, camphor, geraniol and geraniol acetate, heterocyclic components like pyrazine, pyridine, thiazole, furan and tetrahudrofuran derivatives, isocoumarins, coriandrin, dihydrocoriandrin, coriandrons A-E, flavonoids, pthlides, neochidilide, digustilide phenolic acids and sterols.	[89]
7	*Emblica officinalis*	Umbelliferae	Fruit leaves	vitamins, Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants	[89]
8	*Digera muricata* (L.)	Amarantheaceae	Leaf	Phenols, flavonoids, glycosides, tannins and terpenoids and minimum for saponins.	[89]
9	*Chenopodium album* L.	Amarantheaceae	Leaf	Alkaloids, apocarotenoids, flavonoids, phytoecdysteroids xyloside, Limonene (23.2 %),	[90]

				α-terpinyl acetate (13.7 %), α-terpinene (12.3 %) and cis ascaridole (12.2 %)	
10	*Basella alba Linn*	Basellaceae	Leaf	proteins, fat, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B9 (folic acid), riboflavin, niacin, thiamine and minerals such as calcium, magnesium iron, Kaempherol at a basellasaponins , amino acid such as arginine, leucine, isoleucine, lysine, threonine and tryptophan, peptide, phenols	[91]
11	*Basella rubra*	Basellaceae	Leaf	calcium, iron, vitamins A, B, and C saponins A, B, C, and D, oleanane-type triterpenes oligoglycosides, together with betavulgaroside 1, spinacoside C, and momordins IIb and Iic, β-carotene, small amounts of α-carotene s 4-coumaroyl and feruloyl derivatives	[92,93,94]
12	*Physalis philadelphica*	Solanaeae	Leaf, fruit	withanolides are 2,3-dihydro-3beta-methoxyixocarpalactone A ,2,3-dihydro-3beta-methoxyixocarpalactone B, 2,3-dihydroixocarpalactone B ,and 4beta,7beta,20R-trihydroxy-1-oxowitha-2,5-dien-22,26-olide	[95]
13	*Rumex vesicarius*	Polygonaceae	Leaf	Minerals, protein and ascorbic acid, oxalic acid, tocopherol and lipids. Ca ,Cu , Fe, Mg, K, Na, Zn, Lipids, Ascorbic acid, Tocopherol	[96]
14	*Paederia foetida*	Rubiaceae	Leaves	B sitosterol, leupiol, methyl mercaptan, crystalline keto alcohol, paederolone, paederone and hetasitosterol	[97]
15	*Solanum nigrum Linn*	Solanaceae	Leaf	Acetic acid, tartaric acid, malic acid and citric acid, solanine, Alpha, beta gamma chaconines and alpha, beta gamma solanines Solanidine, Solanine, beta 2-solamargine, solamargine and degalactotigonin. five non-saponin namely 6-methoyhydroxycoumarin, syringaresinol-4-O-beta-D-glucopyranoside, pinoresinol-4-O-beta-D-glucopyranoside, 3, 4-dihydroxhbenzoic acid (IV), p-hydroxybenzoic acid and 3-methoxy-4-hydroxyienzoic acid	[98]
16	*Trigonella foenum-gracecum Linn*	Leguminosae	Leaf	Amino acid, fatty acid, vitamins, saponins. folic acid, disogenin, gitogenin, neogitogenin, omorientin saponaretin, neogigogenin, and trigogenin, 4, 5[delta]-cadinene (27.6%), [á]-cadinol, palmitic acid, linoleic acid oleic acid and stearic acid, hexanal, 2-methyl-2-butenal, 3-octen-2-one, flavonoids, polysaccharides, saponins, polysaccharides,fixed oils trigonelline, choline, Quercetin, galactomannan, polysaccharides.	[99]
17	*Brassica oleracea* Capitata	Brassicaceae	Leaf	glucosinolates and their derived products, Flavonoids and other phenolics quercetin 3-Osophoroside-7-O-glucoside, 3-p-coumaroylquinic acid, kaempferol 3-Osophoroside-7-O-glucoside, kaempferol 3-O-(caffeoyl)-sophoroside-7-Oglucoside, sinapoyl	[100]

				glucoside acid, kaempferol 3-O-(sinapoyl)-sophoroside-7-O-glucoside, sinapic acid, kaempferol 3-O-sophoroside, 3 isomeric forms of 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, 1,2,2'-trisinapoylgentiobiose and 1,2'-disinapoyl-2-feruloylgentiobiose. kaempferol 3-O-sophorotrioside-7-O-glucoside, kaempferol 3-O-(methoxycaffeoyl /caffeoyl)sophoroside-7-O-glucoside, kaempferol 3-O-sophoroside-7-O-glucoside,	
18	*Moringa pterygosperma gaerth*	Moringaceae	Leaf	4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate,4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate,niazimicin ,pterygospermin,benzyl isothiocyanate and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate carotenoids (including β-carotene or pro-vitamin A)	[101]
19	*Hibiscus cannabinus L*	Malvaceae	Leaf,	tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids	[102]
20	*Sesbania grandiflora L*	*Fabaceae*	Leaf	galactommannans, linoleic acid, β-Sitosterol and Carbohydrates . vitamin C, and calcium, iodine, Pectin, Saponins, aliphatic alcohol leucocyanidin and cyanidin, oleanolic acid and its methyl ester and kaemferol-3-rutinoside, tannins and gum, Sesbanimide	[103,104,105]
21	*Portulaca oleracea L*	Portulacaceae	Leaf	omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, α-tocopherols, ascorbic acid and glutathione ,free oxalic acids, β-Carotene, omega-3 fatty acids, coumarins, flavonoids, monoterpene glycoside andanthraquinone glycosides	06,107,108]
22	*Murraya koenigii L*	Rutaceae	Leaf	Alkaloid, volatile oil, GlycozolineXanthotoxin and Sesquiterpine	[109,110,111,112,113
23	*Celosia argentea*	Amaranthaceae	Leaf	alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils, steroids, carbohydrates, carotenoids, anthocyanins	114]
24	*Boerhavia diffusa*	Nyctaginaceae.	Leaf	Alkaloids punarnavine, rotenoids (boeravinones A-F), amino acids, lignans (liriodendrons), ßsitosterols and tetracosanoic, esacosanoic, stearic and ursolic acids. rotenoids (known as boeravinones (A - F) Punarnavoside, a phenolic glycoside, 11,12 C-methyl flavone liriodendrin and syringaresinol mono-β-D-glycoside , hypoxanthine 9-Larabinose. 15 dihydroisofuroxanthone-borhavine, phytosterols, punarnavine and punernavoside, potassium nitrate, ursolic acid.fatty acids and allantoin boerhavin and boerhavic acid, aegeline, agelinine, rutine, sterol, tannins, flavonoids, quercetin, volatile oils, ß-sitosterols.	[115,116,117,118,119, 120,121,122]
25	*Eclipta alba*	Asteraceae	Leaf	coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids. The leaves contain	124

				stigmasterol, a-terthienylmethanol, wedelolactone, demethylwedelolactone and emethylwedelolactone-7-glucoside, hentriacontanol and heptacosanol. Polyacetylene, thiophenes. phytosterol, P-amyrin ,luteolin-7-glucoside, P-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone. cystine, glutamic acid, phenyl alanine, tyrosine and methionine,Nicotine and nicotinic acid	
26	*Centella asiatica*,	Apiaecae	Leaf	asiaticoside carotene, ascorbic acid, phenols. madecassic acid	[125]
27	*Phyllanthus amarus*	Euphorbiaceae	Leaf	alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, *geraniin*, hypophyllanthin, phyllanthin, lignans, lintetralins, lupeols, methyl salicylate, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal and tricontanol	[126]
28	*Hibiscus sabdariffa*	Malvaceae	Leaf	Ascorbic acid (Vitamin C) and tocopherol (Vitamin E), flavonoids, polyphenols.	[96]
29	*Curcuma longa*	zingiberaceae	Leaf	Ascorbic-acid rhizome, beta-carotene rhizome, caffeic-acid rhizome, curcumin rhizome, eugenol essential oil , p-coumaric-acid rhizome, protocatechuic-acid leaf, syringic-acid leaf, vanillicacid leaf, camphene, eugenol,curcumin	[127]
30	*Ocimum sanctum*	Labiatae	Leaf	Volatile iol, terpinoids,eugenol, thymol,estragole	[128]
31	*Basella alba*	Basellaceae	Leaf	High in vitamin A, vitamin C, Ca, Iron, phosphours, vitamin B9 (folic acid), calcium, magnesium, flavonoids, polyphenols.	[129]

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Sources and origin of antioxidants

Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. β-carotene is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin and mangoes. Some green leafy vegetables, including collard greens, spinach and kale, are also rich in betacarotene. Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale. Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges and other foods. Estimates suggest 85% of American dietary intake of lycopene comes from tomatoes and tomato products [5].

Types of antioxidants

Antioxidants are grouped into two namely;
(1) Primary or natural antioxidants.
(2) Secondary or synthetic antioxidants.

Primary or natural antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following [6].

1. Antioxidants minerals - These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.

2. Anti oxidants vitamins – It is needed for most body metabolic functions. They include-vitamin C, vitamin E, vitamin B.
3. Phytochemicals - These are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids: These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colours. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. β-carotene, which is rich in carrot and converted to vitamin A, when the body lacks enough of the vitamin. Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source include diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

Secondary or synthetic antioxidants

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compound includes [6]:

i. Butylated hydroxyl Anisole (BHA).
ii. Butylated Hydroxyr Toluene (BHT).
iii. Propyl Gallate (PG) and metal chelating agent (EDTA).
iv. Tertiary Butyl hydro Quinone (TBHQ).
v. Nordi Hydro Guaretic Acid (NDGA).

CLASSIFICATION

- **Enzymatic antioxidants**

1. Primary antioxidants
   Eg: SOD, Catalase, Glutathione Peroxidase.
2. Secondary enzymes
   Eg: Glutathione reductase, Glucose 6-phosphate dehydrogenase.

- **Non-Enzymatic antioxidants**

1. Minerals eg: Zinc, Selenium
2. Vitamins eg: Vitamin A, Vitamin C, Vitamin E,
3. Carotenoids eg: β-carotene, Lycopene, Lutein, Zeaxanthin
4. Low molecular weight Antioxidants eg: glutathione, uric acid
5. Organosulfur compounds eg: Allium, Allyl sulfide, indoles
6. Antioxidant cofactors
7. Polyphenols

Copper/ Zinc and Manganese dependent Superoxide dismutase (SOD):

It is an endogenously produced enzyme present both in prokaryotes and eukaryotes [7]. SOD is a group of metalloenzymes with various prosthetic groups. Three main classes of them differ in their amino acid sequence structure and metallic factors as follows;

(1) Cu-Zinc SOD in the cytoplasm with two sub units and sensitivity to cyanide and hydrogenperoxide.
(2) Mn SOD in the mitochondrial matrix and in prokaryotes and is insensitive to cyanide.
(3) Fe SOD, usually found in prokaryotes and in the chloroplasts of some plants. It is not sensitive to cyanide but is inhibited by hydrogen peroxide.
(4) Al SOD has recently reported [8].

Catalase

H_2O_2 is also metabolized by catalase (CAD), a heam protein with an extremely high turn over rate

$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$

SOD protect from senescence, aging, ischemic tissue damage, lipid peroxidation, protein denaturation and radiation damage.

Glutathione peroxidase

Glutathione carries out the reduction of H_2O_2 which is enzymatic reaction catalyzed by GPx, found in vacuole, cystol and extracellular space. The enzyme has substrate specifity. Peroxidases are involved in

1) Biotic and abiotic stresses
2) Lignin and suberin synthesis
3) Disease and pathogen response [9].

As glutathione peroxidase contains one residue per mole of an unusual aminoacid Selenocysteine that contains selenium in place of sulphur that is why dietary supplimentation with selenium protects cancer. Peroxidases are widely distributed in plants and reduce hydrogen peroxide to water at the expense of oxidation of an organic substrate

$2\ GSH + H_2O_2 \longrightarrow GSSG + 2\ H_2O$

Consequence of H_2O_2 accumulation in glucose-6-phosphodehydrogenase deficiency due to malarial drug primaquine results in to haemolytic anemia due to oxidative stress. In alcoholics, memory loss related to pentose phosphate pathway based mutation in transketolase causes Wernick Korsakoff syndrome.

Glutathione Reductase

Glutathione keeps cystein thiol groups in the reduced state. If two thiol group become oxidized, they can be reduced non-enzymically by glutathione. GSSG is reduced by NADPH-dependent enzyme glutathione reductase.

$GSSG + NADPH + H \longrightarrow 2GSH + NADP^+$

Glutathione S- Transferases

Through the action of this widely distributed enzyme, glutathione participates in detoxification of xenobiotics or foreign organic compounds. Ovithol found in fertilized eggs of Sea urchin, plays a role comparable to

gluathione. It protects eggs against oxidative damage by peroxides. Ovithol is reduced by glutathione.

Glutathione

Glutathione is a tripeptide is present in high concentrations in most eukyrotic cells and reacts with free radicals. It directly quenches lipid peroxides. Vit C and glutathione work interactively [10]. Glutathione present in food prevents cancer due to aflatoxin.

B. Non enzymatic antioxidants- These are biological molecules that can act as antioxidants by either quenching a free radical directly or indirectly by promoting a process responsible for radical scavenging indirectly [11].

a. Selinium

Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. Plant foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Animals that eat grains or plants grown in selenium-rich soil have higher levels of selenium in their muscle. In the United States, meats and bread are common sources of dietary selenium. Brazil nuts also contain large quantities of selenium.

b. Transferrin

Transferrin is a major iron transporting protein in the body. It is normally 20- 30% loaded. The excess storage capacity helps to bind free iron salts that otherwise may cause reactive oxygen species.

c. Lactoferin

Lactoferin is a milk protein similar to transferring that helps in iron binding.

d. Ceruloplasmin-

Ceruloplasmin is a copper containing protein. It catalyses the oxidation of Fe^{++} to Fe^{+++} while oxygen is reduced to water.

e.Vitamin A

Vitamin A is found in three main forms: retinol (Vitamin A1), 3,4-didehydroretinol (Vitamin A2), and 3-hydroxyretinol (Vitamin A3). Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella cheese.

f. Vitamin C (Ascorbic acid)

In the aqueous phase, ascorbic acid may reduce reactive oxygen metabolites directly, with the concurrent formation of dehydroascorbate, and or indirectly by the regeneration of tocopherol from the tocopherol radical [12]. Vitamin C can be found in high abundance in many fruits and vegetables and is also found in cereals, beef, poultry, and fish.

g. Vitamin E

Vitamin E, also known as alpha-tocopherol, is found in almonds, in many oils including wheat germ, safflower, corn and soybean oils, and is also found in mangoes, nuts, broccoli, and other foods [13]. Vitamin E is present in relatively high concentrations in both cells and mitochondrial membranes. It reacts with reactive oxygen metabolites, yielding lipid hydroperoxide, which can be removed by the activity, of the phospholipase- GSPHx system.

h. β-carotene

β-carotene is a lipid soluble precursor of vitamin A. It functions synergistically with tocopherol to prevent lipid peroxidation.

i. Ubiquinol- 10

It is a reduced form of coenzyme Q10, present in lipoprotein at relatively low concentrations. It probably regenerates tocopherol from the tocopheroxyl radical and increases its antioxidant efficiency.

C. Plant derived antioxidants

To protect the cells and organ systems of the body against ROS, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous in origin, that function interactively and synergistically to neutralize free radicals [14].

These components include:

Nutrient derived antioxidants like ascorbic acid, tocopherols and carotenoids and other low molecular weight compounds such as GSH and lipoic acid.

Antioxidant enzymes Eg: SOD, GSHPx and GSH reductase, which catalyze free radical quenching reactions.

Metal- binding proteins such as feritin, lactoferitin, albumin, and ceruloplasmin that sequester free iron and copper ions as these ions are capable of catalyzing oxidative reactions.

Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Antioxidant operation and mechanisms

The word anti-oxidant is used in a general sense to refer to any type of chemical agent which inhibits attack by oxygen or ozone [15]. As applied to vegetable oils, anti-oxidants are compounds which interrupt the oxidation process by preferentially reacting with the fat radical to form a stable radical which does not quickly react with oxygen [16]. When the reference is to food uses, they are grouped as a food additive which has the effect of increasing the shelf life of foods by protecting them against deterioration caused by oxidation which leads to rancidity and color changes. Antioxidants function either by inhibiting the formation of free alkyl radicals in the initiation step or by interrupting the propagation of the free radical chain. In truncating the propagation step, the antioxidants function as hydrogen donors. Generally, the most popular antioxidants are hydroxylphenol compounds with various ring substitutions. They are characterized by possessing low activation energies for the hydrogen donation process. The antioxidant radical which results is stabilized with its local electrons delocalized; hence

antioxidant free radicals do not readily initiate other free radicals. They rather even react with lipid free radicals to form stable and complex compounds. In investigating phenolic antioxidants, it is found that their antioxidative capabilities bear a relationship to the number of phenol groups occupying 1,2 or 1,4 positions in an aromatic ring as well as to the volume and electronic characteristics of the ring substituents present [17]. In elucidating the mechanism of oxidative inhibition, it is generally established that anti-oxidants function as oxygen interceptors in the oxidative process thereby breaking the chain reaction that perpetuates the process [18]. Simply put, conventional antioxidant activity involves the donation of hydrogen to free radicals followed by the formation of a complex between a lipid radical and the antioxidant radical formed as a result of the hydrogen loss. Here the antioxidant radical functions as a free radical acceptor. The general scheme is presented below:

R + AH → RH +A
RO + AH → ROH +A
ROO + AH → ROOH+A
R + A → RA
RO + A → ROA
ROO + A → ROOA
Antioxidant +O2 → Oxidized antioxidant

Mention must be made of synergists – substances which increase the effectiveness of a primary antioxidant. Certain metallic ions such as copper and iron act as pro oxidants, catalyzing the oxidation process. Such metal ions can be sequestered or chelated by certain organic acids. They effectively contribute to lower transition metal activity. Synergists are not as effective when used alone; rather, they work best when combined with an antioxidant. Examples of such compounds are citric acid, phosphoric acid and some of their derivatives. Synergism has been studied, not just in relation to antioxidants alone, but in relation to combinations of antioxidants, anti wear and other additives [19]. An example of a synergist used in conjunction with phenolic antioxidants is citric acid [20].

Estimation of antioxidants

Conjugated diene assay

This method allows dynamic quantification of conjugated dienes as a result of initial PUFA (Poly unsaturated fatty acids) oxidation by measuring UV absorbance at 234 nm. The principle of this assay is that during linoleic acid oxidation, the double bonds are converted into conjugated double bonds, which are characterized by a strong UV absorption at 234 nm. The activity is expressed in terms of Inhibitory concentration (IC_{50}) [20-22].

DPPH Method (1, 1 diphenyl 2, picryl hydrazyl)

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC_{50} [23].

Super oxide radical scavenging activity

In-vitro super oxide radical scavenging activity is measured by rioflavin/light/NBT (Nitro blue tetrazolium) reduction. Reduction of NBT is the most popular method. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formazon that can be measured at 560nm. The capacity of extracts to inhibit the colour to 50% is measured in terms of EC_{50}. Antioxidant activity of Ailanthus, flavanoids and Triphala has been reported in terms of super oxide radical scavenging activity. The super oxide radical can also be detected by oxidation of hydroxylamine, yielding nitrite which is measured colorimetric reaction [24,25]**.**

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves in the *in-vitro* generation of hydroxyl radicals using Fe^{3+} /ascorbate/EDTA/H_2O_2system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods the hydroxyl radicals formed by the oxidation is made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces the intense yellow color with Nash reagent (2M ammonium acetate with 0.05M acetic acid and 0.02M acetyl acetone in distilled water). The intensity of yellow color formed by that reation is measured at 412nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavengering [24].

Nitric oxide radical inhibition activity

Nitric oxide, because of its unpaired electron, is classified as a free radical and displays important reactivities with certain types of proteins and other free radicals. *In vitro* inhibition of nitric oxide radical is also a measure of anti oxidant activity. This method is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in buffer saline and measured by Griess reagent. In presence of scavengers, the absorbance of the chromophore is evaluated at 546nm. The activity is expressed as % reduction of nitric oxide [24].

Reducing Power Method

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In

this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples [26].

Phospho molybdenum Method

It is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phospho molybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH [27].

Peroxynitrite radical scavenging activity

Peroxynitrite is now recognized by researchers as the culprit in many toxic reactions. Hence, an *in vitro* method for scavenging of peroxy radical has been developed to measure antioxidant activity. The scavenging activity is measured by monitoring the oxidation of di hydro rhodamine on a microplate fluorescence spectrophotometer at 485nm [28].

ABTS (2,2-azinobis(3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt)Method

This is a measure of antioxidant activity as opposed to antioxidant concentration which might include a proportion of biologically inactive antioxidants. It also permits the measurement of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects. The antioxidant activity of wines was measured by using this method. The assay is based on interaction between antioxidant and ABTS+ radical cation which has a characteristic color showing maxima at 645, 734 and 815nm [27-29].

DMPD (N, N-dimethyl-p-phenylene diamine dihydrochloride) Method

This assay is based on the reduction of buffered solution of colored DMPD in acetate buffer and ferric chloride. The procedure involves measurement of decrease in absorbance of DMPD in presence of scavengers at its absorption maxima of 505nm. The antioxidant activity of wines was measured by using this method. The activity was expressed as percentage reduction of DMPD [27-30].

Oxygen Radical Absorbance Capacity (ORAC)

ORAC is an exciting and revolutionary new test tube analysis that can be utilized to test "Antioxidant Power" of foods and other chemical substances. It calculates the ability of a product or chemical to protect against potentially damaging free radicals. This analytical procedure measures the ability of a food, vitamin, nutritional supplement, or other chemicals to protect against the attack by free radicals, or to act as an Antioxidant. The test is performed using Trolox (a water-soluble analog of Vitamin E) as a standard to determine the Trolox Equivalent (TE). The ORAC value is then calculated from the Trolox Equivalent and expressed as ORAC units or value. From this assay shows higher the ORAC value, the greater the "Antioxidant Power".

This assay is based on generation of free radical using AAPH (2,2-azobis 2-amido propane dihydrochloride) and measurement of decrease in fluorescence in presence of free radical scavengers. In automated ORAC assay b-phycoerythrin (b-PE) was used as target free radical damage, AAPH as a peroxy radical generator and Trolox as a standard control. After addition of AAPH to the test solution, the fluorescence is recorded and the antioxidant activity is expressed as trolox equivalent [31].

β-Carotene Linoleate model

This is one of the rapid method to screen antioxidants, which is mainly based on the principle that Linoleic acid, which is an unsaturated fatty acid, gets oxidized by "Reactive Oxygen Species" (ROS) produced by oxygenated water. The products formed will initiate the β-carotene oxidation, which will lead to discoloration. Antioxidants decrease the extent of discoloration, which is measured at 434nm and the activity is measured [32].

TRAP Method

This method is defined as total radical trapping antioxidant parameter. The fluorescence of R-Phycoerythrin is quenched by ABAP (2,2'-azo–bis (2-amidino-propane) hydrochloride) as a radical generator. This quenching reaction is measured in presence of antioxidants. The antioxidative potential is evaluated by measuring the delay in decoloration [33].

Cytochrome C test

Superoxide anions were assayed spectrophotometrically by a cytochrome reduction method described by McCord [34]. Xanthine oxidase converts xanthine to uric acid and yields superoxide anions and these radicals directly reduce ferri-cytochrome C to ferro-cytochrme C, having an absorbance change at 550 nm. When test compounds showed superoxide scavenger activity, there was a decrease in the reduction of ferri-cytochrome C [35].

Erythrocyte ghost system

This method involves isolation of erythrocytes ghost cells and the induction of lipid peroxidation using erythrocyte ghosts and the induction of tetra-butyl hydroxy peroxide (t-BHP). TBARS (thio barbituric acid reactive substance) was produced during the reaction is measured at 535 nm [36].

Microsomal lipid peroxidation or Thiobarbituric acid (TBA) assay

TBA test is one of the most frequently used tests for measuring the peroxidation of lipids. Method involves isolation of microsomes from rat liver and induction of lipid

peroxides with ferric ions leading to the production of small amount of Malonaldehyde (MDA). TBA reacts with MDA to form a pink chromagen, which can be detected spectrophotometricaly at 532 nm [37].

The potential role of antioxidants in disease

Oxidative stress and diseases

I. Nephrotic Syndrome

The Nephrotic Syndrome (NS) is defined by heavy proteinuria (urine total protein excretion greater than 3.5 g/d or total proteincreatinine ratio greater than 3.5 g/g) due to abnormal increase of glomerular permeability and following hypoalbuminemia, hyperlipidemia and edema. Peroxidation of lipid membranes raises the concentration of their by product MDA and the consequent lowering of antioxidants as a result of consumption [38]. Total antioxidant activity as the most reliable factor is involved in antioxidation protection with nephrotic syndrome [39]. In the kidney, oxygen radical production has been detected in vascular cells, juxtra glomerular cells, tubular cells, podocytes, mesangial cells and isolated glomeruli. Free radicals have a negative influence on renal tissue in NS [39].The administration of various natural or synthetic antioxidants has been shown to be of benefit in prevention and attenuation of renal scarring in kidney diseases [40].The combined therapy of antioxidants, minerals with B-complex vitamins for treatment of imbalance oxidant /antioxidant status, hyperhomocyst(e)inemia and deficiency of copper and zinc in nephrotic syndrome patients.

II. Oxidative stress and neurodegenerative diseases

The brain is exposed throughout life to OS, and certain diseases of the brain and nervous system are thought to involve free radical processes and oxidative damage, either as a primary cause or as a consequence of disease progression.

1. Alzheimer's disease

Alzheimer's disease (AD) is a progressive neuropsychiatric disorder of unknown etiology. It is characterized by neuronal degeneration and cognitive deterioration, especially in the elderly [41].OS has been implicated in the pathogenesis of AD [42] by the finding of several characteristics, such as enhanced lipid peroxidation, in specific areas of the brain in postmortem studies [43]. Several investigators detected an increase in the activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase in the hippocampus and amygdale. The suggestion that OS causes oxygen radical formation with resultant neurodegeneration and possibly plaque formation in the central nervous system, was supported by the study of Frautschy [44].According to the provided evidence for the hypothesis that amyloid protein, the major constituent of the senile plaque, is neurotoxic and that such toxicity is mediated by free radicals in vitro and in a transgenic mouse model of AD.

2. Cognitive dysfunction in the elderly

Cognitive impairment is a common problem in the over 65year age group, progressing to its most devastating form of clinical dementia, usually Alzheimer's dementia, in about 5% of this population [45]. Goodwin noted a correlation between memory function and vitamin C in the blood of healthy volunteers aged 60 or over [46].Accordingly, Perry found a positive association of memory performance with β-carotene and vitamin C levels in plasma measured twice: 22 years and immediately before the tests. Another study with a larger sample group (n=335) reported that all the subjects with white matter lesions had lower plasma vitamin E levels [47].

3. Parkinson's disease

Data from postmortem studies of brains from patients with Parkinson's disease (PD) suggest that OS plays an important role in neural degeneration of the pigmented dopaminergic neurons in the substantia nigra pars compacta (SNpc) [48]. Apparently, there is a specific chemical fingerprint indicative of the damaging oxidative events: higher levels of cholesterol hydroperoxide, malondialdehyde, and protein adducts of 4- Hydroxy-2-Noneal (HNE) and of 8-hydroxy-2-deoxyguanosine, which point to the presence of ROS-induced DNA nicks. One of the suggested causes of OS in the SNpc is the production of ROS during the normal metabolism of dopamine. In the human SNpc, the oxidation products of dopamine may polymerize to form neuromelanin, which may also be toxic [49]. Several studies have shown that dopamine is toxic to various cell cultures, causing programmed cell death. N-methyl-4-phenyl-1, 2,3,6- tetrahydropyridine (MPTP) is a neurotoxin that produces biochemical and neuropharmacological changes in humans, lower primates and mice, which closely resemble those found in PD and also involve free radical formation [50]. According to postmortem studies, the SNpc of PD patients shows a significant (60%) reduction in GSH and a moderate (29%) increase in oxidized glutathione (GSSG) levels [51,52]. This could be a critical primary event, leading to a weakening or deficiency of the natural antioxidative cellular defense mechanisms and thereby triggering degeneration of the nigral neurons, causing PD.

4. Huntington's disease

Huntington's disease is an autosomal neuronal disorder characterized as a movement disorder and caused by repetition of a CAG trinucleotide sequences encoding for a polyglutamine tract at the N terminus of the gene encoding a protein named huntingtin. There is a progressive, massive loss of neurons, particularly in the striatum [53]. Several postmortem studies showed increased iron levels in the striatum of patients with Huntington's disease[54]. Animals, as well as human postmortem studies, support the theory of metabolic dysfunction with concomitant OS [55.56]. Excessive glutamate activation of excitatory receptors may also be involved and may lead to ROS production.

5. Amyotrophic lateral sclerosis (ALS)

ALS is characterized by a selective and progressive degeneration of the lower motor neurons in the spinal cord and the upper motor neurons in the cerebral cortex, usually beginning in midlife. OS may be involved in all types of ALS (57). Levels of vitamin E and malondialdehyde (MDA) as a measure of lipid oxidation, increased over time in mutant CuZnSOD mice, as compared to controls. In patients with sporadic ALS there was a marked elevation over control levels in plasma 2-thiobarbituric reactive substances, which are products of lipid peroxidation. However, the plasma concentrations of antioxidants (α-tocopherol, β-carotene, ubiquinol-10 and glutathione) and the SOD activity in red blood cells were not significantly different between groups [58].

6. Schizophrenia and tardive dyskinesia

The presence of excess levels of ROS has been described for both schizophrenia and neuroleptic induced tardive dyskinesia [59]. Schizophrenia is a common psychiatric disorder affecting almost 1% of the population. The contribution of oxidative injury to the pathophysiology of schizophrenia is indicated by the increase in lipid peroxidation products in the plasma and CSF, and the altered levels of both enzymatic and non-enzymatic antioxidants in chronic naive first-episode patients [60.61]. Furthermore, male schizophrenic patients were found to have lower levels of uric acid than control subjects, and the plasma levels of uric acid in the patient groups were significantly and inversely correlated with psychosis. Tardive dyskinesia is a movement disorder affecting 20–40% of patients treated chronically with neuroleptic drugs. Tsai hypothesized that neuroleptics such as haloperidol, enhance striatal glutaminergic neurotransmission by blocking presynaptic dopamine receptors, thus promoting neuronal damage caused by OS [62].

7. Chemically-induced neurological disorders

Several neurotoxic chemicals have been shown to elevate the cerebral rate of ROS production in experimental animals. These include methyl mercuric chloride, cadmium, toluene, and other organic solvents [63, 64]. All of these agents are also capable of increasing intracellular levels of calcium ions [65].

8. Brain aging

Aging in mammalian species appears to be the result of normal developmental and metabolic processes responsible for graying of the hair, decreases in the rate of wound healing and increases in susceptibility to disease and death. The most reliable risk factor for neurodegenerative diseases is normal aging. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, and proteins) especially in brains from elderly subjects, supporting the hypothesis that oxidative injury might directly cause the aging process. Additional links between OS and aging focus on mitochondria. Direct biochemical measurements of mitochondrial function demonstrate age-dependent increases in mitochondrial deletions, point mutations, and oxidative damage to the DNA. The mitochondrial DNA in the elderly population is particularly susceptible to OS probably due to its close proximity to the respiratory chain, limited repair mechanisms, few non-coding sequences and absence of histones [66-68].

III. Diabetes mellitus

Diabetes in humans is a disease associated with increased oxidative stress. The cause of this is not yet fully understood but is thought to include mitochondrial dysfunction, direct enzyme inhibition by hyperglycaemia, auto-oxidation of glucose and activation of NADPH-oxidase. The oxidative stress manifests itself as elevated concentrations of lipid peroxidation products, erythrocyte fragility and decreases in the antioxidant enzyme systems (CAT, GSH-PX, SOD) [69, 70]. Recent studies have also shown a positive correlation between blood glucose concentration and oxidantinduced lymphocyte DNA damage [71]. Clinical intervention to control blood glucose concentrations may alleviate oxidative DNA damage, although this has not been reported. Likewise, dietary intervention with antioxidant nutrients, vitamins E, C and taurine may mediate the high level of oxidative stress in diabetics. Certainly, steady state estimates of cellular DNA oxidation indicate a role for antioxidant vitamins in the prevention of DNA oxidative damage. In one study, a combined dietary supplement of 25 mg β-carotene, 100 mg vitamin C and 280 mg vitamin E resulted in a significant decrease in lymphocyte DNA strand breaks over a 4 month period in healthy adult humans [72]. Similar doses of vitamin E in cats and dogs equate to approximately 10 times the current minimum requirement (1.4 IU/MJ diet).

IV. Asthma

Feline asthma closely parallels human asthma, another clinical condition now known to be associated with oxidative stress. Although the pathogenesis of asthma, both human and feline, is not fully defined, a typical feature is an increase in the number of inflammatory cells in the lung. Such cells generate ROS, which are involved in the pathophysiology of asthma, including airway smooth muscle contraction, increased airway reactivity and increased vascular permeability [73].Studies have indicated that there is reduced activity of SOD in the lung cells of asthmatics. SOD activity is reduced by 25% in bronchoalveolar lavage cells and by almost 50% in bronchial epithelial cells. It has also been demonstrated that cells both of peripheral blood and lung from asthmatics generate increased ROS and this increase correlates with disease severity. Despite the evidence implicating oxidative insult in the development of asthma, there are virtually no reported antioxidant intervention studies. *In vitro* studies have demonstrated that taurine can protect against bronchiolar damage induced by NO_2 [74]. Complementary *in vivo* rodent studies have confirmed that taurine at physiological concentrations (1%) protects mammalian

alveolar pneumocytes following exposure to acute free radical insult, preventing both the initial acute inflammatory response and the later development of fibrosis [75].

V. Atherosclerosis:

It has been known that LDL can be oxidized by many kinds of oxidants by different mechanisms and pathways. Some of the oxidant may arise from cells such as microphases, endothelial and smooth muscle cells. Other oxidants may be derived from exogenous sources, such as food and smoking. Free radical mediated lipid peroxidation proceeds by a chain mechanism, where the lipid peroxyl radicals act as chain carrying species. Myelo Peroxidase (MPO) secreted from phagocytes has been implicated in the pathogenesis of atherosclerosis. Reactive nitrogen species are another species, which may contribute in atherosclerosis. Nitric Oxide (NO) is not a string oxidant in itself, but it reacts rapidly with O_2 to give peroxynitrite, which oxidizes LDL to an atherogenic form [76].

VI. Heart failure

Despite advances in treatment, chronic congestive heart failure carries a poor prognosis and remains a leading cause of cardiovascular death. Accumulating evidence suggests that reactive oxygen species (ROS) play an important role in the development and progression of heart failure, regardless of the etiology.

VII. Hemorrhagic shock

Acute hemorrhagic shock causes decreases in the cardiac function and contractility and is associated with an increase in oxygen free radical producing activity of PMN leukocytes. Oxygen free radicals have been shown to depress the Ca2+ transport and Ca2+ - ATPase of sarcoplasmic reticulum and hence, decrease the contractility and the rate of relaxation [77].

VIII. Ischemia- reperfusion

Reactive oxygen-derived radicals and metabolites are known to play important roles in the pathogenesis of ischemia/reperfusion and anoxia/ reoxygenation injury. Free radicals are induced by the reperfusion blood flow in addition the lack of oxygen (O_2) supply to the ischemic cell.

IX. Lung disease

The large endothelial surface is constantly exposed to many atmospheric pollutants including tobacco smoke, fuel emissions, ozone and nitrogen dioxide and given the natural oxidizing nature of the atmosphere (Ex: 21% O_2) the lung is always at risk of oxidative injury [78].

X. Aging

The free radical theory of aging, conceived in 1956, has turned 40 and is rapidly attracting the interest of the maintenance of biological research. These include phenomenological measurements of ageassociated oxidative stress, interspecies comparisons, dietary restriction, the manipulation of metabolic activity and oxygen tension, treatment with dietary and pharmacological antioxidants, in vitro senescence, classical and population genetics, molecular genetics, transgenic organisms, the study of human diseases of aging, epidemiological studies, and the ongoing elucidation of the role of active oxygen in biology [79].

XI. Free radicals and cancer

The complex series of cellular and molecular changes participating in cancer development are mediated by a diversity of endogenous and exogenous stimuli. One type of endogenous damage is that arising from intermediates of oxygen (dioxygen) reduction oxygen free radicals, which attacks not only the bases but also the deoxy ribosyl backbone of DNA. OFR are also known to attack other cellular components such as lipids, leaving behind reactive species that in turn can couple to DNA bases [80].

XII. Inflammation

During phagocytosis, cells consume increased amount of oxygen; a process termed the respiratory burst. Activation results in increased NADPH production via the hexose mono phosphate shunt and the generation of O_2, H_2O_2, OH and hypo chlorous acid (HOCI), Hypoxanthine concentration, xanthine oxidase activity and ROS production are increased in rheumatoid arthritis [81].

XIII. Ocular disease

Oxidative stress is implicated in age-related macular degeneration and cataracts by altering various cell types in the eye either photochemically or nonphotochemically [82]. Under the action of free radicals, the crystalline proteins in the lens can cross-link and aggregate, leading to the formation of cataract [83]. In the retina, long-term exposure to radiation can inhibit mitosis in the retinal pigment epithelium and choroids, damage the photoreceptor outer segments, and has been associated with lipid peroxidation [84].

XIV. Fetus

Oxidative stress is involved in many mechanisms in the development of fetal growth restriction and pre-eclampsia in prenatal medicine. Some reports indicate that blood levels of lipid peroxidation products (F2-isoprostanes, MDA) are elevated in pre-eclamptic pregnancy and intra-uterine growth retardation and it has been suggested that ROS/RNS play a role in the etiology of these diseases [85,86]. In pregnancies complicated by pre-eclampsia, increased expression of NADPH oxidase 1 and 5 isoforms which are the major enzymatic sources of superoxide in the placenta is seen [87].

CONCLUSION

The most important free radical in biological systems is radical derivatives of oxygen with the increasing

acceptance of free radical as commonplace and important biochemical intermediate. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration.The imbalance between ROS and antioxidant defence system increases the oxidation burden and lead to the damage of macromolecules such as carbohydrates or proteins, such processes of various diseases. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. Plants having vitamins (C, E, carotenoids, etc.), flavonoids (flavones, isoflavones, flavonones, anthocyanins and catechins), polyphenols (ellagic acid, gallic acid and tannins) possess remarkable antioxidant activity. Antioxidant activity not restricted to a particular part of plant nor the specific families. Current review reveals the different potential application of antioxidant / free radical manipulations in prevention or control of diseases. Natural products from dietary components such as Indian species and medicinal plants are known to possess antioxidant activity. All plants discussed in this review exhibited significant clinical and pharmacological activity with fewer side effects.

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