Advances in Bioresearch
Volume 3 [4] December 2012: 164- 175
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[ISO 9001: 2008 Certified Organization]
Website: http://www.soeagra.com/abr/abr.htm

A B R
ISSN 0976-4585

Review Article

Reactive Oxygen Species and Natural Antioxidants: A Review

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REACTIVE OXYGEN SPECIES & FREE RADICALS

It is ironic that oxygen, an element indispensable for life [1], under certain situations has deleterious effects on human body [2]). Most of the potentially harmful effects of oxygen are due to the formation and activity of number of chemical compounds known as ROS, which have a tendency to donate oxygen to other substances. The causes of the poisonous properties of oxygen were obscure prior to the publication of Gershman's free radical theory of oxygen toxicity in 1954, which states that the toxicity of oxygen is due to partially reduced forms of oxygen [3].

Oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbits [4]. This unpaired electron(s) usually give a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems [5]. Molecular oxygen (dioxygen) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical(O_2^-)[5]. Superoxide anion, arising either through metabolic processes or following oxygen "activation" by physical irradiation is considered the "primary" ROS, and can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme or metal catalyzed processes [6].

The presence of an unpaired electron in an atomic orbital giving a thorough definition to free radical, results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive, that can either donate an electron to, or accept an electron from other molecules, therefore behaving as oxidants or reductants [7].

Natural process of generation of ROS

Free radicals and other ROS are derived from normal essential metabolic processes in the human body. Due to the continuous process of both enzymatic and non-enzymatic reactions free radical formation occurs continuously in the cells. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P-450 system[8]. Free radicals can also be formed in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

The research of free radicals in biological systems and their enzymatic roles were explored in 1969 when Mc Cord and Fridovich discovered the enzyme superoxide dismutase (SOD), and thus provided convincing evidence about the importance of free radicals in living systems [9]. Further explorations date from 1977, when Mittal and Murad provided evidence that the hydroxyl radicals, OH, stimulate activation of guanylate cyclase and formation of the "second messenger" cyclic guanosine monophosphate (cGMP) [10]. Since then a large body of evidence has been accumulated that living systems have not only adapted to a coexistence with free radicals but have developed various mechanisms for the advantageous use of free radicals in various physiological functions.

Some internally generated sources of free radicals and ROS are [11] as follows :

- Mitochondria
- Xanthine oxidase
- Peroxisomes
- Inflammation
- Phagocytosis
- Arachidonate pathways
- Exercise
- Ischemia / reperfusion injury

The production of superoxide occurs mostly within the mitochondria of a cell [12]. The mitochondrial electron transport chain is the main source of ATP in the mammalian cell, thus essential for life. During energy transduction, a small number of electrons "leak" to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the pathophysiology of a variety of diseases [13] and [14].

When produced *in vivo* OH reacts close to its site of formation. The redox state of the cell is largely linked to an iron (and copper) redox couple and is maintained within strict physiological limits [15]. Under stress conditions, an excess of superoxides releases "free iron" from iron containing molecules O_2^- acts as an oxidant of [4fe-4s] cluster containing enzymes and facilitates OH production from H_2O_2 by making Fe^{2+} available for the Fenton reaction [6] and [16].

The most realistic *in vivo* production of hydroxyl radical according to Fenton reaction occurs when M^{n+} is iron, copper, chromium or cobalt.

Peroxisomes are known to produce H_2O_2, but not O_2^-, under physiologic conditions [14]. Peroxisomes are major sites of oxygen consumption in the cell and participate in several metabolic functions that use oxygen. Oxygen consumption in the peroxisome leads to H_2O_2 production, which is then used to oxidize a variety of molecules. The organelle also contains catalase, which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound. Thus, the peroxisome maintains a delicate balance with respect to the relative concentrations or activities of these enzymes to ensure no net production of ROS. When peroxisomes are damaged and their H_2O_2 consuming enzymes down regulated, H_2O_2 releases into the cytosol, which is significantly contributing to oxidative stress.

If a phagocytic cell such as neutrophil is exposed to a stimulus, it has the ability of recognizing the foreign particle and undergoing a series of reactions called the respiratory burst. Nicotine adenine dinucleotide phosphate (NAP(P)H) oxidase is best characterized in neutrophils, where its production of O_2^- generates the respiratory burst necessary for bacterial destruction [17].

Lipid peroxidation is a free radical process involving a source of secondary free radicals, which further can act as "second messenger" or can directly react with other biomolecule, enhancing biochemical lesions. Lipid peroxidation occurs on polyunsaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction. Hydroxyl radical is thought to initiate ROS and remove hydrogen atom, thus producing lipid radical. Further, by addition of oxygen it forms highly reactive peroxyl radical that attacks another fatty acid forming lipid hydroperoxide (LOOH) and a new radical. Thus lipid peroxidation is propagated, resulting in the formation of compounds like alkanes, malanoaldehyde and isopropanes which are used as markers and have been verified in neurodegenerative diseases [18].

Beneficial effects of ROS

ROS are known for playing a dual role as they can be both deleterious and beneficial species for the living systems. Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to anoxia, as for example, in defence against infectious agents and in the function of a number of cellular responses or signaling systems. One another beneficial aspect of ROS is the induction of a mutogenic response. [19].

Another aspect of ROS is importantly achieved by mechanisms called "redox regulation", that protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox statue *in vivo* [20].

In purine catabolism, the enzyme, xanthine oxidoreductase (XOR) [21] and [22] catalyzes the oxidative hydroxylation of hypoxanthine to xanthine, and subsequently of xanthine to uric acid.

Uric acid acts as a potent antioxidant and free radical scavenger XOR, has, therefore important functions as a "cellular defense enzyme" against oxidative stress. With both XO (Xanthine oxidase) & XD (Xanthine dehydrogenase) forms, numerous ROS and RNS are synthesized [22].

Harmful effect of ROS

The harmful effect of free radical causing potential biological damage, particularly by ROS, is termed oxidative stress [23], [24] and [25]. This occurs in biological systems when there is an over production of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organism. In fact, the free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body, and such normal changes with age are relatively common to all. The harmful aspect is when these changes are superimposed by the influential aspects of genetics and environment, which modulate free radical damage and these are manifested as diseases at certain ages, cancer and atherosclerosis, being the salient ones.

Oxidative damage to DNA, lipids & proteins

The damage to cell structures, nucleic acids, lipids and proteins is distinctly evident at the high concentrations of ROS [19]. The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and the pyrimidine bases, and also the deoxyribose backbone [4]. The most extensively studied DNA lesion is the formation of 8-OH-G. Permanent modification of genetic material resulting from these oxidative damage incidents represents the first step involved in mutagenesis, carcinogenesis and ageing [26], DNA is considered as a major target especially in cancer and ageing.

It is known that metal induced generation of ROS results in an attack not only on DNA, but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation [27]. Once formed, peroxyl radicals (ROO°) can be rearranged via a cyclisation reaction to endoperoxide, the precursors of malanodialdehyde, with the final product of the peroxidation process being malondialdehyde (MDA) [28], [29] and [30]. MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. It appears to be the major toxic product of lipid peroxidation.

Mechanisms involved in the oxidation of proteins by ROS were elucidated by studies in which amino acids, simple peptides and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl / superoxide radicals are formed (Standtman, 2004). Oxidation of cysteine residues by the action of ROS may lead to reversible formation of mixed disulphides between protein thiol groups (-SH) and low molecular thiols, in particular GSH (S-glutathiolation). Besides cysteine, methionine, arginine and histidine seem to be the most vulnerable to oxidation by ROS [33]. Protein oxidation affects the alteration of signal transduction mechanism, enzyme activity, heat stability and proteolysis susceptibility, which leads to ageing.

Advanced glycation end products (AGEs) is a class of complex products, that result from the reactions between carbohydrates and free amino group proteins. The intermediate products are known, variously, as Amadori,. Schiff Base and Maillord products, named after the researchers who first described them [34]. The brown colour of the very unstable and reactive AGEs is probably related to the name of melanoidins, initially proposed by Milliard, and well known from food chemistry.

Cardiovascular diseases

The oxidative events may affect cardiovascular diseases, thus responsible for about half of all the deaths. The ROS induced oxidative stress in cardiac and vascular myocytes has been linked with cardiovascular tissue injury [35].

Polyunsaturated fatty acids occur as a major part of the low density lipoproteins (LDL) in blood and oxidation of, these lipid components in LDL play a vital role in atherosclerosis [36]. Amongst the important cells in the vessel wall are the endothelial cells, smooth muscle cells as well as macrophage which can release free radicals that affect lipid peroxidation [37]. The high level of oxidized lipids lead to the continuation of blood vessel damage to the reaction process resulting in generation of foam cells and plaque, the symptoms of atherosclerosis. Other cardiovascular

disorders like hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure are all ROS induced disorders [38].

Carcinogenesis

The presence and participation of free radicals in carcinogenesis, mutation and transformation is well known. Induction of mutagenesis, the best known of the biological effect of radiation, occurs mainly through damage of DNA by the HO. Radical and other species are produced by the radiolysis, and also by direct radiation effect on DNA. Radicals mainly add to double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety, affecting and causing cell mutagenesis and carcinogenesis. The ROS induced damage can result in either arrest of transcription or signal transduction pathways, replication errors, and genomic instability, all of which are associated with carcinogenesis [39] and [19]. The carcinogenic sources of ROS are like tobacco smoke, arsenic compounds, hexavalent chromium, cadmium etc. It has been clearly demonstrated that ROS interfere with the expression of a number of genes and signal transduction pathways and are thus instrumental in the process of carcinogenesis [19, 40].

Ageing

The various pathological conditions have been implicated in human beings due to ROS and oxidative stress, involving cardiovascular disease, cancer, neurological disorders, diabetes and above all ageing [34], [35], [41] and [42]. The process of ageing may be defined as a progressive decline in the physiological functions of an organisms after the reproductive phase of life. The major mechanism of ageing attributes to DNA or the accumulation of cellular and functional damage [43]. The various diseases in human beings fall, mainly, into two groups:

1. The first group involves diseases characterized by pro-oxidants shifting the thiol / disulphide redox state and impairing glucose tolerance – the so called “mitochondrial oxidative stress” conditions, vis., cancer and diabetes mellitus.
2. The second group involves disease characterized by “Inflammatory oxidative conditions” and enhanced activity of NAD(P)H oxidase leading to atherosclerosis and chronic inflammation , or xanthine oxidase – induced formation of ROS as in ischemia and reperfusion injury.

The process of ageing is to a large extent due to the damaging consequence of free radical action [44].The free radical theory of ageing was first introduced in 1956 by Denham Harman who proposed the concept of free radicals playing a role in the concept of free radicals playing a role in the ageing process [44]. The genesis of ageing starts with oxygen occupying the final position in the electron transport chain [14]. Generally, a role of protein modification in ageing was highlighted by the result that many different enzymes isolated from younger animals were catalytically more active and more heat stable than the enzymes isolated from older animals [32]. It was also proposed that ROS mediated protein damage is involved in ageing.

NATURAL ANTIOXIDANTS IN HUMAN BODY

Exposure to free radicals from a variety of sources had led organisms to develop a series of defense mechanisms. The antioxidants act at different levels in the defense system such as preventive, radical scavenging, repair and adaptation [45].

The preventive antioxidants are included in the first line of defense, which suppress the formation of free radicals. The metal induced decompositions of hydroperoxides and hydrogen peroxide are considered to be the important sources. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathiome peroxidase (GPx), catalase (CAT).

The second line of defense is the antioxidants that scavenge the active radicals to suppress chain initiation and or break the chain propagation reactions. They are hydrophobic and other lipophylic. These include vitamin C, Vitamic E, uric acid, bilirubin, albumin and thiols.

The third line of defense is the repair and de novo antioxidants. The proteolytic enzymes, proteinases, proteases and peptidases present in the cystosol and in the mitochondria of mammalian cells are included.

The DNA repair systems form strong defense systems against oxidative damage. And the fourth line of defense include function called adaptation where signal for the production and reactions of free radicals induce the formation and transport of the appropriate antioxidant to the right side [46].

A wide variety of naturally occurring antioxidants are found in nature which are different in their composition, properties, mechanisms and site of action. Some of the main categories are as follows-

Enzymes

The cells are protected against oxidative stress by an interacting network of antioxidant enzymes [47]. These act as antioxidants by transforming reactive oxygen species and reactive nitrogen species into the stable compounds [48]. Enzymes are important for the repair of damaged DNA, damaged protein, oxidized lipids and peroxides. Lipase, proteases, transferase, methionine sulphoxide reductase etc. help to reconstitute the damaged cell membrane. Some of the important ones are as follows:

- **Superoxide dismutase**

These enzymes (SODs) catalyze the breakdown of the superoxide anion into hydrogen peroxide and oxygen. [49] and [50].These are present in almost all aerobic cells and in extracellular fluids (Johnson, F., Giulin, C., 2005). The three major families of SOD include Cu/Zn, Fe and Mn types, depending on the metal cofactor. In plants, SOD are present in chloroplasts, peroxisomes, apoplast and cystol, and in human beings, SOD1 is localized in the cytoplasm, SOD2 in mitochondria and SOD3 is extra cellular. [52], [53] and [54].

- **Catalase**

It is found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen, which otherwise is a harmful by product of many normal metabolic processes, and to prevent any damage, it must be quickly converted into other, less dangerous substances [55].

- **Gluthathione**

It is a cysteine- containing peptide found in most forms of aerobic life, that are not required in the diet and instead synthesized in the cells from the constituent amino acids.[57]. It has antioxidant property since the thiol group in its cysteine moiety is a reducing agent. Due to its high concentration and central role in maintaining the cells redox state, gluthathione is one of the most important cellular antioxidants [58].

High molecular weight compounds

These include proteins like albumin, transferin, ceruplosmin that restrict the production of metal catalyzed free radicals [59].

Low molecular weight compounds

The low molecular weight compounds include lipid soluble and water soluble antioxidants. Tocopherol, quinines, bilirubin and some polyphenols come under lipid soluble antioxidants; and ascorbic acid, uric acid and some other polyphenols come under water soluble ones [60].

Minerals

Minerals like selenium, copper, manganese, zinc etc. are well known antioxidants. Selenium appears to be necessary for efficient scavenging of peroxides from cystosol and cell membrane. Copper exerts its antioxidant activity through the cystosolic superoxide dismutase [61].Zinc is an element essential for normal growth, reproduction and other functions of the body. Nowadays chromium is also used in antioxidant formulation.

Vitamins

The vitamins play a crucial role in preventing peroxidation damage in the biological system. Thus vitamin A, C, and E act as the popular antioxidants. Vitamin C, along with uric acid, bilirubin, albumin and thiols are hydrophilic radical scavenging antioxidants, while vitamin E and ubiquinol are lipophilic radical scavenging antioxidants [62] and [63]. Vitamin E is the most potent radical scavenging lipophilic antioxidants.

Non-enzymatic antioxidants

Many of the important antioxidants known are non-enzymatic, and the following are the significant ones :

- **Ascorbic acid**

Ascorbic acid, is, in other words, the vitamin 'C' which is a monosaccharide antioxidant found in both animals and plants. It is not synthesized in humans and so must be obtained from the diet. [64]. In cells, it is maintained in its reduced form by reaction with glutathione, which can be

catalyzed by protein disulphide, isomerase and glutaredoxins [56]. Ascorbic acid is a reducing agent and can reduce, thereby neutralize ROS, such as hydrogen peroxide, [65].

- **Tocopherols and tocotrienols (Vitamin E)**

Vitamin E is the collective set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties [66]. It has been reported that the α-tocopherol form is the most important lipid soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [67]. This removes the free radical intermediates and prevents the propagation reaction from continuing. The reaction produces oxidized α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants such as ascorbate, retinol or ubiquinol [68].

- **Uric acid**

Uric acid accounts for roughly half the antioxidant ability of plasma. In fact, it may have substituted for ascorbate in human evolution[69]. However, like ascorbate, uric acid can also mediate the production of active oxygen species.

- **Melatonin**

Melatonin, primarily produced by the pineal gland, is well known for its bleaching effect upon the skin pigment, the 'melanin', and has been widely used as a protective agent against a wide variety of processes and agents that damage tissues via free radical mechanisms. It is uniquely found in human beings, animals, plants and microbes. Melatonin, is, chemically known as N-acetyl-5-methoxytryptamine [70], and is a naturally occurring hormone found in animals and some other living organisms, including algae [71]. It is a powerful antioxidant that can easily cross cell membranes and the blood-brain barrier ([72]. Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant [73].

External antioxidants

The interesting fact is that human body is designed to create its own antioxidants. Indeed, the body has many built-in systems for fighting inflammation, diseases and toxins naturally. We have all been guided by the health community to supplement these internally produced antioxidants with 'external antioxidants' such as a specific set of foods, vitamins or herbal remedies. Externally sourced antioxidants are what we ingest, either through healthy foods such as berries, or supplements, such as vitamin C.

People who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases. [74]. Also, some types of vegetables and fruits may lower risk against some cancers (Food, Nutrition, Physical activity and the prevention of Cancer: a Global Perspective - 2007). It was also reported that people taking Vitamin E supplements had a lower risk of developing heart disease [75]. Many nutraceutical and health food companies sell formulations of antioxidants as dietary supplements and these are widely used in industrialized countries[76]. These supplements may include specific antioxidant chemicals, like the polyphenol, resveratrol (from grape seeds or knotweed roots) [77], combination of antioxidants, like the "ACES" products that contain beta carotene (provitamin A), vitamin C, vitamin E and selenium or herbs that contain antioxidants such as green tea and jiaogulan. Rather, dietary polyphenols may have non-antioxidant roles in minute concentrations that affect cell to cell signaling, receptor sensitivity, inflammatory enzyme activity or gene regulation.

Whole foods represent the simplest examples of functional food which can satisfactorily affect one or more target functions in the body thus reducing the risk of a disease and in maintenance of well health [78] broccoli, carrots and tomatoes come under functional food. Green vegetables and spices like mustard and turmeric, used extensively in Indian cuisine also fall under this category [79]. The food or parts of food that provide medical or health benefits, are known as 'Nutraceuticals'[80]. and may range from isolated nutrients, dietary supplements, herbal products and processed products such as cereals, soups and beverages. The major active nutraceutical ingredients in plants are flavoroids, the phenolic compounds, those act as potent antioxidants and metal chelators,

possessing anti-inflammatory, antiallergic, hepatoprotective, anti-viral and anti-carcinogenic activities [81].

Synthetic antioxidants

Many antioxidants derived artificially from the synthetic sources are used as food additives to help guard against food deterioration and also as the important constituents of a variety of drugs which are known as synthetic antioxidants. Exposure to oxygen and sunlight are the two main factors in the oxidation of food so food is preserved by keeping in the dark and sealing it in containers coated with wax. However, storing plant materials in anaerobic conditions produces unpleasant flavours and unappealing colours [82]. In order to preserve the natural characteristic upto a certain extent, an important class of preservatives are used, which include natural antioxidants such as ascorbic acid and tocopherols, as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylthydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) [83].

Antioxidants are frequently added to industrial products, where they are used as stabilizers in fuels and lubricants to prevent oxidation and in gasolines to prevent the polymerization that leads to the formation of engine fouling residues [84]. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials. (Why use Antioxidants- Retrieved 2007).

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been reported to be dangerous for human health. Many of them, used in a variety of drugs and medicines, are nowadays banned.

ANTIOXIDANTS - FROM NATURAL RESOURCES

Dietary fibres, Vitamins, minerals, antioxidants, oligosaccharides, essential fatty acids (Omega - 3), lactic acid, bacteria and lignins are the prominent ingredients that make food functional. Many of these are present in medicinal plants. Indian systems of medicine believe that complex diseases can be treated with complex combination of botanicals with single drugs. Some medicinal plants & dietary constituents having functional attributes, are spices such as onion, garlic, mustard, red chillies, turmeric, clove, cinnamon, saffron, curry leaves, fenugreek and ginger. Some herbs as *Bixa orellana* and vegetables like amla, wheat grass, soyabean, and Grancinia cambogia have antitumor effects. Other medicinal plants with functional properties include *A. marmelos, A.cepa, Alo vera, A.paniculata, Azadirachta india* and *Brassica juncea* [85]. spices and herbs in food as medicine is a current hot trend in capturing everyone's imagination with images of a new magic bullet or fountain of youth.

Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. Antioxidant constituents of plant materials act as radical scavengers, and convert the radicals to less reactive species. Natural oxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. Because extensive and expensive testing of food additives is required to meet safety standards, synthetic antioxidants have generally been eliminated from many food applications. The increasing interest in the search for natural replacements for synthetic antioxidants has led to the antioxidants evaluation of a number of plant sources.

Antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreaded free radicals and stop oxidation chains *in-vivo*, so they have become viewed by many as natures answer to environmental and physiological stress and other disorders. It is known that compounds belonging to several classes of phytochemical components such as phenols, flavonoids, and carotenoids are able to scavenge free radical such as O2-, OH-, or lipid peroxyl radical LOO in plasma [86].

Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites [87]. Plant derived antioxidants have been reported to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists [88]. The most current research on antioxidant action focuses on phenolic compounds, such as flavonoids fruits and vegetables contain different antioxidant compound such as vitamin C , vitamin E and Carotenoids,

whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants [89]. These components include:

- Nutrient derived antioxidants like ascorbic acid (Vitamin C), tocopherol and tocotrienols (Vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and glutathione reductase.
- Metal binding proteins, such a ferritin, lactoferritin and ceruloplasmin that sequester free iron and copper ions, capable of catalyzing oxidative reactions.
- Numerous other antioxidant phytonutrients are detected in a wide variety of plant foods.

An essential oil defined as the product obtained by hydro distillation, steam distillation or dry distillation, or by a suitable mechanical process without heating (for citrus fruits) of the plant or some parts [90]. They can be synthesized from all plant organs like flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots and therefore extracted from these parts, where they are stored in secretary cells, cavities, canals, epidermis cells or glandular trichomes [91] and [92]. The complex composition of essential oils show a wide range of constituents like terpenes, monoterpenes, subsquiterpenes, allyl and propenyl [93], most of these compounds having antioxidant properties.

EXPERIMENTAL EVIDENCE OF ANTIOXIDANT PROPERTIES OF PLANTS

Much of the literature is available regarding the antioxidant property of several medicinal plants, and yet the further exploration in this direction is remarkable and continuous phenomena.

During the evaluation using the ferric thiocyanate, Yang, S.A., et al., [94] found that *Lavandula angustifolia* Mill. Oil from Australia was very effective against lipid peroxidation than any of the other oils studied. It was predominantly constituted by linalool and lynalyl acetate.

The oils of *Thymus vulgaris L.(thyme), Eugenia caryophyllus (C.Spreng)* and *Ocimum basilicum L. (basil)* had appreciable antioxidant activities, comparable to that of α-tocopherol, the reference chosen by Wei, A., et al., [95] with predominant p-cymene and thynol, eugenol and β-caryophyllene as well as linalool, isoanethole and eugenol respectively, which displayed similar ability for preventing lipidic peroxidation.

The evaluation of antioxidant potential of leaf extracts of *Adiantum capillus veneries Linn*. was carried out by Kumar , A., et al., [97] against hydrogen peroxide induced oxidative damage in peripheral blood lymphocytes with 100 μM H_2O_2 for 2 hours that significantly increased lipid peroxidation and decreased the level of glutathione and the antioxidant enzymes.

The essential oils of *Artimisia herbaalba Asso*., cultivated in southern Tunisia, have been reported antioxidant active by Mighri, H., et al., [98], and four types of oils were detected : β-thujone, α-thujone, thujones (α+β), and 1,8-ineole / camphor / thujones (α+β). These however, exhibited weak antioxidant abilities for preventing the linoleic acid oxidation, attributed to the absence of non-phenolic compounds.

The chemical evaluation of the essential oils of different parts of *Myrtus communis var. italica L*., viz., leaf, stem and flower was carried out by Wannes, W.A., et al., [99]. Leaf and flower oils had the best antioxidant activities, the major components of leaf oil being 1,8-cineole and α-Pinene, and α-teripineol and eugenol being the major components of flower oils.

Hymenocrater longiflorus Benth from Iran was evaluated by Ahmadi, F.et al, [102] for its antioxidant activity, with its main constituents being α-pinene, 1-8-cineole, β-eudesmol, spathulenol, hedycaryol, δ-cadinene and oxygenated sesquiterpinids. These essential oil were able to inhibit the bleaching of β-carotene. The percentage of inhibition was even close (66.4%) to those found for the non-polar-sub-fraction (chloroformic) 69.1%, which presented the best activity.

The antioxidant, anti microbial and antispasmodic activities of *Origanum acutidens (*Hand-Mazz*) Ietswaart* from the Turkish flora were evaluated by Goze, I., et al.,[103]. The major component reported was carvacrol with prominent antioxidant activity, but inferior to that of the reference substance i.e. BHT used.

The oils of *Ageratum conyzoides L*., constituting mainly, precocene I and caryophyllene, possessed good capacity for preventing lipid peroxidation, using lipid substrate as liver homogenate was reported by Patil, R.P., et al.,[104] . It had better results than those found for BHA. The methanolic extracts of the same plants have also been tested but the activities were about 100 times lower than those of the essential oils, attributing to their antioxidant activity.

Amongst the significant Himalayan *Lauraceae* species, only the extracts of *Dodecadenia grandiflora* and *Lindera pulcherrima* were able to inhibit lipid peroxidation, using liver homogenate as lipid substrate. These, along with extracts of *Persea gamblei*, were already reported by Joshi, S.C., et al. [101] as being potent inhibitors of linoleic oxidation measured through the β-carotene bleaching test.

The reports have related the cytotoxicity with antioxidant activity in the zingiberaceous plant in the south-west of China, known as *Amomum tsao-ko* (Crevost & Lemaire), as quoted by Yang, Y., et al.,[94]. Also the essential oils studied had substantialanticancer activity that led to the assay of the samples for their respective antioxidant activity using TBARS. The weak antioxidant activity was reported due to the low phenolic content of the oils.

Aerial parts and seeds of *Foeniculum vulgare* Mill. oils possess different chemical compositions and also their antioxidant activity differs. For higher concentrations of essential oils, a decrease of the antioxidant activity was observed by Miguel., M.G., et al., [100] and [105], suggesting that high concentrations of essential oils possess a pro-oxidant activity, independent of their rich concentration in trans-anethole (aerial parts) or methyl chavicol seeds.

Majorana hortensisi L. oils had appreciable antioxidant activity probably due to the presence carvacrol with positive synergism with other component (Martino, L.,et al,[106])

According to Firuzi, O., et al., [107]. *Heracleum pastinacifolium* and *Heracleum persicum*, showed moderate antioxidant activity, the main constituents of which were myristicin and trans amethole respectively.

Lavender oil of *Lavandula augustifolia* was reported to be the most effective against lipid peroxidation as mentioned by Yang, S.A., et al.,[94]. As far as free radical scavenging activity is concerned lavender essential oil exhibited the highest DPPH-scavenging activity, the major components of it were linalool and linalyl acetate, but limonene also showed similar activity.

CONCLUSION

Since the problem related to ROS is quite natural and we are bound to face the adverse effect of ROS, so before the scientific world this is a big challenge to counter the problem of ROS and its adverse impact. Of course scientific world has exerted a lot to counter the problem but banking on above review it seems that antioxidants from plant origin may prove best suitable. Although , some authors have investigated about phytoremediation but still a in depth and large scale investigation is required to control the problem of ROS.

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[Received 25.07.12; Accepted: 09.10.12]