



Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com



THE IMPORTANCE OF ANTIOXIDANT AND THEIR ROLE IN PHARMACEUTICAL SCIENCE - A REVIEW

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ABSTRACT

The addition of synthetic antioxidants to oils and/or foods is one of the most efficient ways to prevent lipid oxidation. However, the safety of synthetic additives has been questioned stimulating the evaluation of naturally occurring compounds with antioxidative properties. Although there is no assurance of the safety of natural antioxidants, there is some comfort knowing that such antioxidants are purified from natural products that have been consumed for generations. Phenolic compounds in plants are recognized as important compounds in conferring stability against oxidation. Natural antioxidant phenolics can be classified into a lipophilic group, tocopherols, and a hydrophilic group, including simple phenolics, phenolic acids, anthocyanins, flavonoids and tannins. Even though chemists have elucidated the structures of thousands of phenolics, there are still many compounds that have not yet been fully characterized and they are referred as phenolic extracts. In this way berry extracts, aromatic plant extracts, essential oils and their components are gaining interest because of their relatively safe and wide acceptance by consumers. Many authors have reported antioxidant and radical-scavenging properties by berries, spices and essential oils. In this work we reviewed the most important groups of natural antioxidants, with some peculiarities related to chemical composition. Oxidative stress can be caused in result of free radicals formation. Aging and different chronic diseases including diabetes, cancer and cardiovascular diseases could be caused by oxidative stress. Antioxidants are important factor to maintain optimal cellular and human body health. Medicinal plants are an important source of antioxidants¹⁶. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases. four synthetic antioxidants are widely used in foods; namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ).

KEYWORDS

Antioxidants, BHA, TBHQ, Oxidative stress and BHT.

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INTRODUCTION

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. This

review presents some information about the antioxidant/antiradicals and their role in our body and also their presence in spices and herbs¹. Oxygen is absolutely essential for the life of aerobic organism but it may become toxic if supplied at higher concentrations. Dioxygen in its ground state is relatively unreactive; its partial reduction gives rise to active oxygen species (AOS) such as singlet oxygen, super oxide radical anion, hydrogen peroxide etc. This is partly due to the oxidative stress that is basically the adverse effect of oxidant on physiological function.

Free oxygen radicals plays cardinal role in the etiology of several diseases like arthritis, cancer, atherosclerosis etc. The oxidative damage to DNA may play vital role in aging² and the presence of intracellular oxygen also can be responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause damage to critical cell bio-molecules. These radicals are highly toxic and thus generate oxidative stress in plants.

Plants and other organism have in built wide range of mechanism to combat with these Free Radical problems. Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals.

In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of plant materials act as radical scavengers, and convert the radicals to less reactive species³. Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. The antioxidative system includes both enzymatic and non-enzymatic systems. The non enzymatic system includes ascorbic acid (vitamin C); α -tocopherol, carotenes etc. and enzymic system include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and polyphenol oxidase (PPO) etc. The function of this antioxidant

system is to scavenge the toxic radicals produced during oxidative stress and thus help the plants to survive through such conditions. Spices and herbs in food as medicine is a current hot trend that is capturing everyone's imagination with images of a new magic bullet or fountain of youth. The intake of antioxidant compounds present in food is an important health-protecting factor. Natural antioxidants 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 antioxidant evaluation of a number of plant sources².

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 nature's answer to environmental and physiological stress, aging, atherosclerosis, and cancer. The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating antioxidant activity of purified individual compounds, mixed extracts, and endogenous food matrices and optimizing applications⁴. It is well known that Mediterranean diet, which is rich in natural antioxidants, leads to a limited incidence of cardio- and cerebrovascular diseases⁴. 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 peroxy radical LOO in plasma⁵. The effective intake of single food antioxidants and their fate in the human body have been defined only for a few compounds⁶⁻⁷. It is reasonable that the higher the antioxidant content in foods is, the higher the intake by the human body will be. Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids,

vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites⁸. Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists⁹. The most current research on antioxidant action focuses on phenolic compounds such as flavonoids. Fruits and vegetables contain different antioxidant compounds, 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 antioxidants¹⁰⁻¹¹.

These components include

1. 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.
2. Antioxidant enzymes, e.g., super oxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
3. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
4. Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, 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. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being¹².

History of Antioxidants

Antioxidants have been used for the first time in the nineteenth century in the rubber industry, when it was observed that some molecules, identified empirically, could slow the degradation and allow optimization of the process of vulcanization. Today we know that the production and use of rubber reactions take place involving free radicals and oxygen and antioxidants is still a useful tool in the hands of those who need to optimize the performance of our tires. In the twentieth century antioxidants are then entered in the arsenal of the emerging food industry, as a key tool to curb the oxidative degradation of stored food. In that regard, it must be said that, since at that time known mechanisms of oxidation nor antioxidant effect, the connotation of "antioxidant" could only be empirical, grouping any compound or procedure that led to the result of slow degradation and rancidity¹³. This has resulted in an inaccuracy semantics which often leads today to a different definition of antioxidant between chemists, biologists and food technologists. If also did not know the free radicals, was known already at the end of the eighteenth century that oxygen, had an extremely important role for life, but also for degradation of biological material. The most significant demonstration of the role in modern biology radical oxidation is given in

the early 50's when in Buenos Aires Gerchman Rebecca and Daniel Gilbert found that the toxic effect of radiation was greatly enhanced by the presence of oxygen. In terms of antioxidants, a milestone in the evolution of knowledge is the discovery of Albert Szent-Györgyi, in the thirties, which, starting from the study of the reasons dell'imbrunimento apples, discovered vitamin C. The same researchers also studied the antioxidant effect of polyphenols found in plants and proposed a function vitamin (vitamin P) but could not withstand the criticism of the absence of deficiency syndrome, as fundamental to the definition of a function vitamin. The cultural boundary was beginning to be quite defined in 50-60 years: it was known that breathing (which is a form of controlled combustion) required oxygen activation and that breathing could generate free radicals. On this basis, supplemented by observations of acute medical and patho physiological, Denim Harman proposed the free radical theory of aging: if we use oxygen to convert our energy, we cannot escape its toxicity which slowly deteriorates there. The 'sharpness of the proposal has been confirmed in experimental biology, it is now generally accepted that the maximum health potential of a species is determined largely¹⁴ though not entirely, the ability to defend against oxidation.

The 'scientific information is then available today to interpret phenomena from the cellular level to that of quantum mechanics. But what is the medical and nutritional aspect of it all? The first answers cannot be that epidemiology in recent years has seen an explosion of meaning, quality and therefore of relevance. In practice, epidemiological studies of various kinds agree that "taking antioxidants in the diet is good" with respect to the reduction of risk of chronic degenerative diseases-mainly atherosclerosis and possibly reduce the risk of cancer¹³.

The syllogism that were due to the mechanisms of chemical reactivity of oxide-reductive antioxidants was then supported by a significant number of studies in vitro or cellular models showed different effects of different molecules-generally-polyphenols. A more careful examination, however,

concentrations obtainable in the body compared to those necessary to obtain an increase in antioxidant defense seems to exclude any realistic in vivo antioxidant effect of different dietary polyphenols. In practice, the only food antioxidants that reasonably can have an effect in vivo antioxidant are Vitamin C and Vitamin E. Their deficiency syndrome, however, is typical dell'avitaminosi and certainly not what you would expect from a deficiency of antioxidant defense¹⁵.

On 'other side, it is now shown that many natural substances of polyphenolic nature, and functionally antioxidants, may act by modulating the expression of genes, a mechanism that little or nothing to do with transitions oxide-reductive typical antioxidant effect. The acquisition of this type of information is in its infancy, being supported by complex expression analysis today only possible as a result dell'elucidazione genome. In other words phenolic antioxidants food regulatory elements are configured as "nutrigenomics," to use a practical how ugly neologism¹⁶.

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:⁸

1. A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mito-chondrial electron transport system.
2. Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.

3. Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of “leaky gut” syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body’s oxidant load⁹.

ANTIOXIDANT PROTECTION

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. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

These components include

1. Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
2. Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
3. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
4. Numerous other antioxidant phytonutrients present in a wide variety of plant foods¹⁷.

DIETARY ANTIOXIDANTS

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane

where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues.

Research suggests beta carotene may work synergistically with vitamin E. A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E⁹.

PHYTONUTRIENTS

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant-derived substances, collectively termed “phytonutrients,” or “phytochemicals,” are becoming increasingly known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plant kingdom: approximately 3,000 flavonoid substances have been described¹⁰. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as “biological response modifiers”¹⁸.

Flavonoids have been demonstrated to have anti-inflammatory, anti-allergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages¹¹. The best way to ensure an adequate intake of phytonutrients is to eat a diet rich in a wide variety of fresh fruits and vegetables. Phytonutrient supplements are also now widely available¹⁹.

ENDOGENOUS ANTIOXIDANTS

In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help

protect against free radical-induced cell damage. The antioxidant enzymes-glutathione peroxidase, catalase, and superoxide dismutase (SOD)-metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms⁹. Research indicates that consumption and absorption of these important trace minerals may decrease with aging. Intensive agricultural methods have also resulted in significant depletion of these valuable trace minerals in our soils and the foods grown in them²⁰. Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. Exposure of the liver to xenobiotic substances induces oxidative reactions through the upregulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase². When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation (a key step in the body's detoxification process) making it less available to serve as an antioxidant. Research suggests that glutathione and vitamin C work interactively to quench free radicals and that they have a sparing effect upon each other². Lipoic acid, yet another important endogenous antioxidant, categorized as a "thiol" or "biothiol," is a sulfur-containing molecule that is known for its involvement in the reaction that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha-ketoglutarate, in the Krebs cycle. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains and as such has been called a "universal antioxidant"¹⁶. Lipoic acid may also exert its antioxidant effect by chelating with pro-oxidant metals.

Research further suggests that lipoic acid has a sparing effect on other antioxidants^{16,21}. Animal

studies have demonstrated supplemental lipoic acid to protect against the symptoms of vitamin E or vitamin C deficiency¹⁶.

THE IMPORTANCE OF BALANCE

Although much of the research to date focuses on the potential benefit of single antioxidant nutrients, it has become clear that the best protection against oxidative stress comes from a wide assortment of interrelated antioxidants and antioxidant cofactors². In other words, the human body utilizes an integrated antioxidant system composed of several players that work together as a team. The reducing potential of each individual member of the antioxidant defense team is enhanced when a full complement of players is available. For example, some evidence suggests a poor concentration of any single one of the antioxidants vitamin C, vitamin E, or beta carotene, appears to increase the risk of cardiovascular disease. Additionally, the combination of several suboptimal concentrations may have an additive or even synergistic effect on increasing risk. (82 Conversely, it has been suggested that, under certain conditions, an excess of any one type of antioxidant in the absence of balance with the others may actually be counter-protective²². Moreover, the relative importance of a given antioxidant may vary with different disease conditions because the type or types of ROS generated are likely to differ, and because varying levels of specific antioxidants exist within the different tissues of the body²³.

Food Sources of Antioxidants and classification

Vitamin C Also called ascorbic acid, vitamin C is a water-soluble vitamin found in all body fluids, so it may be one of our first lines of defence. This powerful antioxidant cannot be stored by the body, so it's important to get some regularly not a difficult task if you eat fruits and vegetables. Important sources include citrus fruits, green peppers, broccoli, green leafy vegetables, strawberries, raw cabbage, and potatoes¹³.

Vitamin E A fat-soluble vitamin that can be stored with fat in the liver and other tissues, vitamin E (tocopherols, tocotrienols) is promoted for a range of purposes-from delaying aging to healing sunburn.

While it's not a miracle worker, it's another powerful antioxidant. Important sources include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil, and fish-liver oil²⁴.

Beta-carotene The most studied of more than 600 different carotenoids that have been discovered, beta-carotene (lycopene, carotenes) protects dark green, yellow and orange vegetables and fruits from solar radiation damage. It is thought that it plays a similar role in the body. Carrots, squash, broccoli, sweet potatoes, tomatoes, kale, collards, cantaloupe, peaches, and apricots are particularly rich sources of beta-carotene²⁵.

Selenium Selenium probably interacts with every nutrient that affects the antioxidant balance of the cell. This mineral is thought to help fight cell damage by oxygen-derived compounds and thus may help protect against cancer. It is best to get selenium through foods,²⁶ as large doses of the supplement form can be toxic. Good food sources include fish, shellfish, red meat, grains, eggs, chicken, and garlic. Vegetables can also be a good source if grown in selenium-rich soils²⁷.

Polyphenol antioxidants Polyphenol antioxidants (resveratrol, flavonoids) are characterized by the presence of several phenol functional groups. In human health these compounds, are thought to be instrumental in combating oxidative stress. The main source of polyphenol antioxidants is nutritional, since they are found in a wide array of phytonutrient bearing foods. For example, most legumes; fruits such as apples, blackberries, cantaloupe, cherries, cranberries, grapes, pears, plums, raspberries, and strawberries; and vegetables such as broccoli, cabbage, celery, onion, and parsley are rich in polyphenol antioxidants. Red wine, chocolate, green tea, olive oil, bee pollen, and many grains are alternative sources²⁰. The principal benefit of ingestion of antioxidants seems to stem from the consumption of a wide array of phytonutrients; correspondingly, the role of dietary supplements as a method of realizing these health benefits is the subject of considerable discussion.

Glutathione Glutathione protects cells from toxins such as free radicals. The human body produces

glutathione from the synthesis of three key amino acids-cysteine, glycine, and glutamic acid. Food sources with the highest amounts of naturally occurring glutathione include; asparagus, avocado, grapefruit, squash, potato, cantaloupe, peach, zucchini, spinach, broccoli, watermelon, and strawberries. Fish, meat, and foods which yield sulfur containing amino acids (e.g., eggs) are the preferred sources for maintaining and increasing bodily glutathione levels. Supplemental glutathione is only available in one active form, GSH²⁸.

Peroxidase An enzyme occurring especially in plants, milk, and leukocytes and consisting of a protein complex with heme groups that catalyzes the oxidation of various substances. Food sources of peroxidase include horseradish root, soybean, mango fruit, and turnip²⁹.

Cysteine Cysteine is an important free radical antioxidant in cellular systems. It blocks oxidants of the free radical type that may be engaged in certain forms of cellular pathology, including aging, carcinogenesis, diabetes mellitus, and the development of heart disease. Cysteine is incorporated in the cellular glutathione, which works along with vitamin E to protect cells against free radical oxidant damage. Cysteine is a nonessential amino acid, which means that it is manufactured from other amino acids in the liver; it does not have to be obtained directly through the diet¹⁸. It is synthesized in the liver from methionine. Animal protein is known to be higher in sulfur amino acids such as cysteine than vegetable proteins. Of the vegetable proteins, beans are generally higher in sulfur amino acids than grains. Therefore, a balanced vegetable protein of grains and beans would be useful in providing adequate cysteine intake in the diet. Excessive intake of cysteine can result in liver damage, kidney stone formation, or even some forms of schizophrenia²⁹.

Flavonoids Flavonoids promote antioxidant activity, cellular health and normal tissue growth and renewal throughout the body. They also work with vitamin C to reduce oxidative stress for the water based portion of the cell and may slow down some of the effects of aging. There are more than 4,000 unique flavonoids

and they are most effective when several types are consumed together. Food sources include: cranberries, kale, beets, berries, red and black grapes, oranges, lemons, grapefruits, and green tea³⁰.

BASIC CHARACTERISTICS OF DIETARY ANTIOXIDANTS

Dietary antioxidants are mainly secondary metabolites that plants synthesize to protect themselves against oxidative stress. According to their chemistry, they may be grouped into four classes: vitamin C (ascorbic acid); vitamin E (tocopherols); carotenoids (e.g. a and b-carotenes, lycopene, lutein) and polyphenolic antioxidants. The latter are a very diverse group among which phenolic acids and flavonoids such as anthocyanins and quercetin are the most important antioxidants. The four classes of dietary antioxidants differ dramatically in their mean antioxidant potency, absorbability and environmental availability. Flavonoids and phenolic acids are the antioxidants with the highest environmental availability, being most concentrated in all food categories. Flavonoids also have the highest antioxidant potency *in vitro*, followed by carotenoids, vitamin E and vitamin C³¹.

Mechanisms of Action Dietary antioxidants reduce oxidative stress by scavenging free radicals by three main mechanisms (variations are possible depending on the reactive species involved). Tocopherols and most polyphenols donate a hydrogen ion, carotenoids quench oxygen singlets and ascorbate transfers electrons (reviewed in Vertuani et al. 2004; Halliwell and Gutteridge 2006). The end result of these actions is that the free radical is neutralized. The efficiency with which an antioxidant destroys free radicals is called anti-oxidant potency and is measured in TEAC nM (Trolox equivalent antioxidant capacity; Trolox is a synthetic analogue of tocopherol). After reducing the free radicals, antioxidants are oxidized. At this stage, most antioxidants are fairly stable and relatively innocuous molecules that are subsequently catabolized and excreted. In contrast, carotenoid radicals are rather noxious pro-oxidants that may oxidize other biologically important molecules.

How- ever, if other antioxidants are present (usually vitamin C or E), they usually reduce the oxidized carotenoids, which are thereby recycled. Therefore, the function of carotenoids as antioxidants is debatable and always contingent on the presence of other antioxidants^{32,33,18}.

According to modern theory of free radical biology and medicine, 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. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers and neurodegenerative diseases. 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, have been reported to possess good antioxidant properties. Moreover, the essential oils and various extracts of aromatic plants have been of great interest for their potential antioxidative effects for the preservation of the foods from the toxic effects of the oxidants²⁶. As shown in Table No.1.

Food Choice

Antioxidant concentrations differ up to five orders of magnitudes both within and between food categories. Food selection is thus important for determining the intake of antioxidants. If animals select food items that have high contents of rare antioxidants, they may mitigate the severity of the constraints outlined above. Food selection is a fundamental process that has been documented in most organisms³⁴.

It has been mainly related to differences in macro nutritional contents in optimal foraging theory. Surprisingly, although fundamental for our understanding of the role of carotenoids in evolutionary ecology, no study has investigated the hypothesis that animals might select carotenoid-rich food. Recently, however, Schaefer et al. (2008) and Catoni et al. (in press) have shown that birds select food rich in anthocyanins and flavonoids, respectively,^{16,35} if given a choice between two otherwise identical foods. The proximate

mechanisms of distinguishing between foods with different antioxidant contents may vary. Antioxidants that function as pigments, such as anthocyanins and carotenoids, may be detected visually. In fruits, however, only anthocyanins are visually discernible because they usually mask the contents of carotenoids owing to their higher concentrations. Today, no study has investigated whether the concentration of carotenoids or polyphenolic antioxidants is visually discernible in seeds or arthropods. Like other micronutrients, nonpigmentary antioxidants may be selected by means of taste or physiological feedbacks. A plausible but still untested idea is that inters individual differences in the ability to discern antioxidants, or food quality in general, might result in large differences in antioxidant intake.

Differences in antioxidant intake may arise as a by-product from food selection directed at nutrients. Such interindividual differences in food selection have been demonstrated for a broad range of taxa in captivity, and may well be present in free-ranging animals. The ability to select food rich in antioxidants might be particularly important during periods of sickness. Several species of mammals and birds select food for self-medication to reduce noxious effects of target parasites and toxins. Thus far, the concept of 'nutraceutical' self-medication has not been applied to dietary antioxidants and it would be easily overlooked, unless it is specifically taken into consideration in experimental setups.

Food Interactions

Because most food items contain several different antioxidants, animals commonly absorb a cocktail of antioxidants which might interact positively or negatively. During absorption, competitive, negative interactions might occur between antioxidants with similar solubility. Carotenoids and vitamin E, for example, may compete for absorption so that supplementation with high doses of either antioxidant class may result in lower absorption and consequently in lower organismal levels of the other antioxidant class. Similar competitive interactions may also occur between flavonoids and vitamin C.

After ingestion, antioxidants found in the same tissues usually interact positively, independent of antioxidant solubility characteristics. Typical examples of such interactions are the regenerative actions of vitamins E and C on carotenoids or of polyphenols on vitamins E and C. Positive interactions between polyphenols and vitamin C are found in both directions, so that either class of antioxidants may regenerate the other, depending on the situation. Considering interactions among antioxidants is important because it explains, for example, why carotenoids are effective antioxidants only in the presence of vitamins E and C and why supplementation with these two antioxidants may result in greater development of carotenoid based sexual signals.

However, further studies are necessary to determine the net outcome of positive and negative interactions for wild consumers and the relevance of such interactions for the expression of various life history traits.

The Importance of Antioxidants in Fruits and Vegetables

Fruits and vegetables are packed with powerful antioxidants that can lower your risk of heart disease, cancer, diabetes-related damage and even slow down the body's natural aging process. What exactly are antioxidants and why do we need them? Antioxidants are nature's way of fighting off potentially dangerous molecules in the body. Such molecules come in the form of synthetic chemicals such as pesticides, plastics, and chlorine by products and are called free radicals. Free radicals are unstable molecules that essentially feed off of otherwise healthy molecules in order to survive⁷. Every day tens of thousands of free radicals are generated within the body, causing cell damage that can lead to chronic and degenerative diseases if left unchecked. The body sometimes creates its own free radicals in order to destroy viruses or bacteria. To balance out these unruly molecules, the body also creates antioxidants, which have the sole purpose of neutralizing free radicals. The body is only designed to create a certain amount of antioxidants on its own however, and as we are faced with an ever-growing

number of environmental toxins, the body is less capable of fighting off the unwanted harmful invaders. Fruits and vegetables provide the body with an added source of antioxidants that is needed to properly wage war against free radicals.

Without the necessary intake of healthy fruits and vegetables, free radicals can spread and eventually lead to stroke, heart attack, arthritis, vision problems, Parkinson's disease, Alzheimer's disease and various types of cancer. The benefits of getting your daily dose of fruits and vegetables are numerous! The antioxidant, Vitamin E, is wonderful for your heart. Vitamin E has the ability to essentially "mop up" the LDL ("bad") cholesterol in your arteries, allowing for the necessary elasticity and blood pressure levels to keep your heart pumping safely. Cholesterol, if left untreated, builds up as plaque on the inside walls of the arteries, impeding blood flow LDL and forcing the heart to work overtime to continue functioning. Eventually, plaque buildup can become so severe that it can create a blockage in the artery, leading to heart attack or stroke. (5) By getting enough Vitamin E in your diet you can give your body the necessary antioxidants to prevent your LDL cholesterol levels from getting out of control. Antioxidants can protect you against diabetes related damage. Free radicals thrive in the altered metabolic states of diabetics. But with the necessary antioxidants that fruits and vegetables can provide, free radicals can be neutralized, protecting your kidneys, blood vessels, eyes and heart from harmful damage.

Free radicals cause cancer cells to grow. Many studies have linked cancer, including those of the stomach, prostate, colon, breast, bladder, esophagus and pancreas, to free radicals. Eating your fruits and vegetables may not prevent cancer altogether, but can give your body the fighting chance that it needs. Antioxidants can neutralize cancer cells before they develop into a mass.

A recent study at Harvard University found that men who ate the most tomato based foods (rich in antioxidants) had a 35% lower risk of developing prostate cancer than those who ate the least amount of tomato based foods. Antioxidants slow the effects

of aging! Free radicals damage the cells within our body that are vital to a youthful appearance and good health. Eating fruits and vegetables can slow down the loss of muscle elasticity that leads to wrinkles, boost your immunity making you less susceptible to illness, and put the breaks on memory failure, as free radicals injure the brain cells necessary for retaining information. Antioxidants are available in supplement form but are the most powerful when found in whole foods⁷.

The best practice is to combine a "greens" supplement (containing vitamins, minerals and antioxidants found in produce) with the recommended 5 to 9 servings of fruits and vegetables each day. Leafy vegetables, like spinach and collard greens, and orange colored fruits and vegetables such as mangos, oranges, cantaloupe, sweet potatoes and carrots are all excellent sources of the antioxidant beta-carotene. Fruits and vegetables containing lycopene, such as tomatoes, watermelon, guava, papaya, apricots and pink grapefruit, are also packed with antioxidants. It is important that when you are purchasing fruits and vegetables for yourself and your family that you shop in the organic section. The produce available in most stores no longer contains the level of nutrients that it did 100 years ago. Because of pesticides and the diminished mineral levels in soils used today, eating non-organic produce will not provide you with the antioxidants (or vitamins and minerals) that your body needs. On average, organic produce contains nearly 30% more nutrients than non-organic and is grown without using harsh chemicals that can lead to further free radical exposure²⁰. Autumn is a great time to start a new healthy habit that your family will love, and eating 5 to 9 servings a day of scrumptious organic fruits and vegetables really can keep the doctor away.

Antioxidant activity of aromatic plants

Consumer interest in natural food additives, have reinforced the interest in natural antioxidants. Herbs and spices are harmless sources for obtaining natural antioxidants. 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 molecule³⁶. Oregano is particularly rich, 55000 ppm, in this compound and peppermint and lemon balm also contain high amounts, about 30000 and 37000 ppm, respectively¹⁷. *Curcuma zedoaria* (Berg.) Rosc. (Zingiberaceae) has long been used as a Chinese 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-picrylhydrazyl radical 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 properties³⁷. However, natural curcuminoids were also isolated from *Curcuma longa* and showed reducing antioxidant activities³⁸.

Antioxidant activity from aroma compounds and essential oils

Some volatile compounds from aroma and/or essential oils possess the potential as natural agents for food preservations. The antioxidant activities of aroma extracts obtained from spices, herbs, brewed coffee and beans have been investigated in various model systems. Some known natural aroma components, such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone, maltol, and 5-hydroxy methylfurfural have been reported to possess appreciable antioxidant activities. There is now evidence of beneficial influence of essential oils on lipid metabolism, ability to stimulate digestion and antioxidant properties. Eugenol (clove), linalool (coriander) and cuminaldehyde (cumin) were found to be effective antioxidants.

These compounds inhibited lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase. At the same time, there was no alteration in the fatty acid composition of membrane lipids and the levels of endogenous antioxidants, vitamin E, ascorbic acid and glutathione. Aroma extract from dried clove buds (*Syzygium aromaticum* (L.) Merr. et Perry,

Myrtaceae) and its two major aroma chemicals, eugenol and eugenyl acetate, showed antioxidative activity in two different assays: oxidation of hexanal and inhibition malonaldehyde formation from cod liver oil. Their activity is not as strong as α -tocopherol and BHT. *Eucalyptus polyanthemos* Schauer was examined for its inhibitory effect on the system malonaldehyde formation from horse blood plasma oxidized with Fenton's reagent. Thus, the chemicals from aroma of *Eucalyptus* inhibited malonaldehyde formation by 23%, at level of 400 $\mu\text{g/ml}$, whereas α -tocopherol and BHT inhibited malonaldehyde formation by 52 and 70% respectively, at the same level³⁹.

The antioxidant activity of eugenol has been reported several times on various systems. As eugenol and thymol have a phenolic group, the phenolic group plays an important role in their antioxidant activities. Benzyl alcohol that does not contain any phenolic group displayed reasonable antioxidant potential. The radical formed on benzyl carbon is stabilized by the adjacent benzene ring. This radical consequently abstracts a hydroxyl radical to form benzaldehyde. The essential oil of *Salvia tomentosa* Miller (Lamiaceae) was particularly found to possess low antioxidant activity. However, the free radical scavenging activity of aqueous methanol extract was superior to all other extracts of *S. tomentosa* (IC₅₀ = 18.7 $\mu\text{g/ml}$). The glycosidically bound volatiles amounted to 20 mg kg⁻¹ in dried leaves and flowers of oregano (*Origanum vulgare* ssp. *hirtum*). Thymoquinone was identified as the major component. Other important aglycones were benzyl alcohol, eugenol, 2-phenyl-ethanol, thymol, 3-hexen-1-ol and carvacrol. It was found that all of the aglycones have an antioxidant effect when tested by measuring peroxide values of lard stored at 60°C. The free radical scavenging activity of the essential oil of *Thymus sipyleus* subsp. *sipyleus* var. *rosalans* was superior to var. *sipyleus* oil (IC₅₀ = 220 and 2670 $\mu\text{g/ml}$, respectively). However, in the case of linoleic acid system, oxidation of linoleic acid was effectively inhibited (92%) by *T. sipyleus* subsp. *sipyleus* var. *rosalans*, while the var. *sipyleus* oil had no activity^{24,40,41}.

The values of IC₅₀ for *S. cryptantha* and *S. multicaulis* were 17.8 and 14.5 µg/ml, whereas BHT exhibited values of 7.8 µg/ml²¹. Among the constituents of essential oils, thymol, carvacrol, 4-allylphenol and eugenol exhibited potent antioxidant activities; those aroma chemicals inhibited hexanal oxidation by 95-99 % at 5 µg/ml over 30 days, which is comparable to that of α-tocopherol or BHT. The hexanal oxidation was almost completely inhibited by 4-allylphenol at 5 µg/ml over 30 days. Among other aromatic components tested for their antioxidant activity, benzyl alcohol showed slight antioxidant activity at the level of 50 µg/ml over 30 days.

At the lowest level of 1 µg/ml, eugenol displayed the highest antioxidant activity among the essential oil components tested. Linalool, methyl-salicylate, estragol, 1,8-cineole, 4-terpineol and benzylaldehyde showed slight antioxidant activity at a level of 50 µg/ml, they inhibited hexanal oxidation among 23% and 10%²⁰. Anethole (10 mM) and related compounds inhibited DPPH among 40 - 90%. The introduction of hydroxyl groups in the double bond of the lateral chain of anethole molecule, increased antioxidant activity⁴².

Isoeugenol, eugenol and anethole present a methoxybenzene moiety and a propenyl group. The conjugated double bonds of anethole and isoeugenol are known for stabilizing the reactivity of the phenyl and benzyl groups and contribute for the antioxidant properties of these molecules^{43,30}. *Lippia alba* showed antioxidant protecting effects within of range of 5-20 g/l³². γ-Terpinene, a monoterpene hydrocarbon present in essential oils, retards the peroxidation of linoleic acid. The peroxidation of γ-terpinene has been shown to yield p-cymene as the only organic product in a chain reaction in which the chain carrier is the hydroperoxyl radical. The peroxidation of linoleic acid is well known to be a chain reaction in which the chains are carried by linoleyl peroxy radicals, and the products are linoleyl hydroperoxides.

The retardation of linoleic acid peroxidation by γ-terpinene has been found to be due to rapid chain termination via a very fast cross-reaction between

hydroperoxyl radical and linoleyl peroxy radicals⁷. Oxidation of LDL (low-density lipoprotein) is believed to play a key role in atherogenesis. For this reason, a sufficient protection of LDL by antioxidants may provide protection from atherosclerosis. γ-Terpinene showed inhibition of LDL oxidation⁸. The monoterpene terpinolene from the oil of *Pinus mugo L.* also prevents oxidation of LDL⁹. Inhibition of LDL oxidation by eugenol compounds is due to the suppression of free radical cascade of lipid peroxidation LDL by reducing copper ion^{10,11}. The most important mechanism is their 5 reaction with lipid free radicals, forming inactive products. The mechanisms of antioxidant activity are shown in Table No.1. Antioxidants can be classified into two groups based on the way they inhibit or retard oxidation. The first group is primary (chain-breaking) antioxidants, which react directly with lipid radicals and convert them into stable products. The second group is secondary (preventive) antioxidants, which can lower the rate of oxidation by different mechanisms as shown in the Table No.2^{44,20,45}.

Essential oils as antioxidants in foods

Aromatic herbs have a long history of culinary use. Not only are many of the flavors and aromas distinctively pleasant, but they can be used to conceal off-flavors and odors. The high susceptibility to oxidation of the fat and oil polyunsaturated fatty acids used in human foods requires the application of antioxidants^{16,26,46}. Thus, in the last years essential oil research has concentrated on two primary areas: the antimicrobial^{21,47} and the antioxidant activities. However, Smith et al⁴⁸ have developed a guide to evaluate the safety of essential oils, for their intended use as antioxidants and/or flavor ingredients. Enzymatic browning in fruit and vegetable tissues can cause undesirable quality changes during handling, processing and storage. This reaction results mostly from polyphenol oxidase and peroxidase. Both enzymes catalyse more than one reaction and act on a number of substrates, not only causing browning of foods but also leading to discoloration, off-flavors and nutritional damage²⁸. Essential oils of

Eucalyptus globulus, Melaleuca alternifolia, Melissa officinalis, Rosmarinus officinalis, Syzygium aromaticum and Citrus limonum reduced peroxidase activity of organic leafy vegetables extracts. Thus, essential oil of clove was more effective than the other oils³³. Botsoglou et al.^{24,31,42} examined the effects of dietary oregano essential oil on the susceptibility of raw and cooked turkey breast, thigh meat and chicken breast to lipid oxidation during refrigerated storage for 9 days. Oregano oil at 200 mg kg⁻¹ was significantly more effective in delaying lipid oxidation compared to the level of 100 mg kg⁻¹, equivalent to α -tocopheryl acetate at 200 mg kg⁻¹, but inferior to oregano oil plus α -tocopheryl acetate at 100 mg kg⁻¹ each, which in turn was superior to all dietary treatments, indicating a synergistic effect. The effect of dietary dried oregano (*Origanum vulgare* subsp. *hirtum*) leaves supplementation on performance and carcass characteristics was determined by Bampidis et al.^{3,26,49,15}.

The oregano diet for growing of lamb did not affect their performance and carcass characteristics. Application of antioxidants is one of the technically simplest ways of reducing fat oxidation. Thus, Tomaino et al.⁴³ studied the effect of heating on antioxidant effectiveness of essential oils. When maintained at room temperature, basil, cinnamon, clove, nutmeg, oregano and thyme essential oils tested appeared endowed with good radical scavenger properties in the DPPH assay, with order effectiveness

clove >> cinnamon>nutmeg>basil> oregano >> thyme.

When heated up to 180°C, nutmeg oil, but not the rest of essential oils, showed a significantly higher free radical - scavenger activity. Ageing is the progressive accumulation of changes with time, associated with or responsible for the ever-increasing likelihood of diseases and death that accompanies advancing age. There is growing evidence that this process may be the consequence of free radical reactions. Although, the removal of damaging oxygen species is catalysed by antioxidant enzymes, from foods numerous non-enzymatic defence are also employed to provide protection, these include

vitamin E, vitamin C and essential oils^{19,20,29}.

Natural antioxidants

Medicinal plants are an important source of antioxidants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark. In nature there are a wide variety of naturally occurring antioxidants which are different in their composition, physical and chemical properties, mechanism and site of action. There is a long list of antioxidant plants of which, some have been discussed in Table No.3^{20,50,51}.

Due to toxicological concerns of synthetic antioxidants^{24,31}, there have been increasing interests in identifying phenolic compounds in plants to minimize or retard lipid oxidation in lipid-based food products. Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs³³.

Hundreds of natural phenolic compounds have been reported to possess high antioxidant properties. Their use in foods, however, is limited by certain requirements not the least of which is adequate proof of safety. Only a few of them can be commercially applied in foods. The main lipid-soluble antioxidants currently used in food are monohydric or polyhydric phenols with various ring substitutions. For maximum efficiency, primary antioxidants are often used in combination with other phenolic antioxidants or with various metal sequestering agents, e.g. tocopherols with citric acid and isopropyl citrate⁴². Most important commercially available natural antioxidants are tocopherols (vitamin E), ascorbic acid (vitamin C) and rosemary extract^{3,43}. Compounds such as β -carotene, ascorbic acids have demonstrated to have antioxidant and synergistic activity in despite of their non-phenolic structure^{30,32,50}.

Methods for testing antioxidative activity

Total antioxidant activities of the plant extracts cannot 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 vegetables^{14,18,52,53}. The BCB (β -carotene bleaching method) employs an emulsified lipid, which introduces a number of variables influencing antioxidant activity of samples. The BCB method can be helpful especially for investigations of lipophilic antioxidants and it is appropriate for the investigation of the antioxidant activity of essential oils. On the other hand if polar compounds as ascorbic acid, rosmarinic acid, etc, are tested only by the BCB method they would be considered as weak antioxidants²⁸. The DPPH method is faster than BCB method and it can be helpful in investigation of novel antioxidants for a rapid estimation and preliminary information of radical scavenging abilities. The method is sensitive and requires small sample amounts²⁶. TBA method is also sensitive and achieves reproducible results. This method is preferable in order to obtain useful data in an environment similar to the real-life situation. Both methods, DPPH and TBA, similarly allow testing of both lipophilic and hydrophilic compounds^{28,34}. However, the antioxidant power depends on the chosen method, on the concentration and on the nature and physicochemical properties of studied antioxidants³⁴.

FUTURE RESEARCH DIRECTIONS

To develop a more complete framework for the relevance of dietary antioxidants for the expression of life history traits and trade-offs, we suggest that future studies should consider the full range of available antioxidants, their possible interactions, their environmental availability, and the potential for interindividual differences in anti-oxidant intake and uptake. This is because more dietary antioxidants than traditionally perceived may affect crucial life history traits, such as fertility, growth, immunity, senescence and the expression of sexually selected traits. Surprisingly, the most common and potent antioxidants, flavonoids and phenolic acids, are those that have received the least attention. Given their ubiquity in food items, we urge researchers to include these potentially important antioxidants in

evolutionary ecology as well as ecological immunology studies^{44,35}.

Since antioxidants differ in their chemistry and tissue distribution, they may not be equally relevant for each trait, and we have highlighted one fundamental distinction between antioxidant types: those that act directly upon traits and those that affect traits indirectly. To date, the concept that the relevance of single antioxidants for various life history traits is likely to differ has hardly been accounted for in evolutionary ecology^{29,46}.

To address this concept we suggest that the absorption and tissue distribution of several antioxidant classes should be studied in different animal taxa. In addition, to establish the relevance of single antioxidants for given life history traits, the effect of supplementation should be compared for different kinds of antioxidants¹⁸.

Experiments should also ideally use different mixtures of antioxidants, mimicking a more natural situation, to account for interactions among antioxidants. Owing to the large differences of antioxidant contents between food items, animals might select food to optimize their intake of specific antioxidants or antioxidants in general. To assess the relevance of food selection, the environmental availability of antioxidants can be compared with the actual intake of antioxidant in free-ranging animals. Selection for increasing antioxidant intake can be unequivocally demonstrated in captivity by measuring the consumption of various otherwise identical food types differing in antioxidant content (similar to Schaefer et al. 2008; Catoni et al., in press)⁴⁸.

By modifying visual and taste cues between food types, we can establish the proximate mechanisms that animals use to discern antioxidants in food. Environmental variation in the availability of antioxidants and individual variation in food selection may result in interindividual differences in antioxidant intake that may explain, at least partially, intra and interspecific differences in resource allocation to self-maintenance or reproduction³⁵.

Table No.1: Synthetic antioxidants used in pharmaceutical sciences

S. No	Synthetic antioxidants	References
1	Butylated hydroxyanisole	Reische DW <i>et al</i> , 1998
2	Butylated hydroxytoluene	Reische DW <i>et al</i> , 1998
3	Tert-butylhydroquinone	Pratt DE <i>et al</i> , 1990
4	Propyl gallate	Pratt DE <i>et al</i> , 1990
5	Erythorbic acid	Pratt DE <i>et al</i> , 1990
6	Ascorbyl palmitate	Pratt DE <i>et al</i> , 1990
7	Tocopherols	Micova K <i>et al</i> 2001
8	Gum guaiac	Micova K <i>et al</i> 2001
9	Ethoxyquin	Micova K <i>et al</i> 2001
10	Phosphates	Micova K <i>et al</i> 2001
11	Ethylene diamine tetra acetic acid	Micova K <i>et al</i> 2001
12	Tartaric acid	Micova K <i>et al</i> 2001
13	Citric acid	Micova K <i>et al</i> 2001
14	Lecithin	Micova K <i>et al</i> 2001
15	Ascorbic acid	Micova K <i>et al</i> 2001
16	Sulfites (as sulphur dioxide)	Micova K <i>et al</i> 2001
17	Ascorbyl stearate	Micova K <i>et al</i> 2001

Table No.2: Antioxidant classification and Mechanism of antioxidant Activity

S. No	Antioxidant class	Mechanism of antioxidant activity	Examples of Antioxidants
1	Proper antioxidants	Inactive lipid free radicals	Phenolic compounds
2	Hydroperoxides Stabilizers	Preventing decomposition of hydroperoxides into free radicals	Phenolic compounds
3.	Synergists	Promoting activity of proper antioxidants	Citric acid, ascorbic Acid
4	Metal chelators	Binding heavy metals into inactive compounds	Phosphoric acid, maillard reaction compounds, citric acid
5	Singlet oxygen Quenchers	Transforming singlet oxygen into triplet oxygen	Carotenes
6	Substances Reducing hydroperoxides	Reducing hydroperoxides in a non-radical way	Proteins, amino acids

Table No.3: Botanical family name, common name and part used

S. No	Botanical/family Name	Common name	Part used	References
1	Curcuma longa Linn. (Zingiberaceae)	Turmeric	Leaf	Sawasha SG <i>et al</i> 2003
2	Cuscuta reflexa Roxb. (Convolvulaceae)	Akashabela	Stem	Yadav SB <i>et al</i> 2001
3	Daucus carota Linn. (Apiaceae)	Carrot	Root	Bishayee A <i>et al</i> 1995
4	Embllica officinalis Gaertn. (Euphorbiaceae)	Amla	Fruit	Khopde SM <i>et al</i> 2001
5	Foenicullum vulgare Mill. (Apiaceae)	Saunf, Fennel	Fruit oil	Ruberto G <i>et al</i> 2000
6	Glycyrrhiza glabra Linn. (Fabaceae)	Mulethi, Liquorice	Root	Morteza Semnani K <i>et al</i> 2003
7	Mangifera indica Linn. (Anacardiaceae)	Aam	Root,	Martinez G <i>et al</i> 2000
		Mango	leaf, fruit	
8	Momordica charantia Linn. (Cucurbitaceae)	Karela, Bitter melon	Root, leaf, fruit,	Vohra SB <i>et al</i> 1973

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 defence system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration. Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system. 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. It involves a variety of components, both endogenous and exogenous in

origin, that function interactively and synergistically to neutralize free radicals. Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems.

ACKNOWLEDGMENT

The author is gratefully acknowledged the Management of Department of Pharmacy, Manav Bharti University, Solan, Himachal Pradesh, India for providing the facility.

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