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16 Antioxidants: Their Health Benefits and Plant Sources

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16.1 Introduction

An important field of research today is the control of 'free radicals' generation or redox' status with the properties of food and food components. Reactive oxygen species (ROS) may interact with cellular macromolecules and modify several cellular proteins, lipids and DNA, which results in altered target cell functions. Oxidative stress occurs in a cell or tissue when the ROS generation level exceeds the antioxidant capability of that cell (Kumar *et al*., 2011). ROS can be produced both endogenously and exogenously. Endogenous oxidative stress can be the result of normal cellular metabolism and oxidative phosphorylation. Exogenous sources of ROS can also impact on the overall oxidative status of a cell. Drugs, hormones and other xenobiotic chemicals can produce ROS by either direct or indirect mechanisms (Kakkar and Singh, 2007). Several human chronic disease states, including cancer, have been associated with oxidative stress produced through either an increased free radical generation and/or a decreased antioxidant level in the target cells and tissues (Rice-Evans and Burdon, 1993). Natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health. It has been reported that a diet rich in antioxidant phytochemicals, such as polyphenolics, carotenoids, terpenoids and flavonoids, protects against cellular damage due to ability to quench oxygen-derived free radicals (Dhakarey *et al*., 2005; Singh, P., 2008; Singh, B.N., 2009a). If antioxidant defence systems are not sufficiently present in critical situations like oxidative stress, contamination, UV exposure etc., the production of free radicals increases significantly (Singh, U. *et al*., 2008). Non-enzymatic (vitamin E, vitamin C, glutathione (GSH), etc.) and enzymatic (superoxide dismutase, GSH peroxides, glutathione-S-transferase and catalase) antioxidant levels in the cell can be decreased through modification in gene expression, decreased antioxidant uptake in the diet, or can be overloaded in ROS production, which creates a net increase in the amount of oxygen free radicals present in the cell. It has been reported that with the administration of antioxidants, cells are protected against carcinogen-induced damage (Kumar *et al*., 2011). Mechanisms of protection could be effective against a wide

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range of dietary carcinogens possibly influencing several cancer sites. Antioxidant enzymes are detoxification/biotransformation enzymes that are involved in the detoxification of toxic substances such as xenobiotics, carcinogens, free radicals and peroxides by conjugating these substances with GSH (Tripathi *et al*., 2010).

Traditional medicine all over the world is nowadays being revalued by an extensive amount of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals (FR) and ROS can be involved in a high number of diseases (Richards and Sharma, 1991). As plants produce a lot of antioxidants to control the oxidative stress caused by sunlight and oxygen, they can represent a source of new compounds with antioxidant activity. One of the clinical specialities of Ayurveda is Rasayana. Rasayana is not only a drug therapy but is a specialized procedure practised in the form of rejuvenating recipes and dietary regimen promoting good habit. The purpose of Rasayana is two-fold: prevention of disease and counteraction of ageing processes which result from optimization of homeostasis. The meaning of the word Rasayana (rasa: essence, water; ayana: going) essentially refers to nutrition and its acquisition, movement, circulation and perfusion in the body tissues (Singh, 1992). With regard to Rasayana drug therapy, Sharma *et al*. (1992) reported the strong antioxidant activity of any Rasayana: these compounds were found to be 1000 times more potent than ascorbic acid, α-tocopherol and probucol.

16.2 Antioxidants

In living cell, two antioxidant defence system are present against free radical damage. The first line of defence includes antioxidant enzymes (such as superoxide dismutase, catalase, GSH peroxidase), whereas the second defence system includes low molecular non-enzymatic antioxidants (thioredoxin, GSH, vitamins A, C, E, lycopene, lutein, quercetin etc.). These antioxidants inhibit the formation of FRs by breaking the chain reaction or can reduce the concentration of FR by donating hydrogen and an electron. They also act as peroxide decomposer (vitamin E), enzyme inhibitor, singlet oxygen quencher (vitamin E), synergist and metal-chelating agents (tranferritin). To provide maximum intracellular protection, antioxidants are strategically compartmentalized throughout the cell. So that FR is produced intracellular and extracellular during metabolism, both enzymatic and non-enzymatic antioxidants are able to detoxify FRs.

Certain antioxidant enzymes (superoxide dismutase, catalase and GSH) are produced within the body. Other antioxidant agents are found in foods, such as green leafy vegetables, and it is believed that diets rich in antioxidant (such as β-carotene and vitamins A, C and E) are beneficial to human health (Halliwell and Gutteridge, 1989). Therefore, antioxidant naturally present in body or supplied in the form of diet (phytonutrients) plays an important role to control various diseases resulting from oxidative stress. Fresh fruits and vegetables are of more importance than cooked, because of the high concentration and maximum absorption of antioxidants. In recent years, researchers have been researching the relationship between antioxidants and prevention of some diseases, such as cardiovascular disease and cancer (Kubola and Siriamornpun, 2008).

As soon as these FRs are generated in the body, they are trapped by antioxidant present in extracellular and intracellular defence system. If the generation of free radicals is much more than the concentration of antioxidants then oxidative stress arises. As a result of oxidative stress, arthritis in joints, emphysema and bronchitis in lungs, atherosclerosis or heart disease in the blood vessels, peptic ulcer in the stomach, ageing and wrinkling in the skin are caused. In the nucleus, it also alters the sequence of nucleotide base pair, strand break etc. in the DNA resulting in transformed and mutated DNA. Mutated DNA will produce diseases like cancer, leukaemia and lymphoma (Prakash *et al*., 2012).

16.2.1 Antioxidant enzyme

Three groups of enzymes play significant roles in protecting cells from oxidative stress.

Superoxide dismutase

Superoxide dismutase (SOD) has been recognized to play an important role in the body defence mechanism against the deleterious effect of superoxide FR in the biological system. It acts on two superoxide molecules and converts it into hydrogen peroxide and oxygen. The beneficial aspect of this reaction is that it produces less toxic hydrogen peroxide. The organisms that resist oxygen toxicity must have the SOD enzyme. On the basis of metal cofactor, the organism has three distinct types of SOD. In eukaryotes, cytosol has the copper- and zinc-containing form of SOD while mitochondria and bacterial cells have the manganese-containing form of SOD (Table 16.1). Iron-containing SOD is found in bacteria, cynobacteria and some plants. Newly discovered forms of SOD, also found in bacteria, contain nickel as a cofactor. Interestingly, SODs are inducible enzymes, i.e. with the increase in the concentration of oxygen in the environment of the cell, the concentration of SOD enzyme also increases. The main source of naturally occurring SOD enzyme is green vegetables such as in barley, broccoli, Brussels sprouts, cabbage, wheat and most green plants (Gassen and Youdim, 1999).

Catalase

The catalase activity of mammalian tissue varies greatly. It is highest in liver and kidney and low in connective tissue. In the cell, it is mainly particle bound (in mitochondria and peroxisomes) whereas in erythrocytes it exist in soluble state. Catalase activity received much attention for its role in oxidative metabolism as well as protective function by acting as H_2O_2 scavenger. Catalase located in the organelles acts as regulator of H_2O_2 levels and, on the other hand, in erythrocytes, catalase and GSH peroxidase jointly exert a protective function for haemoglobin and other SH-protein. It degrades hydrogen peroxide to water and oxygen, and hence finishes the detoxification reaction started by SOD (Gassen and Youdim, 1999).

Glutathione peroxidase

GSH peroxidase is a member of family of GPx enzymes, whose function is to detoxify

Table16.1. Important enzymatic and non-enzymatic physiological antioxidants.

Antioxidants	Location	Properties
Enzymatic		
Superoxide dismutase	Mitochondria, cytosol	Dismutase superoxide radicals
Glutathione peroxidase	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxides
Catalase	Mitochondria and cytosol	Removes hydrogen peroxide
Non-enzymatic		
Vitamin E	Cell membrane	Chain-breaking antioxidant in cell membrane
Vitamin C	Aqueous phase of cell Sap	Acts as free radical scavenger and recycles vitamin E
α-Lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
Carotenoids	Membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
Bilirubin	Blood	Extracellular antioxidant
Ubiquinones	Mitochondria	Reduced forms are efficient antioxidants
Metals ions sequestration: transferrin, ferritin, lactoferrin		Chelating metals ions, responsible for Fenton reactions
Nitric oxide		Free radical scavenger, inhibitor of LP

peroxide in the cell. Peroxides decompose to form highly reactive free radicals, which can damage the macromolecules like protein, DNA and lipid. GPx enzyme plays an important role in the protection of cell from this damage, particularly lipid peroxidation. GSH peroxidase contains selenium as a cofactor. The synthesis of GSH peroxidase in humans appears to be very important in scavenging H_2O_2 (Cheng *et al*., 2003).

16.2.2 Antioxidant phytochemicals

There are more than a thousand phytochemicals that have been identified with antioxidant properties. Plants produce these chemicals to protect themselves from microorganism and oxidative stress, but now several evidences suggest that these phytochemicals also protect humans against various diseases caused by FRs. Some of the well-known phytochemicals are lycopene (tomatoes), isoflavones (in soy), flavanoids (in fruits, vegetables), allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots) and polyphenols (tea, grapes). Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Shukla *et al*., 2009). The antioxidant activity of phytochemicals is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides.

Flavonoids

Flavonoids are the most common secondary metabolites in higher plants, and can directly scavenge the superoxide ion, hydroxyl radical and H_2O_2. These include more than 4000 phenolic compounds that occur naturally in plants.

Flavonols

The main flavonol is quercetin, followed by myricetin, kaempferol, laricitrin, isorhamnetin and syringetin. The main sources of flavonols are onion, kale, broccoli, lettuce, tomato, apple, grape, berries, tea and red wine. High contents of flavonols are present in greener leaves (Manach *et al*., 2004). Flavonols have multiple biological health benefits. It reduces risk of cardiovascular diseases, cancer, improve endothelial function and reduce platelet activity. This property is mainly attributed due to their antioxidant properties (Patel, 2008). Furthermore, flavonols also help to prevent oxidative damage to cells, lipids and DNA. The antioxidant properties of flavonols are drawn from the presence of aromatic rings of the flavonoid molecule, which allows the donation and acceptance of electrons from FR species.

Anthocyanins

Anthocyanins are violet, blue and purple pigments, which are mainly present in fruits, berries and flowers. The major dietary anthocyanins include cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin (Manach *et al*., 2004). Anthocyanins and their derivatives have the capacity to scavenge FRs through a number of mechanisms, thereby reducing the oxidative stress. Anthocyanins present in red cabbage reduce the oxidative stress caused by the toxin paraquat (Igarashi *et al*., 2000). Tsuda (2000) reported that cyanidin, which is found in most fruit sources, has potential antioxidant activity under *in vivo* conditions. In another animal study, Tsuda (1998) reported that cyanidins protect cell membrane lipids from oxidation by a variety of harmful substances.

Tannins

Tannins are commonly present in fruits (grapes, persimmon, blueberry, etc.), tea, chocolate, legume forages and legume trees (*Acacia* sp., *Sesbania* spp. etc.) and grasses (sorghum, maize, etc.). Tannins include proanthocyanidins, gallotannins and ellagitannins. At high temperatures in alcohol solutions or in a strong mineral acid, proanthocyanidins release anthocyanidins, which have antioxidant properties. Gallotannins and ellagitannins are both hydrolysable tannins. Gallotannins constitute galloyl esters of glucose or quinic acid whereas ellagitannins are derivatives of hexahydroxydiphenic acid (HHDP). Another form of tannin is phloroglucinols, which are subunits of phlorotannins and present in marine brown algae only. Tannins give an astringent or bitter taste to foods and beverages (e.g. some red wines, teas and unripe fruits). The basic function of tannin is not as a primary antioxidant (i.e. they donate hydrogen atom or electrons) but they act as secondary antioxidants (i.e. interfere with the chain reaction or by chelating the metal ions such as Fe(II) thereby retarding oxidation or Fenton reaction). Zhang *et al.* (2004) showed that the inhibition of lipid peroxidation by tannin constituents can act via the inhibition of cyclooxygenase.

Phenolic acids

Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom. Predominant phenolic acids include hydroxybenzoic acids (e.g. gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid) and hydroxycinnamic acids (e.g. ferulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid and sinapic acid) (Wrigstedt *et al.*, 2010). Ferulic, caffeic and *p*-coumaric acid are present in many medicinal herbs and dietary spices, fruits, vegetables and grains. Wheat bran is a good source of ferulic acids. Hydroxycinnamic acids (non-flavonoid phenolics) are characterized by the C6–C3 structure. Plants use these compounds in both structural and chemical defence strategies against microbial flora as well as oxidative stress (Cartea *et al.*, 2011). Naturally occurring hydroxycinnamic acids possess high level of antioxidants in comparison to hydroxybenzoic acid due to increased possibilities for delocalization of the phenoxy radical (Beer *et al.*, 2002). Phenolic compounds have the potential to function as antioxidants by scavenging the superoxide anion, hydroxyl radical, peroxy radical or quenching singlet oxygen and inhibiting lipid peroxidation in biological systems (Izunya *et al.*, 2010). At low temperatures during the maturity of leaves, the leaves have been shown to increase the phenols and flavonoids content (Singh, P. *et al.*, 2008; Singh, B.N., 2009c).

16.3 Antioxidant Nutrients

16.3.1 Vitamin E

Vitamin E is the main lipid-soluble antioxidant and plays a vital role in protecting membranes from lipid peroxidation. Primary function of vitamin E is to trap peroxy radical formation during lipid peroxidation in cellular membranes. It is mainly present in nuts, seeds, vegetables, fish oils, whole grains (especially wheat germ), fortified cereals and apricots (Glenville, 2006). Current recommended daily allowance (RDA) is 15 IU day^{-1} for men and 12 IU day^{-1} for women.

16.3.2 Vitamin C or ascorbic acid

Vitamin C or ascorbic acid is a water-soluble antioxidant that can reduce a variety of free radicals. It acts as a synergist for tocopherol by converting the oxidized tocopherols back to their reduced status. Ascorbic acid can also act as a pro-oxidant under certain circumstances and helps regeneration of membrane-bound oxidized vitamin E. Vitamin C reacts with the α-tocopheroxyl radical and is oxidized to dehydroascorbic acid. Humans lack L-gulono-γ-lactone oxidase, which is a key enzyme in ascorbic acid synthesis, hence it cannot be synthesized in the body and must be acquired from dietary sources. Ascorbic acid is mainly present in citrus fruits and juices, kiwi, cabbage, green peppers, spinach, broccoli, kale, cantaloupe and strawberries. The RDA for vitamin C is 60 mg day^{-1}. If taken in high dosages it may be excreted out due to its water-soluble nature but may cause adverse side effects in some individuals. The efficiency of ascorbic acid as scavenger of superoxide in mammalian tissue is not less than the SOD enzyme. The ascorbic acid level in extracellular fluids is higher than those of glutathione. So, ascorbate probably plays a predominant role in extracellular antioxidant protection. Vitamin C

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reacts with the superoxide radical to form dehydroascorbic acid and it returns to its original state (vitamin C) with the help of gluthathione (Prakash *et al.*, 2012).

16.3.3 Glutathione

Glutathione, a tripeptide (glutamyl-cysteinyl-glycine) antioxidant, is the most important intracellular defence against damage by ROS. It is widely distributed among living cells and apparently involved in many biological functions. Glutathione present in the oxidized (GSSH) form is converted to the reduced GSH by enzyme glutathione reductase. It has been reported that reduced GSH is mainly present in tissue. The free sulfhydryl (SH) is a very reactive group in cysteine, providing target for radical attack. Reduced glutathione is oxidized when it reacts with free radicals and it gets back to the reduced state by redox cycle involving GSH reductase and the electron acceptor NADPH (Gassen and Youdim, 1999).

16.3.4 Selenium

Selenium, an essential element for antioxidation reactions, is required only in small amounts in humans and animals (Thomson, 2004). Selenoproteins (proteins containing selenium) are important antioxidant enzymes. There are nearly 30 known selenoproteins, mainly containing selenocysteine. The active site of GSH peroxidase (the most abundant selenoprotein in mammals) and thioredoxin reductase enzyme has selenocysteine. Thioredoxin reductase not only maintains cell proteins in a reduced state but also provides deoxyribonucleases required for DNA synthesis (Holmgren, 1989). At low concentrations it acts as an antioxidant, inhibiting lipid peroxidation, whereas at higher concentrations it behaves as pro-oxidant, enhancing the accumulation of lipid peroxidation products. The antioxidant properties of selenoproteins help to regulate thyroid function, play important role in the immune system and prevent cellular damage from free radicals (Corvilain *et al.*, 1993). Selenium deficiency may cause a form of heart disease, hypothyroidism and a weakened immune system (Zimmerman and Kohrle, 2002).

16.3.5 β-Carotene

β-carotene (precursor to vitamin A, retinol) is present in liver, egg yolk, butter, milk, spinach, squash, carrots, broccoli, tomato, yams, cantaloupe, peaches and grains. β-carotene is converted to Vitamin A by the body. The carotenoids (fat-soluble antioxidant) are one of the most common pigments found in nature (Daun, 1988). β-carotene (one of the best known carotenoids) is necessary for the synthesis of vitamin A. Some other related pigments include α-carotene, lutein, lycopene and astaxanthin. There is evidence that diet containing fruit and vegetables is associated with lower incidences of cancer (Giovannucci, 1999). β-carotene has the capacity to quench reactive oxygen (stop oxidative mechanisms), making them chemoprotective against cancer. There is strong evidence that β-carotene increases the detoxification of carcinogens present in the liver, thereby reducing the development of cancer (Solomons, 2001).

16.3.6 Metal-binding protein

Transition metals are tightly bound to various proteins that prevent them from reacting with peroxides to form free radicals. These include the following.

Ceruloplasmin

Ceruloplasmin is an effective antioxidant with potent peroxidase property. It decomposes hydrogen peroxide in the presence of reduced glutathione. Ceruloplasmin is expressed mainly in the liver but has been found to be expressed in the lungs (Fleming *et al.*, 1991) and mammary glands. The role of ceruloplasmin as antioxidant is against organic and inorganic oxygen radicals from iron and ascorbate. It contains 90–95% of the circulating copper in normal mammals. The concentration of ceruloplasmin increases by a factor of 2 to 3 during pregnancy and hormonal conditions. It also inhibits lipid peroxidation induced by ferrous ion by way of decomposing lipid peroxides (Verma *et al*., 2005).

Lactoferrin

Lactoferrin belongs to the iron transporter or transferrin family of glycoproteins and is mainly present in whey and exocrine secretions from mammals and is released from neutrophil granules during inflammation. Human breast milk may contain as much as 15% lactoferrin while cow's milk may have only 0.5% to 1.0%. It has two important roles: (i) it shows antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities; and (ii) lactoferrin plays an important role in the uptake and absorption of iron through the intestinal mucosa. Its ability to bind iron probably contributes to both its antioxidant properties and its antibacterial action (Gupta *et al*., 2012).

Metallothionein

Metallothionein (MT) consists of four low-molecular-weight (6000–7000), metal-binding proteins with high cysteine content. Metallothioneins (MTs) are sulfhydryl-rich proteins, which specifically neutralize hydroxyl radicals (Viarengo *et al*., 2000). Antioxidant properties of MTs are mainly due to sulfhydryl nucleophilicity. *In vitro* studies have revealed that it reacts directly with ROS including superoxide and hydroxyl radicals and hydrogen peroxide. Binding of transition metals (Fe, Cu) to the protein reduce the Fenton reactivity, resulting in reduced oxidative stress.

Transferrin

Transferrin (iron-binding blood plasma glycoprotein) has a molecular weight of approximately 80 kDa and bind iron very tightly but reversibly and hence control the level of free iron in biological fluids (Crichton and Charloteaux-Wauters, 1987). It has two specific high-affinity Fe(III) binding sites. Iron present in the body is always found in protein-bound form and never in a free state. If iron is being transported or stored it must be chelated in very specific ways by transferrin or ferritin. Transferrin is mainly present in serum, but it is also found in other body fluids at lower concentrations (Chauhan *et al*., 2004). The antioxidant activity of transferrin is due to its reducing properties. It reduces the concentration of free ferrous ion that catalyses the conversion of hydrogen peroxide to highly toxic hydroxyl radical by Fenton reaction. Transferrin is a universal iron carrier and is able to deliver iron to cells without formation of free radicals.

Ferritin

Ferritin (a globular protein complex consisting of 24 protein subunits) is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. Ferritin is synthesized by almost all living organisms, including algae, bacteria, higher plants and animals. Intracellular iron is stored in the ferritin in both prokaryotes and eukaryotes and released into cells when needed; hence it acts as buffer against iron deficiency. Ferritin that is not combined with iron is called apoferritin. Ferritin converts ferrous (Fe^{2+}) to ferric (Fe^{3+}) form by ferroxidase activity, thereby reducing the chance of the deleterious reaction that occurs between ferrous iron and hydrogen peroxide known as the Fenton reaction, which produces the highly damaging hydroxyl radical (Sarma *et al*., 2010).

16.4 Some Commonly Measured Analytes with Antioxidant and Pro-oxidant Activities

16.4.1 Gamma-glutamyltransferase

Gamma-glutamyl transpeptidase (also known as γ-glutamyltransferase, GGT, GGTP, gamma-GT) (EC 2.3.2.2) is an enzyme that transfers γ-glutamyl functional groups. It is the first enzyme of the γ-glutamyl cycle that regulates the antioxidant glutathione; hence it is a critical enzyme in glutathione homeostasis. GGT is present in the cell membrane of many tissues, including the kidney, bile duct, pancreas, gallbladder, spleen, heart, brain and seminal vesicle (Sarma *et al*., 2010).

16.4.2 Uric acid

Uric acid, the end product of purine metabolism, works as an antioxidant. It is the most abundant aqueous antioxidant in humans and contributes as much as two-thirds of all free-radical scavenging capacity in plasma. It is particularly effective in quenching hydroxyl, superoxide and singlet oxygen and peroxynitrite radicals and may play a protective physiological role by preventing lipid peroxidation. The major antioxidant role of uric acid is its ability to bind and inactivate peroxynitrite. At physiological concentrations, urate protects erythrocyte ghosts against lipid peroxidation leading to lysis of erythrocytes. Urate is found to be about as effective an antioxidant as ascorbate in these experiments. Urate is much more easily oxidized than deoxynucleosides by singlet oxygen and is destroyed by hydroxyl radicals at a comparable rate (Nieto *et al*., 2000).

16.4.3 Bilirubin

Bilirubin, the end product of haem metabolism, has the ability to function as an antioxidant in the brain, scavenging free radicals and reducing oxidative damage. It is reported that bilirubin protects oxidation of lipids such as linoleic acid and vitamin A. Stocker *et al*. (1987) demonstrated that bilirubin has more of an antioxidant effect than vitamin E towards lipid peroxidation. It has also been experimentally proved that higher concentration of serum bilirubin increases its antioxidant capacity.

16.4.4 High-density lipoprotein

High-density lipoprotein (HDL) has long been known as the 'good cholesterol', protecting against heart disease and atherosclerosis. It has been experimentally found that HDL has powerful antioxidant properties, similar to vitamin C and vitamin E. An enzyme related to synthesis of HDL cholesterol, lecithin-cholesterol acyltransferase, is a powerful antioxidant enzyme that blocks the oxidization of low-density lipoprotein (LDL) cholesterol. Cholesterol is beneficial if it is not oxidized. Barter *et al*. (2007) suggested that a low level of HDL increases the risk of diseases even in people with very low LDL levels. Jafri *et al*. (2010) suggested that there is an inverse relationship between high HDL and cancer occurrence.

16.4.5 Nitric oxide

Nitric oxide is an uncharged lipophilic molecule that behaves like amphoteric molecule, i.e. NO could function as an electron donor (oxidant) or an electron acceptor (antioxidant) (Drew and Leeuwenburgh, 2002). It contains a single unpaired electron (NO•), which reacts with other molecules, such as oxygen, GSH and superoxide radicals. They prevent free radicals from stealing electrons from other molecules.

16.5 Sources of Natural Antioxidants

Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties. Sources of tocopherols, carotenoids and ascorbic acid are well known and there are plenty of publications related to their roles in health. Exogenous dietary antioxidants capable of scavenging free radicals are of great interest in combating oxidative stress-induced cell damage. Plants containing a high content of polyphenols and flavanoids are considered as potential antioxidants and can be used as adjuvant therapy. These plant polyphenols and flavanoids are multifunctional and can act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal ion chelators (Gassen and Youdim, 1999).

Several natural antioxidants such as silymarin, grape seed extract, resveratrol, curcumin etc., are known to reduce oxidative stress and protect from hepatic damage. Ulusoy *et al*. (2012) reported antioxidant and anti-apoptotic effects of proanthocyanidine from grape seed extract. Silymarin, a flavonoid complex from *Silybum marianum*, has been used in the treatment of hepatitis, liver cirrhosis, viral hepatitis and fatty liver. It has been shown to have antioxidant, antilipid peroxidative, anti-inflammatory and liver regenerative effects. Lupeol, a pentacyclic triterpenoid, found in many plants such as crataeva, mango, olive etc., received much attention due to its wide spectrum of medicinal properties that include antiprotozoal, anti-inflammatory, anticarcinogenic, cardioprotective and antimicrobial activities. Hepatoprotective action of lupeol against aflatoxin B1-induced toxicity has been reported by Preetha *et al*. (2006).

Cymbopogon citratus D. Stapf., commonly known as lemongrass, contains volatile oil consisting of citral, a monoterpene (a mixture of two isomeric aldehydes, neral and geranial in the ratio of 2:3), as a major component, which is used in various perfume and cosmetic industries (Rauber *et al*., 2005). The plant is reported to possess antifungal, mosquito repellent, insecticidal, antidiabetic, antiseptic, antimutagenic and anticarcinogenic activity (Masuda *et al*., 2008).

Fumaria parviflora Lam. (Fp) is used for dermatological diseases, stimulation of liver function and gall bladder, as antiscabies, antiscorbite, antibronchite, diuretic, expectorant, antipyretic, diaphoretic, appetizer and antineoplastic agent. Its antinoceceptive effect has also been worked out (Heidari *et al*., 2004). Phytochemical analysis of Fp indicated presence of organic acids and isoquinoline alkaloids, namely: fumaric acid, protropine, cryptopine, sinactine, stylopine, dihydrofumariline, per-fumidine and dihydrosanguirine (Suau *et al*., 2002). Acetyl-cholinesterase and butyrylcholinesterase inhibitory activity of Fp has also been reported (Orhan *et al*., 2004). Significant oral antipyretic activity has been shown by hexane-chloroform and water-soluble extracts of Fp in rabbits (Akhtar *et al*., 1984). A 50% ethanolic extract of Fp was also tested to discover the role of mitochondria and ROS/oxidative stress in cytotoprotective and anti-apoptotic effects against nimesulide-induced hepatotoxicity (Tripathi *et al*., 2010).

Glycyrrhiza glabra (liquorice) possesses triterpene, saponins, glycyrrhizin/glycyrrhizic acid and glycyrrhetic acid. Glycyrrhizic acid (GA), a biologically active constituent of liquorice root with a structure of 20b-carboxy-11-oxo-30-norolean-12en-3-b-yl-2-o-b-D-glucopyranosiduronic acid, is believed to be partly responsible for anti-ulcer, anti-inflammatory, antidiuretic, anti-epileptic, anti-allergic, antidote, antitumour, antiviral, antihypotensive and several other properties of the plant (Baltina, 2003). Hypocholesterolaemic and hypoglycaemic activities have also been reported (Sitohy *et al*., 1991).

Bacopa monnieri Linn. (syn. *Herpestis monnieri* Linn. H.B. and K) is used as a nerve tonic, brain tonic, memory enhancer, laxative, astringent, antipyretic, anti-inflammatory and leprosy healer. It is also useful in renal disorders, blood diseases, cough, anaemia and poisoning. The plant also finds various applications in central nervous system depressant activity. Its major constituents including two saponins (bacoside A and bacoside B) have been isolated and characterized (Chowdhuri *et al*., 2002).

Geraniol, an acyclic monoterpenoid, is an important constituent of essential oils of ginger, lemon, lime, lavender, nutmeg, orange, rose and palmarosa. It is reported to prevent cancer. Camphene, another component, is a bicyclic monoterpene with a pungent smell. It constitutes a minor part of many essential oils including turpentine oil, cypress oil, citronella oil, ginger oil etc., and is known to possess antilithic and expectorant properties. Camphene is also present in apricot, carrots, cinnamon, ginger, cumin seed, nutmeg, cardamom and turmeric. It is used as a food additive for flavouring as well as in the preparation of fragrances, plasticizers for resins and lacquers (Verschueren, 2001).

Free radicals generated in diabetes may lead to several kinds of diabetic complications including nephropathy, neuropathy, cardiopathy and many more. Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes mellitus due to the fact that they are less toxic than oral hypoglycaemic agents such as sulfonylureas, metformin, etc. (Ponnachan *et al*., 1993).

Anthocyanins have been shown to be natural anti-inflammatory agents and pain relievers. Chronic inflammation has also been associated with an increased risk of cancer, but anti-inflammatory drugs are not effective for reducing this type of inflammation (Singh, B.N. *et al*., 2009b). Some important sources of antioxidants are presented in Table 16.2.

16.6 Roles of Antioxidants in the Prevention of Diseases

Plants have numerous natural antioxidants to control the oxidative stress induced by these free radicals (Pacher *et al*., 1997; Sarma *et al*., 2010). Free radicals have been implicated in the pathogenesis of over 100 human diseases such as cancer, heart disease, stroke, Alzheimer's disease, diabetes, premature ageing, high blood pressure and sepsis, to name a few.

16.6.1 Cancer

Antioxidants protect DNA thereby reducing the oxidative DNA damage caused by the free radical and ultimately control the increased abnormal cell division, the main characteristic of carcinogenesis. Experimental evidence using cell culture and animal models indicate that antioxidants either slow or prevent the development of cancer through its action as free-radical scavenger (Rock *et al*., 1996). Using *in vitro* and an animal model system, it was experimentally found that plant-derived phytochemicals, such as allyl sulfides, isothiocyanates and sulforaphene, inhibit the various step of tumour development (Milner, 1994). Blot *et al*. (1993) and Sardas (2003) reported that a combination of β-carotene, vitamin E and selenium significantly reduces the chance of cancer development especially in the case of gastric cancer. Experimental evidence also suggests that β-carotene with α-tocopherol/retinol significantly reduced the chance of lung cancer (Omenn *et al*., 1994).

16.6.2 Alzheimer's disease

Alzheimer's disease (AD) is characterized by progressive loss of memory as the major clinical manifestation. Studies on free radicals suggest that oxidative stress causes neurodegenerative disorders, including AD. Metal ion also plays an important role in the development of AD. Nutraceutical antioxidants such as β-carotene, curcumin, lutein, lycopene, turmerin etc., showed positive effects by reducing oxidative stress, mitochondrial dysfunction and various forms of neural degeneration (Glenville, 2006). It has been observed that a lower activity of antioxidant enzyme such as superoxide dismutase is related to occurrence of Alzheimer's disease in humans (Thome *et al*., 1997). Kontush *et al.* (2001) reported that supplementation with vitamins E and C to the patient significantly increases the concentration of vitamins in plasma and decreases the oxidation of lipoprotein, while vitamin E alone does not have any significant effects. High intake of nutraceutical postpones the development of dementias such as AD (Haider and Bhutta, 2006).

16.6.3 Atherosclerosis

Atherosclerosis is a common cardiovascular disease, which occurs due to deposition of oxidized fatty acid to the arteries in the form of plaque. Approximately two-thirds of the serum cholesterol pool in a normal subject is low-density lipoprotein-cholesterol (LDL-C), which is believed to play an important role in the development of atherosclerosis (Shukla *et al*., 2011).

Flavonoids and other plant-derived polyphenols, present in fresh fruits and vegetables, have been shown to be powerful antioxidants capable of preventing LDL oxidation induced by free radicals. Recommended daily allowance for the flavonoids is 1 g in an ordinary diet, which is sufficient for the antioxidant defence system. Interestingly, it has been found that the antioxidant activity of some of flavonoids synergistically increases when they are supplemented with ascorbic acid to prevent LDL oxidation. The beneficial properties

Table 16.2. Some important sources of antioxidants.

Plant	Antioxidants	References
Medicinal plants		
Terminalia chebula (Bahera)	Casuarinin, chebulanin and chebulinic acid	Cheng *et al.*, 2003
Cassia fistula (Amaltas)	Lupeol, β-sitosterol, hexacosanol, kaempferol, proanthocyanidin, bianthraquinone glycoside, anthraquinones, flavonoids, flavan-3-ol derivatives, sennoside A, sennoside B	Akiremi *et al.*, 2000
Withania somnifera (Ashwagandha)	Withanolides, cuscohygrine, anahygrine, tropane, pseudotropine, anaferine, dl-iso-pllatierine, withanine, withasominine, withaninine, somniferin, pseudowithanine, tropanol, pseudotopanol, cuscokygrene, 3-tigioyloxytropana, isopelletierine	Sangwan, 2004; Mohammad and Elisabeth, 2009; Kushwaha and Karanjekar, 2011
Fruits		
Berries (Sarashphal)	Flavanols, hydroxycinammic acids, hydroxybenzoic acids, anthocyanins	Wang and Lin, 2000; Yanishlieva-Maslarova and Heinonen, 2001
Citrus fruits	Flavanones, flavonols, phenolic acids	Yanishlieva-Maslarova and Heinonen, 2001; Manach *et al.*, 2004
Black grapes	Anthocyanins, flavonols	Belitz and Grosch, 1999; Yanishlieva-Maslarova and Heinonen, 2001
Cherries	Hydroxycinnamic acids, anthocyanins	Belitz and Grosch, 1999; Yanishlieva-Maslarova and Heinonen, 2001
Plums (Jamun), apples, pears	Hydroxycinnamic acids, catechin	Belitz and Grosch, 1999; Yanishlieva-Maslarova and Heinonen, 2001
Vegetables		
Allium sativum (Garlic)	Aliin, allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, S-allylcysteine, S-allyl mercaptocysteine and saponins	Kemper, 2000; Amagase, 2006
Allium cepa (Onion)	Phenolic acids, flavonoids, cepaenes, thiosulfinates, anthocyanins, sulfur compounds, saponins, quercetrin	Singh, B.N. *et al.*, 2009a; Panduranga Murthy *et al.*, 2011
Trigonella foenum-graecum (Fenugreek)	Coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, trigonelline, L-tryptophan-rich proteins and saponins	Yoshikawa *et al.*, 1997
Daucus carota (Carrot)	Carotol, daucene, germacrene D, bergamotene, selinene, carotol, daucol, copaenol	Ozcan and Chalchat, 2007
Sweet potato leaves	Flavonols, flavones,	Chu *et al.*, 2000
Yellow onion	Flavonols	Manach *et al.*, 2004
Beans	Flavanols	Manach *et al.*, 2004
Spinach	Flavonoids, *p*-coumaric acid	Bergman *et al.*, 2001

Flours		
Oats, wheat, rice	Caffeic, ferulic acids	Yanishlieva-Maslarova and Heinonen, 2001
Drinks		
Orange juice	Flavanols	Manach *et al.*, 2004
Coffee	Hydroxycinnamic acids	Manach *et al.*, 2004
Chocolate	Flavanols	Manach *et al.*, 2004
Red wine	Flavan-3-ols, flavonols, anthocyanins	Manach *et al.*, 2004
Herbs and spices		
Sage, carnosol	Carnosic acid, lateolin, rosmanul, rosmarinic acid	Yanishlieva-Maslarova and Heinonen, (2001)
Foeniculum vulgare (Fennel)	Essential oil (trans-anethole, α-phellandrene, α-pinene), dipentene, methyl chavicol, feniculun, anisaldehyde and anisic acid	Piccaglia and Marotti, 2001; Mimica-Dukic *et al.*, 2003; Araque *et al.*, 2007
Rosemary	Carnosic acid, carnosol, Rosmarinic acid rosmanol	Yanishlieva-Maslarova and Heinonen, 2001; Ibanez *et al.*, 2003
Thyme	Thymol, carvacrol, flavonoids, lubeolin	Exarchou *et al.*, 2002
Ginger	Gingerol and related compounds	Moure *et al.*, 2001; Yanishlieva-Maslarova and Heinonen, 2001

of certain plants may be explained by the presence of some especially effective flavonoids like resveratrol, which has also been found in red wines. Probucol, a hypocholesterolaemic drug, has significant antioxidant activity and *in vivo* study on rabbit showed that it has protective effects against atherosclerosis. In animal studies, aspirin has also been shown to prevent atherosclerosis (Jaichander *et al.*, 2008).

16.6.4 Heart diseases

There are several factors such as high cholesterol level, hypertension, diabetes, cigarette smoking etc. that provide a platform for the development of heart disease. Oxidation of low density lipoprotein (LDL-cholesterol) causes deposition of fatty acid in arteries leading to development of atherosclerosis, which ultimately causes heart disease (Anderson *et al.*, 1995). Heart disease is acquired with age because oxidized fatty acid gets more 'sticky' and easier to adhere to the artery walls. It is believed that high intake of ascorbic acid reconstitutes the endothelial dysfunctions (Ting *et al.*, 1997) and protects the circulating lipoprotein from free radicals.

16.6.5 Diabetes

Diabetes mellitus (DM) is characterized by hyperglycaemia (Grill and Bjorklund, 2000). Oxidative stress due to lack of antioxidant defences may also cause diabetes (Cross *et al.*, 1987; Maxwell *et al.*, 1997; Keaney and Loscalzo, 1999; Bonnefont-Rousselot *et al.*, 2000; West, 2000). It is hypothesized that if ROS are involved in the genesis of diabetes, then antioxidants may be an effective approach in prevention of diabetes (Giugliano *et al.*, 1996). Reaven (1995) revealed that supplementation of vitamin E reduces the sensitivity of LDL to *in vitro* oxidation and availability of oxidized LDL in type-2 diabetics as well as in healthy subjects (Liao *et al.*, 1995). It is hypothesized that imbalance between generation and scavenging of free radicals is the main cause associated with diabetes. Insulin increases the uptake of vitamin C in to the cell but in hyperglycaemic conditions this process is inhibited resulting in a condition known as 'tissue scurvy'. Supplementation of vitamin C alone controls the blood glucose level, improves endothelium-dependent vasodilation and increases the resistance of lipoprotein towards oxidation in the patient with either type-1 or type-2 diabetes mellitus (Ting *et al.*, 1996; Timimi *et al.*, 1998; Kawano *et al.*, 1999).

16.6.6 Parkinson's disease

Parkinson's disease (PD) results from damage in neuronal cells in certain regions of the brain, and is characterized by muscle rigidity, shaking and difficulty in walking (Losso, 2003). Latif *et al.* (2007) reported that vitamin E in food may be protective against PD. Glutathione has also shown some promising results in preliminary studies to treat PD but appropriate long-term dosing, side-effects and the most effective method of administration are not yet clear.

16.7 Conclusions

Antioxidants may be a promising source for the prevention and or treatment of free radical-generated diseases such as atherosclerosis, hypertension, diabetes, cancer, Parkinson's and Alzheimer's diseases etc. Evidence also indicates that antioxidants protect/cure the diseases by involving a number of biological processes, including signal transduction pathways, activation of antioxidant defences, cell proliferation, cell survival-associated gene expression, differentiation and preservation of mitochondrial integrity. 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 antioxidant components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals. Increasing dietary intake of antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of human beings.

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