2.0 LITERATURE REVIEW

2.1 Free radicals

Free radicals are atoms or group of atoms with an odd or unpaired number of electrons in the outer orbits (Gilbert, 2000) and can be formed when oxygen interacts with certain molecules. The odd or unpaired electron, free radicals has great affinity to acquire an electron from other molecules. This in turn, changes the structure of the molecules, in turn causing them to become free radicals. Free radicals are inherently unstable and very reactive since they contain extra energy that readily lead to uncontrolled reactions, resulting in oxidative damage of important biological macromolecules such as nucleic acids, proteins and lipids (Stephen *et al.,* 1997).

2.1.1 Types of Free Radicals

Table 2.1: Types of free radicals

Oxygen-containing Radicals	**Oxygen-containing Non radicals**	**Nitrogen-containing radicals**	**Nitrogen-containing Non-radicals**
Superoxide (O_2^-)	Hydrogen Peroxides (H_2O_2)	Nitrite Oxide ($NO^\bullet$)	Nitrous Acid (HNO_2)
Hydroxyl ($^\bullet OH$)	Hydrochlorous Acid (HOCl)	Nitrogen Dioxide ($NO_2^\bullet$)	Dinitrogen Tetroxide (N_2O_4)
Hydroperoxy ($HO_2^\bullet$)	Ozone (O_3)		Dinitrogen Trioxide (N_2O_3)
Alkoxyl ($RO^\bullet$)	Singlet Oxygen ($O^\bullet$)		Peroxynitrite ($ONO_2^\bullet$).
			Peroxynitrous
			Nitronium cation (NO_2^-)

There are four types of free radicals (Table 2.1). Free radicals that contain oxygen are referred to as reactive oxygen species (ROS), whereas those free radicals containing nitrogen are referred to as reactive nitrogen species (RNS). Free radicals also include

non-radical species. Reactive chlorine species are produced in animals and humans under physiologic and pathologic conditions (Halliwell, 2009). Thus ROS and RNS include radical and non-radical species. Once formed, these highly reactive radicals can start chain of reactions. These create a self-propagating chain reaction in which radical will continue to breed as shown below:

$$O_2 + e^- \rightarrow O_2^{\bullet}$$

$$O_2^{\bullet} + H^+ \rightarrow HO_2^{\bullet}$$

$$HO_2^{\bullet} + H^+ + e^- \rightarrow H_2O_2$$

$$H_2O_2 + e^- \rightarrow OH^{\bullet} + HO^-$$

Below are examples of free radicals explained in detail:

(i) Superoxide anion

The superoxide free radical anion (O_2^-) is formed when oxygen is reduced by the transfer of a single electron to its outer shells. Cellular superoxide radicals are generated during various enzymatic reactions, by ionizing radiation and mostly during mitochondrial oxygen metabolism in the mitochondria (Cadenas & Davies, 2000).The major source of superoxide *in vivo* is the electron leakage that results from the electron transfer chain of the mitochondria.

On its own superoxide anion is not particularly damaging. However, it appears to play a central role as other reactive intermediates are formed from it. Its main significance lies in its being a main source for the generation of hydrogen peroxide and as a reductant of transition metals, which are precursors to the formation of the lethal hydroxyl radical.

(ii) Hydrogen peroxide

Hydrogen peroxide (H_20_2) is not a free radical but falls in the category of reactive oxygen species. It is an oxidising agent that is not particularly reactive but its main significance lies in that it is the main source of hydroxyl radicals in the presence of transition metal ions.

Hydrogen peroxide can be generated from the two electron reduction of oxygen. In biological systems hydrogen peroxide is generated from the reaction of superoxide: two superoxide molecules (O_2^-) (Storz, 2007).

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The above reaction is called a *dismutation reaction* as the radical reactants produce nonradical products.

(iii) Hydroxyl radical

The hydroxyl radical (OH^-) is an extremely reactive oxidising radical that will react to most biomolecules at diffusion controlled rates (Cheng *et al*., 2002), which means that reactions will occur immediately with biomolecules. The hydroxyl free radical is important in radiobiological damage and is several orders of magnitude more reactive towards cellular constituents than superoxide radicals (and many orders more reactive than hydrogen peroxide).

Around 1933, Fritz Haber and Joseph Weiss first proposed that hydroxyl free radicals (·OH) were produced when superoxide and hydrogen peroxide react together:

$$O_2^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^- \quad (1)$$

This formula was coined the *Haber-Weiss* reaction.

About 100 years ago, Henry Fenton had observed that the reducing agent, ferrous iron (Fe^{2+}), together with hydrogen peroxide could oxidize some organic compounds. The mechanism is now known to involve hydroxyl radicals, with a key step analogous to reaction (1) but with the electron donor, $O_2\cdot^-$ replaced by Fe^{2+}:

$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+} \qquad (2)$$

The above reaction is more complicated than is stated above and is most commonly referred to as the iron catalysed Haber-Weiss reaction or the superoxide-driven *Fenton reaction* (Halliwell, 1992a).

These reactions are significant as the substrates can be found within the body and could easily interact. Excessive generation of ·OH have been implicated in the onset of many illness including Parkinson disease (Jenner, 2003), Alzheimer disease, cancer (Lee & Jeong, 2007) and atherosclerosis (Laggner *et al*., 2005)

(iv) Singlet oxygen

It is a nonradical (does not have an unpaired electron) reactive oxygen species often associated with oxygen free radicals that has strong oxidising activity. Singlet oxygen ($^1O_2$) is an electronically excited and mutagenic form of oxygen. It is generated by input of energy, example radiation, but can also be generated enzymatically by the action of peroxidases or lipoxigenases or by the reaction of hydrogen peroxide with hypochlorite or peroxynitrite (Di Mascio *et al.,* 1994; Di Mascio *et al.,* 1996), thermo-decomposition of dioxetanes (Briviba *et al.,* 1996), or during the respiratory burst of phagocytes (Steinbeck *et al.,* 1992). They are also generated in biological systems in a number of pigment reactions including chlorophylls, retinal and flavins when they are illuminated in the presence of oxygen.

(v) Nitric Oxide (N0)

It is a common gaseous free radical. It is now recognised to play a role in vascular physiology and is also known as endothelium derived relaxing factor. Vascular endothelium produces nitric oxide, as do neutrophils and macrophages from arginine using the enzyme nitric oxide synthetase. This event can be stimulated by cytokines, tumour necrosis factor, or interleukins (Beckman *et al.,* 1993; Moncada *et al.,* 1991). Inhibition of production is known to reduce microbicidal and tumouricidal activities of macrophages.

(vi) Peroxynitrite

It is produced by the reaction of nitric oxide with superoxide. The result is a radical-radical reaction in which peroxynitrite ($ONOO^-$) is formed:

$$O_2^{\bullet -} + NO\cdot = ONOO^-$$

(vii) Hypochlorous acid

Activated polymorphonuclear cells produce hypochlorous acid (HOCl) as a major bactericidal agent. It is generated by the action of myeloperoxidase on chloride ions in the presence of H_2O_2.

$$H_2O_2 + Cl^- \rightarrow HOCl + OH^-$$

This reaction occurs in the neutrophils phagocytic vacuole after fusion with the myeloperoxidase-containing lysosomal vesicles.

Hypochlorous acid can cross cell membranes and, in the presence of transitional metal ions, generate hydroxyl radicals (Aruoma, 1994). Highly reactive hydroxyl radicals can be formed from HOCl/OCl^- on reaction with reductants that are one-electron donors. Important examples include superoxide radicals and ferrous iron:

$$HOCl + O_2^{\bullet -} \rightarrow \cdot OH + Cl^- + O_2$$

$$HOCl + Fe^{2+} \rightarrow \cdot OH + Cl^- + Fe^{3+}$$

HOCl has been shown to be capable of initiating lipid peroxidation (Panasenko *et al.,* 1995), combining with H_2O_2 to damage DNA and DNA repair processes (Vanrensburg *et al.,* 1994) and altering intracellular free Ca^{2+} and pH (Kuroda *et al.,* 1995). It may contribute to tissue damage during the inflammatory process. This latter event may result from the activation of collagenases or the inactivation of alpha-1 antiproteinase (Ching *et al.,* 1994).

2.2 Beneficial roles of free radicals

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have dual roles as both deleterious and beneficial species to living system: they can be either harmful or beneficial. Beneficial effects of reactive oxygen species (ROS) occur at low or moderate concentrations. Reactive oxygen species (ROS) involve physiological roles in cellular responses to noxia, such as in defense against infectious agent, wound healing and in the function of cellular signalling systems and the induction of mitogenic response (Valko *et al.,* 2007). In an inflammatory response, massive reactive oxygen species (ROS) production results in oxidative burst, which plays an important role in defense against pathogens (Keisari *et al*., 1983).

Nitric oxide (NO·) is a reactive radical that is important in oxidative biological signalling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation. Nitric oxide radical also has antimicrobial, antitumor and cytotoxic effect in higher concentration (Bergendi *et al*., 1999).

2.3 Deleterious effects of free radicals

Oxidative stress is the term given to harmful effect of free radicals that cause potential biological damage. Reactive oxygen species (ROS) are also involved in the development of many degenerative diseases such as diabetes, cirrhosis, atherosclerosis, neurodegeneration and cancer (Duan *et al*., 2007; Marwah *et al*., 2007).

Nitrosative stress is the term given to over production of reactive oxygen species (Klatt & Lamas, 2000; Ridnour *et al*., 2004). This may occur if the generation of reactive nitrogen species in the system exceed the limit the ability of the system to eliminate them. Nitrosative stress thus, may lead to nitrosylation reactions that can alter the structure and inhibit the normal function of proteins (Valko *et al*., 2006).

Oxidative and nitrosative stress occurs in biological systems when there is overproduction of reactive oxygen species (ROS) or reactive nitrogen species (RNS) on one side and deficiency of enzymatic and non-enzymatic antioxidants on the other. In other words, oxidative stress represents a disturbance in the equilibrium status of prooxidant/antioxidant reaction in living organisms. The excess ROS can damage cellular components such as lipids, proteins or DNA, thus inhibiting their normal function. Because of this, oxidative stress has been implicated in aging and pathogenesis of certain diseases. The delicate balance between both beneficial and harmful effects of free radicals is very important in the living organisms and is achieved by mechanisms

called “redox regulation”. Redox regulation maintains “redox homeostasis” by controlling the redox status *in vivo* and also protects living organisms from various oxidative stresses (Valko *et al*., 2006).

2.3.1 Damage to biomolecules

(i) Oxidative nuclear and mitochondrial DNA damage

At high concentrations, reactive oxygen species (ROS) can result in damage to cell structures, nucleic acids, lipids and proteins which are extremely sensitive to oxidation (Valko *et al*., 2006). The hydroxyl radical (·OH) can react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Chen & Gow, 2007; Dasgupta & De, 2007; Valko *et al*., 2007).

DNA damage induced by reactive oxygen species (ROS) result in permanent modification of genetic material such as single or double stranded DNA breaking, modification of purine, pyrimidine, deoxyribose and cross links of DNA. DNA damage can result in arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, which are associated with mutagenesis and carcinogenesis (Chen & Gow, 2007; Dasgupta & De, 2007; Valko *et al*., 2006, 2007).

Reactive nitrogen species (RNS) such as nitrogen oxide (NO·) and peroxynitrites ($ONOO^-$) can also contribute to DNA damage. Peroxynitrite react with guanine to form nitroguanine. This adduct has the potential to induce transformation from G: C to T: A (Valko *et al*., 2006).

Mitochondrial oxidative DNA damage include mutations and altered expression in mitochondrial genes encoding for complexes I, III, IV, V and in the hyper variable regions of mitochondrial DNA (Inoue *et al.,* 2003; Valko *et al*., 2006). Mitochondrial

DNA is more susceptible to oxidation than nuclear DNA because (i) under physiological conditions, mitochondria convert some consumed oxygen to superoxide anion and then hydroxide peroxide; (ii) mitochondria lack the feature of nucleotide excision repair and so DNA repair capacity is limited; (iii) mitochondrial DNA is not protected by histones proteins. This impaired DNA repair capacity may lead to mitochondrial dysfunction and onset of degenerative disease (Valko *et al.,* 2006).

(ii) Lipid peroxidation

Hydroxyl radical (·OH) generated via Fenton reaction is able to initiate lipid peroxidation. The whole process of lipid peroxidation consists of three stages: initiation, propagation and termination (Valko *et al.,* 2006). The hydroxyl radical (·OH) can obtain an electron from polyunsaturated fatty acid and give rise to carbon centered lipid radical ($L^{\bullet}$). The lipid radical can react with molecular oxygen to give rise to lipid peroxyl radical ($LOO^{\bullet}$) (Valko *et al*., 2007). Once formed, the peroxyl radicals ($LOO^{\bullet}$) can be rearranged via cyclisation reaction to form endoperoxide, which are the precursors of malondialdehyde (MDA). The final product of lipid peroxidation is malondialdehyde (MDA). Malondialdehyde (MDA) is mutagenic in bacterial and mammalian cells and found to be carcinogenic in rats. Lipid peroxidation is an autocatalytic reaction, which is terminated by recombination of radicals or depletion of substrate (Valko *et al.,* 2006). Lipid peroxidation can lead to rupture of cell and this is significant in cells such as neurons (Wickens, 2001).

(iii) Protein damage

Hydroxyl radicals react with proteins and leads to abstraction of a hydrogen atom from protein polypeptide backbone to form a carbon-centered radical. Under aerobic conditions, the carbon-centered radical reacts with molecular oxygen to form peroxyl

radicals (Stadtman, 2001, 2004; Valko *et al.,* 2006). The peroxyl radical (LOO·) are then converted to alkyl peroxides. In the absence of ionising radiation, proteins are resistant to damage by hydrogen peroxide (H_2O_2) and other simple oxidant unless transition metals are present. Metal-catalyzed damage to proteins includes oxidative scission, lost of histidine residues, introduction of carbonyl groups and formation of protein-centered alkyl (R·), alkoxyl (RO·), and alkylperoxl (ROO·) radicals. The alkoxyl radical derives from protein are able to undergo peptide-bond cleavage. Peptide-bond cleavage can also occur by initiating hydroxyl radicals attack on glutamic acid and praline residues. Damage to protein is likely to be repairable and non-lethal event for cell, but to mitochondrial proteins, aconitase and adenine nucleotide translocase may be important target of long-term oxidative damage. The side chains from all amino acids are susceptible to ionizing radiation and reactive oxygen species (ROS), reactive nitrogen species (RNS) oxidation (Stadman, 2004; Valko *et al.,* 2006).

Nitric oxide (NO·) reacts rapidly with superoxide radical ($O_2\cdot^-$) to form peroxynitrite anion ($ONOO^-$), a highly toxic compound which is able to nitrosate cysteine sulphydryl groups of proteins to nitrate tyrosine, tryptophan residues and oxidize methionine residues to methionine sulphoxide. Protein modification by peroxynitrate ($ONOO^-$) is strongly inhibited by physiological concentration of carbon dioxide (CO_2) because it reacts rapidly with carbon dioxide (CO_2) to form nitrosoperoxycarbonate ($ONOOCO_2^-$). Nitration of tyrosine, an irreversible process, may prevent the phosphorylation or adenylation of regulatory proteins tyrosine (Valko *et al.,* 2006). Oxidation of protein is related to a number of age-related diseases and aging, the process of aging is often related to the accumulation of oxidized form of proteins. The accumulation of oxidized proteins in living system may be: (i) due to increase in the level of reactive oxygen species (ROS), reactive nitrogen species (RNS) and/or decrease in antioxidant capacity, (ii) decrease in ability to degrade oxidized

proteins, due to decrease in protease concentration and/or increase in protease inhibitors (Valko *et al.,* 2006).

2.4 Antioxidant from plants

As the name implies, antioxidants are substances that are capable of counteracting the damaging, effects of the physiological process of oxidation in animal tissue. Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. Antioxidants play an important role not only physiologically but also found in food industry especially in health-care products. Antioxidants can neutralize the free radicals by reducing the energy of the free radical and interrupting an oxidizing chain reaction to minimize the damage caused by free radicals.

The body has developed several endogenous antioxidant systems to deal with the production of reactive oxygen species (ROS). These systems can be divided into enzymatic and non-enzymatic antioxidants. Superoxide dismutase, catalase and glutathione peroxidase are the most efficient enzymatic antioxidants (Mates *et al.,* 1999). The non-enzymatic antioxidants include the lipid-soluble vitamins, vitamin E and vitamin A or provitamin A (beta-carotene), and the water-soluble vitamin C.

The antioxidant action of non-ezymatic antioxidants and antioxidant enzymes play an essential role in balancing the effect of reactive oxygen and nitrogen species. Such antioxidant defences are extremely important as they represent the direct removal of free radicals (pro-oxidants), thus providing maximal protection for important molecules.

A good antioxidant should: (a) specifically quench free radicals; (b) chelate redox metals; (c) interact with (regenerate) other antioxidants within the "antioxidant network"; (d) have a positive effect on gene expression; (e) be readily absorbed; (f) have a concentration in tissues and body fluids at a physiologically relevant level (Reed, 1993).

The enzymatic and non-enzymatic antioxidant systems are intimately linked to one another and appear to interact with one another (Figure 2.1).

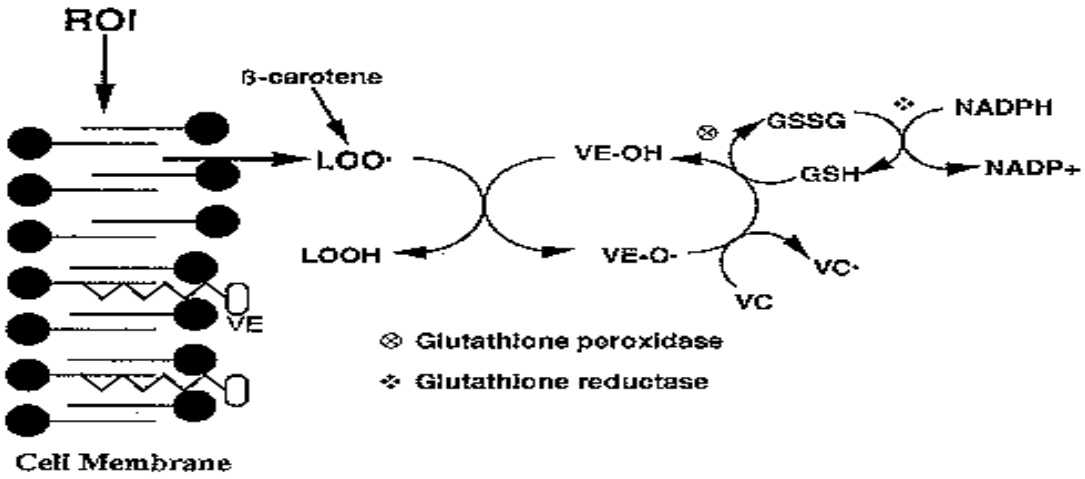

Figure 2.1: Interactions among antioxidants (Seifried *et al*., 2007)

Both vitamin C and GSH have been implicated in the recycling of alpha-tocopherol radicals (Reed, 1993). In addition, the trace elements selenium, manganese, copper, and zinc also play important roles as nutritional antioxidant cofactors. Selenium is a cofactor for the enzyme glutathione peroxidase, and manganese, copper, and zinc are cofactors for SOD. Zinc also acts to stabilize the cellular metallothionein pool, which has direct free radical quenching ability (Bray & Bettger, 1990). The complex interactions of these different antioxidant systems may imply that therapeutic strategies will depend on combination therapy of various antioxidants rather than single agent.

Reactive oxygen intermediates (ROI) induce membrane lipid peroxidation resulting in a chain reaction that can be interrupted by the direct scavenging of lipid peroxyl radicals by vitamin E (VE) and beta-carotene. Vitamin E can then be recycled by both vitamin C (VC) and glutathione (GSH). The reducing ability of GSH is catalyzed by the enzyme glutathione peroxidase (GSSG). Glutathione is then recycled by NADPH, which is facilitated by glutathione reductase (GSSG - the reduced form) (Bulger & Helton, 1998).

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in food, cosmetics and pharmaceutical materials (Kumaran & Karunakaran, 2007). Organisms have developed a series of defense mechanisms to fight exposure to free radicals from variety of sources. Defense mechanisms against free radical-induced oxidative stress involve: (i) preventive mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. Antioxidants are divided into 2 categories: enzymatic and non-enzymatic antioxidant. Examples of enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants include glutathione (GSH), ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids, flavonoids (phenolic compound) and other antioxidants (Dusgupta & De, 2007; Tung *et al.,* 2007; Turkoglu *et al.,* 2007; Valko *et al.,* 2007).

In recent years, plant-derived antioxidants have raised considerable interest among food scientists, manufactures, and consumers. Working on plants was a good start, since plants are of enormous importance in the free radical/antioxidant field. First, they supply us with the essential biradical, O_2. As they do, plants expose themselves to high levels of O_2 and so are rich in antioxidant defences and repair systems against

oxidative damage to help them deal with O_2 toxicity (Halliwell, 1981, 1987, 1999a, 2007). Second, plants supply a range of antioxidants to humans; some known to be important in vivo (ascorbate, α-tocopherol) and others suspected to be so (other tocopherols, tocotrienols, flavonoids, carotenoids). Plants, expecially chloroplasts, are enormously rich in ascorbate (Law, Charles, & Halliwell, 1983). We were able to show that one reason for this is that ascorbate participates in a cycle of reactions that enables chloroplasts to remove H_2O_2. We further found that removal of H_2O_2 is essential because of its ability to inactivate Calvin cycle enzymes essential to CO_2 fixation (Law *et al.*, 1983; Charles & Halliwell, 1980, 1981; Foyer & Halliwell, 1976). Third, diets rich in plants are associated with lower risk of developing many age-related diseases (e.g., some cancers, diabetes, atherosclerosis, dementia) and most people in "advanced" countries would have better health if they ate more fruits and vegetables.

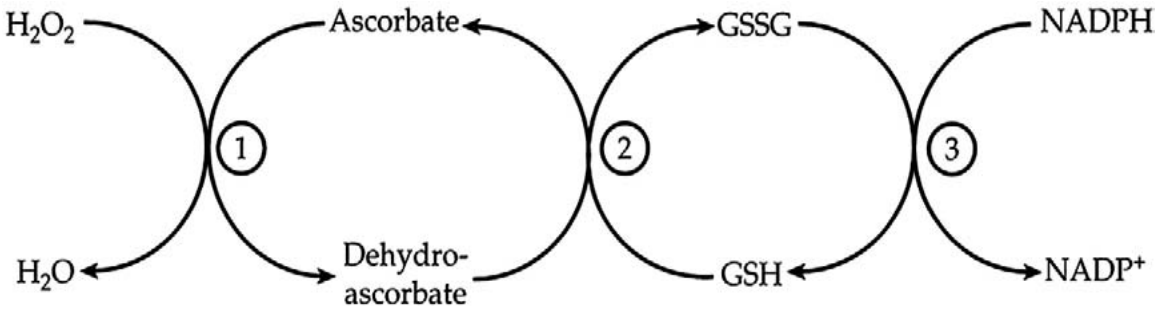

Figure 2.2: The ascorbate – glutathione cycle in chloroplasts

As shown in Figure 2.2, first enzyme involved is ascorbate peroxidase (reaction 1); second is dehydroascorbate reductase (reaction 2 can also occur nonenzymically at high pH - the pH of the stroma during photosynthesis may rise to as high as 8 (Halliwell, 1981), due to formation of a proton gradient); and third is glutathione reductase (reaction 3). The initial product of oxidation of ascorbate by ascorbate peroxidase is semidehydroascorbate (SDA); two SDA radicals can undergo disproportionation to form ascorbate and dehydroascorbate. NAD(P)H-dependent and

ferredoxin-dependent mechanisms for reducing SDA also exist in chloroplasts (Asada, 2000). The cycle shown above has been called the Foyer-Halliwell-Asada cycle after the names of the two scientists who first proposed it (Foyer & Halliwell, 1976) and the third (Asada) who did much to establish evidence for its occurrence (Asada, 2000). However, Groden and Beck first reported ascorbate peroxidase in chloroplasts (Groden & Beck, 1979). The ascorbate-glutathione cycle also operates in plant mitochondria, the root nodules of leguminous plants, and the intestines of some insects (Halliwell & Gutteridge, 2007).

Many spices and culinary herbs are common sources of phenolic compounds which have been reported to show superior antioxidant capacity to fruits, vegetables cereals, and nuts (Carlsen *et al*., 2010; Pellegrini *et al*., 2006). Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH^-) or superoxide radical (O^{2-}) scavengers. They are also strong chelators of metal ions (Marwah *et al.,* 2007).

2.5 Antioxidant

2.5.1 Enzymatic Antioxidants

Enzymatic antioxidants include superoxide dismutase, catalase, and peroxidases.

(i) Superoxide dismutase (SOD)

SOD is an endogenously produced intracellular enzyme present in essentially every cell in the body. It is the antioxidant enzyme that catalyses the dismutation of $O_2^{\cdot -}$ to O_2 and to the less reactive H_2O_2 (McCord & Fridovich, 1969).

Cellular SOD is actually represented by a group of metalloenzymes with various prosthetic groups. The prevalent enzyme is cupro-zinc (CuZn) SOD, which is a stable dimeric protein (32,000 D).

SOD appears in three forms: (1) Cu-Zn SOD in the cytoplasm with two subunits, and (2) Mn-SOD in the mitochondrion (Mayes, 1993; Warner, 1994). A third extracellular SOD recently has been described contains Copper (Cu-SOD).

(ii) Glutathione peroxidase enzyme

The glutathione redox cycle is a central mechanism for reduction of intracellular hydroperoxides (Figure 2.2). Gluthathione peroxidase is a tetrameric protein 85,000-D. It has 4 atoms of selenium (Se) bound as seleno-cysteine moieties that confer the catalytic activity. One of the essential requirements is glutathione as a cosubstrate.

Glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing glutathione (GSH) rereduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase. These enzymes also require trace metal cofactors for maximal efficiency, including selenium for glutathione peroxidase; copper, zinc, or manganese for SOD; and iron for catalase (Halliwell, 1995a).

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

$$2GSH + ROOH \rightarrow GSSG + ROH + H_2O$$

(iii) The catalase enzyme

Catalase enzyme is a protein enzyme present in most aerobic cells in animal tissues. Catalase is present in all body organs being especially concentrated in the liver and erythrocytes. The brain, heart, skeletal muscle contains only low amounts. An increase in the production of SOD without a subsequent elevation of catalase or glutathione

peroxidase leads to the accumulation of hydrogen peroxide, which gets converted into the hydroxyl radical. Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani *et al*., 1996).

Catalase deficiency may increase the likelihood of developing Type II Diabetes. Some human beings have very low levels of catalase (acatalasia), yet show few ill effects. It is likely that the predominant scavengers of H_2O_2 in normal mammalian cells are peroxiredoxins rather than catalase. Human catalase works at an optimum temperature of 37 °C, which is approximately the temperature of the human body (Ho *et al*., 2004; László *et al*., 2001).

2.5.2 Non-Enzymatic antioxidants

(i) Alpha tocopherol (vitamin E)

Alpha tocopherol is the major lipid soluble antioxidant found in cells. Vitamin E is a generic term that includes all entities that exhibit the biological activity of natural vitamin E, d-alpha-tocopherol. In nature, eight substances have been found to have vitamin E activity: d-alpha-, d-beta-, d-gamma- and d-delta-tocopherol (which differ in methylation site and side-chain saturation (Kell & Kaprelyant, 1996); and d-alpha-, d-beta-, d-gamma- and d-delta-tocotrienol. Also, the acetate and succinate derivatives of the natural tocopherols have vitamin E activity, as do synthetic tocopherols and their acetate and succinate derivatives. Of all these, d-alpha-tocopherol has the highest biopotency, and its activity is the standard against which all the others must be compared. It is the predominant isomer in plasma.

Vitamin E is more appropriately described as an antioxidant than a vitamin. This is because, unlike most vitamins, it does not act as a co-factor for enzymatic reactions. Deficiency of vitamin E does not produce a disease with rapidly developing symptoms such as scurvy or beriberi. Overt symptoms due to vitamin E deficiency occur only in cases involving fat malabsorption syndromes, premature infants and patients on total parenteral nutrition. The effects of inadequate vitamin E intake usually develop over a long time, typically decades, and have been linked to chronic diseases such as cancer and atherosclerosis.

Its main function is to prevent the peroxidation of membrane phospholipids, and avoids cell membrane damage through its antioxidant action. The lipophilic character of tocopherol enables it to locate in the interior of the cell membrane bilayers (Halliwell & Gutteridge, 1992b). Tocopherol-OH can transfer a hydrogen atom with a single electron to a free radical, thus removing the radical before it can interact with cell membrane proteins or generate lipid peroxidation (Halliwell & Gutteridge, 1992b). By this process, an aggressive ROI is eliminated and a weak ROI (dehydroascorbate) is formed, and tocopherol-OH is regenerated. Despite this complex defence system, there are no known endogenous enzymatic antioxidant systems for the hydroxyl radical.

Vitamin E also stimulates the immune response. Some studies have shown lower incidence of infections when vitamin E levels are high, and vitamin E may inhibit cancer initiation through enhanced immunocompetence. Vitamin E also has a direct chemical function. It inhibits the conversion of nitrites in smoked, pickled and cured foods to nitrosamines in the stomach. Nitrosamines are strong tumour promoters (Bray & Bettger, 1990).

Alpha-tocopherol has been shown to be capable of acting as pro-oxidant by reducing ferric iron to ferrous iron. The ability of alpha-tocopherol to act as a pro-oxidant (reducing agent) or antioxidant depends on whether all of the alpha-tocopherol becomes consumed in the conversion from ferric to ferrous iron or whether, following this interaction; residual alpha-tocopherol is available to scavenge the resultant ROI (Yamamoto & Niki, 1988).

(ii) Beta Carotene

Carotenoids are precursors of vitamin A and have antioxidant effects. While over 600 carotenoids have been found in the food supply, the most common forms are alpha-carotene, beta-carotene, lycopene, crocetin, canthaxanthin, and fucoxanthin. Beta-carotene is the most widely studied. It is composed of two molecules of vitamin A (retinol) joined together. Dietary beta-carotene is converted to retinol at the level of the intestinal mucosa.

The antioxidant function of beta-carotene is due to its ability to quench singlet oxygen, scavenge free radicals and protect the cell membrane lipids from the harmful effects of oxidative degradation (Santamaria *et al.,* 1991). The quenching involves a physical reaction in which the energy of the excited oxygen is transferred to the carotenoid, forming an excited state molecule (Krinsky, 1993). Quenching of singlet oxygen is the basis for the therapeutic efficacy of beta-carotene in erythropoietic protoporphyria (Matthews-Roth, 1993). The ability of beta-carotene and other carotenoids to quench excited oxygen, however, is limited, because the carotenoid itself can be oxidized during the process by auto oxidation. Burton and Ingold (1985) and other researchers have shown that beta-carotene auto oxidation *in vitro* is dose-dependent and dependent upon oxygen concentrations. At higher concentrations, it may function as a pro-oxidant and can activate proteases.

In addition to singlet oxygen, carotenoids are also thought to quench other oxygen free radicals. It is also suggested that beta carotene might react directly with the peroxyl radical at low oxygen tensions. This may provide some synergism to vitamin E which reacts with peroxyl radicals at higher oxygen tensions (Cotgreave *et al.*, 1988).

(iii) Ascorbic acid (vitamin C)

Ascorbic acid is a water-soluble, antioxidant present in citrus fruits, potatoes, tomatoes and green leafy vegetables. Humans are unable to synthesize *l*-ascorbic acid from *d*-glucose due to absence of the enzyme L-gulacolactone oxidase (Ensinger *et al.,* 1995). Hence, humans must obtain ascorbic acid from dietary sources.

The chemopreventive action of vitamin C is attributed to two of its functions. It is a water-soluble chain-breaking antioxidant. As an antioxidant, it scavenges free radicals and reactive oxygen molecules, which are produced during metabolic pathways of detoxification. It also prevents formation of carcinogens from precursor compounds. The structure of ascorbic acid is reminiscent of glucose, from which it is derived in the majority of mammals.

One important property is its ability to act as a reducing agent (electron donor). Ascorbic acid is a reducing agent with a hydrogen potential of + 0.08 V, making it capable of reducing such compounds as a molecular oxygen, nitrate and cytochromes a and c.

2.5.3 Mechanisms of Antioxidant

Mechanisms of antioxidant include prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging. Reducing properties are generally associated with

the presence of reductones (Duh, 1998; Farhoosh *et al*., 2007), such as ascorbic acid (a potent reducing agent), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Farhoosh *et al*., 2007; Gordon, 1990). Reductones are also reported to react directly with peroxides (Shimada *et al.*, 1992) and also with certain precursors of peroxides, thus preventing peroxide formation (Xing *et al*., 2005).

Free radical scavengers exert their effects by donating hydrogen to the unstable and active free radicals (Tung *et al.,* 2007). Metal chelating ability was significant because it reduce the concentration of the catalyzing transition metal in lipid peroxidation. Chelating agents will form a bond with metal and are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of metal ion (Gulcin *et al*., 2003).

A brief description of mechanism of actions of some antioxidants in different disease is described in Table 2.2. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (Stanner *et al*., 2004). This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area.

Table 2.2: Mechanism of action of various antioxidants against different disease

Compound	Pathology	Mechanism of action	References
Catalase(CAT)	Cancer, diabetic retinopathy	Destroy hydrogen peroxide in high concentration by catalyzing its two-electron dismutation into oxygen and water	Schonbaum & Chance, (1976)
Proanthocyanidin (GSPE)	Cardiovascular disorders	Inhibitory effects on proapoptotic and cardioregulatory genes. Modulating apoptotic regulatory bcl-XL, p53 and c-myc genes	Bagchi *et al*., (2003)
Superoxide dismutase (SOD)	Neurodegenerative diseases	Catalyse the one-electron dismutation of superoxide into hydrogen peroxide and oxygen	Fridovich, (1997)
Alkaloids	Cancer, Neurodegenerative diseases, chronic inflammation	Shoen a variety of biological activities such as inhibition of topoisomerase I and II:cytotoxicity against different tumour cell lines	Gunasekera *et al*., (2003) and Radisky *et al*. (1993)
Catechins	Neurodegenerative diseases	Enhance activity of SOD and catalase	Levites *et al*., (2001)
Carotenoids	Cancer, diabetic retinopathy, chronic inflammation	Mainly act as physical quenchers of reactive oxygen	Sundquist *et al*., (1994)
α -tocopherol	Cancer, Neurodegenerative diseases, chronic inflammation	Scavenges lipid peroxyl radicals(LOO) through hydrogen atom transfer	Burton & Ingold, (1981)
(-)·EGCG	Neurodegenerative conditions	Decreases the expression of proapototic genes (bax, bad, caspase-1 and-6, cyclin dependent kinase inhibitor) thus maintaining the integrity of the mitochondrial membrane	Levites *et al*., (2003)
(-)·EGCG	Cancer, diabetic retinopathy, chronic inflammation	Supression of angiogenesis by inhibiting growth factor triggered activation of receptors and PKC. Downregulation of VEGF production in tumour cells. Repression of AP-1, NF-_B and STAT-1 transcription factor pathways	Wollin & Jones, (2001)
Ferulic acid	Diabetes	Decreases lipid peroxidation and enhances the level of glutathione and antioxidant enzymes	Balasubashini *et al*., (2004)
Tannins	Cardiovascular disorders	Tannins are known to enhance synthesis of nitric oxide and relax vascular segments precontracted with norepinephrine	Dwivedi, (2007)

Table 2.2, continued

Glutathione	Cancer	Glutathione in the nucleus maintainsthe redox state of critical protein sulphydryls that are necessary for DNArepair and expression	Gerard Monnier & Chaudierre, (1996)
Glutathione peroxidase (GPx)	Neurodegenerative diseases	Catalyse the reduction of hydroperoxides at the expense of GSH. In this process, hydrogen peroxide is reduced to water whereas organic hydroperoxides are reduced to alcohols	Ursini *et al*., (1995)
Phenolics	Cancer, diabetic retinopathy, chronic inflammation	Inhibit the oxidation of lipids, fats, and proteins (RH) by donation of a phenolic hydrogen atom to the free radical	Aruoma *et al*., (1993)
Quercetin, Kaempferol, genistein, resveratrol	Colon cancer	Suppresses COX-2 expression by inhibiting tyrosine kinases important for induction of COX-2 gene expression	Lee *et al*., (1998)

2.6 Selected Local Vegetables

2.6.1 *Allium* tuberosum (Garlic chives)

Garlic chive is an allium grown for its leaves, and not its little bulb. The tough, fibrous bulb is elongate and originates from a stout rhizome (underground stem). The gray-green leaves are flat and grasslike, to 15 inch (38 cm) long, and about 0.3 inch (0.8 cm) wide. The plant grows in a clump and the leaves bend down under their own weight. The showy inflorescence stands above the leaf clump on 1-2 ft (0.3-0.6 m) stalks and consists of a rounded umbel, 2 inch (5 cm) across, with many small creamy white, star-shaped, fragrant flowers (Figure 2.3). Each perianth segment (petal and sepal) has a brown stripe. The unique flavor of garlic chives is both sweet and garlicky. Several cultivars are available in Asia, including some grown for the flower stalks, which are also eaten.

Figure 2.3: Flowering garlic chives

The cultivated form is *Allium tuberosum* while the wild form is placed as *A. ramosum*. Older references listed it as *A. odorum* but that is now considered a synonym of *A. ramosum*. Some botanists would place both wild and cultivated forms in *A. ramosum* since many intermediate forms exist. Naturally occurring sulphur-containing compounds present in the Allium family may influence plasma cholesterol and atherosclerosis (Banerjee & Maulik, 2002). These substances are found especially in garlic, onion and leeks, the most prominent of these being garlic (Block, 1985). The garlic preparations showed significant reduction on serum cholesterol levels in clinical (Andrianova, Demidova, & Medvedeva, 2004) as well as experimental studies (Chetty, Calahan, & Harris, 2003).

Usage

Garlic chive spreads by rhizomes and by self-seeding, and makes an excellent ground cover or edging plant. Garlic chive is equally at home in the herb garden, the vegetable garden, a flower bed, or as an edging along a mixed border or along a path. It takes the heat better than true chives (*Allium schoenoprasum*). Whether grown as an ornamental

or for food, garlic chives is usually treated as a semi-permanent crop, and left in place for several years.

The flavour is useful in salads, stir fries and soups. It goes well in egg dishes and with fish.The flavour is best in winter, especially after a few frosts. Younger leaves are tenderer than older ones. In China, garlic chives is usually cooked as a vegetable potherb rather than used as flavouring in other dishes. The Chinese often blanch alternate crops of garlic chives. Blanching causes the garlic chives to yellow and gives them a softer texture. The flowers smell like violets and are well suited for use in both fresh and dried arrangements. In Japan and China, the flowers are dried and ground to make a flavoring spice.

Allium sativum has been reported to exhibit beneficial effects in atherosclerosis and ischemic heart disease in experimental animals and human beings (Bordia & Verma, 1980; Arora & Gupta, 1981). Most commonly used species of garlic in India is *Allium sativum*, while *Allium tuberosum* species is mainly consumed and cultivated in South-east Asia, China and North-east part of India. Since these plants form the constituent of customary diet in Indian and Chinese food, their chronic ingestion is safe and is expected to be of benefit largely in persons prone to hyperlipidemia and atherosclerosis.

2.6.2 *Apium graveolens* (Celery)

Apium graveolens is a plant species in the family Apiaceae commonly known as celery or celeriac depending on whether the petioles (stalks) or roots are eaten. Celery was described by Carolus Linnaeus in Volume One of his Species Plantarum in 1753. The

closely related *Apium bermejoi* from the island of Minorca is one of the rarest plants in Europe.

The plants are raised from seeds, sown either in a hot bed or in the open garden according to the season of the year, and after one or two thinnings out and transplantings they are, on attaining a height of 15-20 cm, planted out in deep trenches for convenience of blanching, which is affected by earthing up to exclude light from the stems. *Apium graveolens* grows to 1 m tall. The leaves are pinnate to bipinnate leaves with rhombic leaflets 3-6 cm long and 2-4 cm broad. The flowers are creamy-white, 2-3 mm diameter, produced in dense compound umbels. The seeds are broad ovoid to globose, 1.5-2 mm long and wide. The wild form of celery is known as smallage. It has a furrowed stalk with wedge-shaped leaves, the whole plant having a coarse, earthy taste, and a distinctive smell.

Usage

Celery (*Apium graveolens*) is commonly used in making salads, spicing up foods containing meat, etc. However, the roots, leaves and celery seeds are used for therapeutic purposes in treating and preventing diseases. *Apium graveolens* initially grew on humid grounds of Europe and Asia. With the start of the 19th century, celery began to be grown in United States. Nowadays this vegetable is more commonly used in foods, due to the richness of its taste.

The stalks are not usually eaten (except in soups or stews in French cuisine), but the leaves may be used in salads, and its seeds are sold as a spice. With cultivation and blanching, the stalks lose their acidic qualities and assume the mild, sweetish, aromatic taste particular to celery as a salad plant.

In the past, celery was grown as a vegetable for winter and early spring; it was perceived as a cleansing tonic, welcomed to counter the salt-sickness of a winter diet. By the 19th century the season for celery had been extended, to last from the beginning of September to late April.

In traditional medicine celery was used to eliminate intestinal parasites. Chinese medicine recommended celery to be used as a medicine to treat high blood pressure due to its stabilizing components. In medicine, celery has been used as far back as Ancient Greece when it was highly valued for its properties as an aphrodisiac as well as its medicinal properties. It is also useful in stomach illnesses, rheumatism, obesity and diseases of the urinary bladder; celery keeps its therapeutic properties the best when it is consumed raw. Either used as a snack or as medicine, celery detoxifies the body, stimulates the nervous system and mineralizes the body.

Celery has the ideal quantities of iron and magnesium to stop oncological diseases from progressing. This plant has diuretic properties and dichloridic effects which are useful in renal afflictions (like renal colic and renal lithiasis) and heart disorders. In this purpose celery root is used. Due to its depurative properties, the consumption of celery is recommended for individuals who have a fast paced lifestyle and are unable to maintain a healthy diet. Because of these properties - diuretic and depurative - celery is the ideal ingredient for diets based on weight-loss. Diets based on celery have energizing and fortifying effects on the body due to the stimulation of the renal glands, reduction of the stress hormone and decreasing of the heart rate. But celery also has hypoglycemic properties, which means it can be used in treating sugary diabetes. Other properties: expectorant, emollient (effective against bronchitis), it adjusts hormonal

dysfunctions (dysmenorrhea, infertility, disorders caused by menopause, various forms of acne, allergic dermatosis); cicatrizing (good for wounds, scratches, ulcerations).

2.6.3 *Ipomoea batatas* (Sweet potato leaves)

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant which belongs to the family Convolvulaceae. Amongst the approximately 50 genera and more than 1,000 species of this family, only *I. batatas* is a crop plant whose large, starchy, sweet tasting tuberous roots are an important root vegetable (Purseglove, 1991; Woolfe, 1992). The young leaves and shoots (Figure 2.4) eaten as greens. The sweet potato is only distantly related to the potato (*Solanum tuberosum*). It is commonly confused with a yam in parts of North America, although it is not even distantly related to the yam (in the *Dioscoreaceae* family), which is native to Africa and Asia.

Figure 2.4: Young leaves and shoots of sweet potato

The genus Ipomoea that contains the sweet potato also includes several garden flowers called morning glories, though that term is not usually extended to *Ipomoea batatas*. Some cultivars of *Ipomoea batatas* are grown as ornamental plants.

This plant is an herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between red, purple, brown and white. Its flesh ranges from white through yellow, orange, and purple.

Besides simple starches, sweet potatoes are rich in complex carbohydrates, dietary fiber, beta carotene (a vitamin A equivalent nutrient), vitamin C, and vitamin B6. A 100 g root is reported to contain from 108 to 121 calories, 68.5 g to 72.3 g water, 1 g to 1.7 g protein, 0.2 g to 0.4 g fat, 25 g to 31.0 g total carbohydrate, 0.7 g to 1.0 g ash, 21 mg to 36 mg Ca., 38 mg to 56 mg P, 0.7 mg to 2.0 mg Fe, 10 mg to 36 mg Na, 210 mg to 304 mg K, 35 µg to 5,280 µg beta-carotene equivalent, 0.09 mg to 0.14 mg thiamine, 0.04 mg to 0.06 mg riboflavin, 0.6 mg to 0.7 mg niacin, and 21 mg to 37 mg ascorbic acid.

The peptic substance (total, 0.78; soluble, 0.43 percent) present in fresh tubers contain: uronic acid, 60%; and methoxyl, 4-5%. Other constituents in the tubers: phytin (1.05%), two mono-amino-phosphatides (probably lecithin and cephalin), organic acids (oxalic acid), phytosterolin, phytosterol, resins, tannins, and coloring matter. Sweet potato contanins calcium, 30; magnesium, 24; potassium, 373; sodium, 13; phosphorus, 49; chlorine, 85; sulphur, 26; iron 0.8 mg/ 100 g; iodine, 4.5 µg/kg; manganese, copper and zinc are present in traces (Hug *et al*., 1983).

Usage

In South America, the juice of red sweet potatoes is combined with lime juice to make a dye for cloth. By varying the proportions of the juices, every shade from pink to purple to black can be obtained. All parts of the plant are used for animal fodder. Sweet potatoes or camotes are often found in Moche ceramics.

Several selections are cultivated in gardens as ornamental plants for their attractive foliage, including the dark-leafed cultivars 'Blackie' and 'Ace of Spades' and the chartreuse-foliaged 'Margarita'. Taiwanese companies are making alcohol fuel from sweet potato. In Malaysia, the leaves of sweet potatoes are usually stir-fried with only garlic or with “sambal belacan” and dried shrimp by the Malaysian Chinese.

2.6.4 *Murraya koenigii* (Curry leaves)

The curry tree is a tropical to sub-tropical tree in the family Rutaceae, which is native to India. It produces the leaves known as curry leaves (Figure 2.5) or sweet neem leaves.

Figure 2.5: Curry Leaves

It is a small tree, growing 4-6m tall, with a trunk up to 40 cm diameter. The leaves are pinnate, with 11-21 leaflets, each leaflet 2-4 cm long and 1-2 cm broad. They are highly aromatic. The flowers are small white, and fragrant. The small black, shiny berries are edible, but their seeds are poisonous. The species name commemorates the botanist Johann König. The botanical name *M. koenigii* refers to two 18th century botanists: a Swede, Johann Andreas Murray (1740-1791) and a German, Johann Gerhard Konig (1728-1785) (Seidemann, 2005).

The shrub is of common occurrence in Himachal Pradesh in areas lying between 800 and 1,450 metres above the sea level. Almost every part of this plant has a strong characteristic odour. The people of the plains, particularly of southern India, use the leaves of this plant as a spice in different curry preparations.

Curry tree is commonly found in the outer Himalayas, from the Ravi eastwards, ascending to 5,000 feet, in Assam, Chittagong, North and South Burma. It is also found in evergreen and deciduous forests of peninsular India, often as underwood (Brandis, 1906).

Usage

The leaves of *Murraya koenigii* are used as an herb in Ayurvedic medicine. Their properties include much value as an antidiabetic (Arulselvan, Senthilkumar, Sathish Kumar & Subramanian, 2006), antioxidant (Arulselvan & Subramanian, 2007), antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc. Curry leaves are also known to be good for hair, for keeping them healthy and long.

The leaves, the bark and the roots of *Murraya koenigii* can be used as a tonic and a stomachic. The bark and the roots are used as a stimulant. They are also used externally to cure eruptions and the bites of poisonous animals. The leaves are highly valued as seasoning in South Indian and Sri Lankan cooking, much like bay leaves and especially in curries with fish or coconut milk. In their fresh form, they have a short shelf life though they may be stored in a freezer for quite some time; however, this can result in a loss of their flavour. They are also available dried, though the aroma is much inferior.

The green leaves are stated to be eaten raw for curing dysentery, and the infusion of the washed leaves stops vomiting (Dastur, 1962; Kirtikar & Basu, 1935; Watt, 1891). The leaves are also used to flavour a range of dishes and typically these are fried in oil until crisp to impart flavour to all types of curry preparations. Fresh leaves release strong aroma while cooking. However, in cities, its dry powder is also used. The plant has also been used in traditional Indian medicine systems for a variety of ailments (Chevallier, 1996; Sivarajan & Balachandran, 1996).

The oil derived from the curry leaves is also used in the perfume and soap industry. The chemical examination of this oil has been made by Nigam and Purobit (1961). Gautam and Purobit (1974) reported that this essential oil exhibited a strong antibacterial and antifungal activity. Fresh juice of curry leaves, mixed with lime juice and sugar, cures morning sickness, nausea and vomiting due to indigestion. A glass of buttermilk with a pinch of salt and a spoonful of ground curry-leaf paste, taken on an empty stomach, relieves stomachache. Chewing the tender leaves helps control loose motions whereas fully grown curry leaves are beneficial in controlling diabetes and weight loss. Leaves cooked in milk and ground to a paste, when applied to poisonous insect bites and other wounds and cuts, relieve pain and swelling. Leaves ground with turmeric, and taken daily, are an effective remedy for allergic reactions. Also their paste, applied on the foot, prevents cracking.

Curry leaves and black pepper beaten with sour curd are beneficial for indigestion (Brahmananda, 2000). Leaves boiled in coconut oil are often used as a hair tonic for stimulating hair growth and retaining natural pigmentation. Several workers have extensively evaluated the aqueous extract of *M. koenigii* leaves for their hypoglycemic activity without any side effects or toxicity (Kesari *et al*., 2005; Math & Balasubramaniam, 2005; Santhakumari *et al*., 1985; Vinuthan, *et al*., 2004). The leaves

have been shown to considerably reduce blood sugar levels, where Cr, V, Mn, Zn, Cu and Se are known to play an important role in biochemical processes and especially in diabetics. Ramsewak, Nair, Strasburg, DeWitt, and Nitiss (1999) isolated three bioactive carbazole alkaloids, mahanimbine, murrayanol and mahanine, having antimicrobial and mosquitocidal activities. Dasgupta, Rao, and Yadava (2003) studied the anticarcinogenic potential of the curry-leaf extract and suggested its use for the prevention of stomach and skin cancers. Tachibana, Kikuzaki, Lajis, and Nakatani (2003) evaluated antioxidative properties of 12 carbazole alkaloids.

Organic constituents of Curry Leaves

Most studies on curry leaves reported in the literature pertain to the organic constituents, namely essential oils, coumarins, terpenoids and carbazole alkaloids, known for their antioxidant properties. Wang *et al.* (2003) isolated two carbazole alkaloids, murrayanine and 8, 8"-biskoenigine, by oxidative coupling, using a solid-state reaction. Sukari *et al*. (2001) extracted murrayazoline and murrayacine from the $CHCl_3$ extract and elucidated their structure using high-resolution NMR (1H NMR, 13C NMR, HMQC and HMBC), IR and mass spectrometry. Chowdhury *et al*., (2001) isolated two alkaloids (1-formyl-3-methoxy-6-methylcarbazole and 6,7-dimethoxy-1-hydroxy-3-methylcarbazole). Adebajo and Reisch (2000) reported eight furocoumarins (xanthotoxin, isobyakangelicol, phellopterin, gosferol, neobyakangelicol, byakangelicol, byakangelicin and isogosferol) and b-sitosterol from *M. koenigii* seeds. Palaniswamy *et al*. (2003) determined the levels of the antioxidant vitamins, a-tocopherol, b-carotene and lutein, in fresh curry leaves by using reversed phase gradient HPLC. Leaves are rich in minerals, vitamins A and B, and are a rich source of carbohydrates, proteins, amino acids and alkaloids (Kong *et al.*, 1986; Tee & Lim, 1991).

Earlier, it has been reported three novel organic compounds, 3-methylthiopropanenitrile, 1,2-benzenedicarboxylic acid, mono (2 ethylhexyl) ester and 1-pentene-3-ol, in the ethanolic extract of curry leaves (Choudhury, Jain, & Garg, 2006). Only scanty reports are available on the minor and trace element composition which plays a significant role in biochemical and enzymatic processes (Herber & Stoeppler, 1994; Kariyanna, 2003). These suggested dark and light green-coloured leaves as an indicator for high Mn and Fe contents, respectively. Gopalan *et al.* (1999) compiled eight essential elements, along with organic compounds, in curry leaves, which play a vital role in human metabolism. Narendhirakannan, Subramanium, and Kandaswamy (2005) analyzed the elemental composition in the leaves of *M. koenigii*, widely used in the treatment of diabetesrelated metabolic disorders, by using atomic absorption spectrophotometry (AAS). Ray, Nayak, Rautray, Vijayan, and Jena (2004) determined K, Ca, Fe, Cr, Mn, Cu, Zn, Rb, Sr and Pb using an energy dispersive X-ray fluorescence technique (EDXRF). Rajurkar and Pardeshi (1997) reported essential and trace elements in curry leaves by NAA and AAS. NAA has been widely employed for determining 10-20 elements in curry leaves from Tirupati in Andhra Pradesh (Balaji *et al.,* 2000), Nagpur in central India (Singh & Garg, 2006) and other parts of the country (Choudhury *et al.*, 2006). An alkaloid, murrayacinine, is also found in this plant (Chakraborty *et al.,* 1974).

2.6.5 *Psophocarpus tetragonolobus* (Winged bean)

The winged bean (*Psophocarpus tetragonolobus*) also known as the goa bean (kacang botol in Malaysia) and asparagus pea and winged pea (*Lotus tetragonolobus*), is a tropical legume plant native to Papua New Guinea. It grows abundantly in hot, humid equatorial countries, from the Philippines and Indonesia to India, Burma, Thailand and Sri Lanka. It does well in humidtropics with abundant rainfall. There are also varieties

than can be grown in most areas of the United States. The plant is one of the best nitrogen fixers with nodulation accomplished by the soil bacterium Rhizobium. Because of its ability to fix nitrogen from the atmosphere, the plant requires very little or no fertilizers (Venketeswaran *et al.,* 1990).

Figure 2.6: Winged beans

The winged bean plant grows as a vine with climbing stems and leaves, 3-4 m in height. It is an herbaceous perennial, but can be grown as an annual. It is generally taller and notably larger than the common bean. The bean pod is typically 15-22 cm (6-9 inch) long and has four wings with frilly edges running lengthwise (Figure 2.6). The skin is waxy and the flesh partially translucent in the young pods. When the pod is fully ripe, it turns an ash-brown color and splits open to release the seeds. The large flower is a pale blue. The beans themselves are similar to soybeans in both use and nutritional content (being 29.8% to 39% protein). Being a tropical plant, it is sensitive to frost. It will not flower if day length is less than 12 hours. The seeds have a hard coat and it helps to presoak the seeds before planting to hasten germination. The plant grows very quickly, reaching a length of four meters in a few weeks. (Wilson *et al*., 1992).

Usage

The leaves, flowers, pods, green seeds, dried seeds, and (in some varieties) tuberous roots of winged bean are all edible and nutritious. In Indonesia, “tempeh” and tofu are made from winged bean seeds. A winged bean "milk" and flour are used as dietary treatments for protein-deprived children. Winged bean stems and leaves are used as cattle forage. Being a powerful nitrogen-fixing legume, and it is used as a “green manure” for intercropping with bananas, sugarcane, taro, and other tropical crops. The tender young shoots and leaves of winged bean may be eaten raw or cooked as green vegetables. Add young stems and leaves to soups and curries. The half-ripe seeds can be removed from the pod and cooked like peas or kidney beans. The flowers can be eaten raw, fried or steamed. Tubers should be dug at the end of the season. Winged beans are air dried for a few days and peeled before cooking. Roasting is said to be the best way to prepare the tubers (http://www.floridata.com/ref/p/psop_tet.cfm).

2.6.6 *Sauropus androgynus* (Sweet leaves)

Sauropus androgynus, also known as katuk, star gooseberry, or sweet leaf (Figure 2.7), is a shrub grown in some tropical regions as a leaf vegetable. Its multiple upright stems can reach 2.5 m high and bear dark green oval leaves 5-6 cm long.

Figure 2.7: Sweet leaves

The species is highly mycorrhizal-dependent, is adapted to acid soils and will grow in heavy clay soils. The only report of pests or disease is some damage overseas from the Chinese rose beetle (*Adoretus sinicus*; http://agrss.sherman.hawaii.edu/onfarm /veg/veg0000b.html). In its natural state as an under-storey plant in lowland rainforest, Sauropus grows to 6 m; when grown as a vegetable crop it requires regular pruning to 1-2 m tall for best results (http://www.newcrops.uq.edu.au/newslett/ncnl9191.htm).

Usage

It is one of the most popular leaf vegetables in South Asia and Southeast Asia and is notable for high yields and palatability. The shoot tips have been sold as tropical asparagus. In Vietnam, people cook it with crab meat, minced pork or dried shrimp to make soup. In Malaysia, it is commonly stir-fried with egg and dried anchovies. (Kanchanapoom *et al.*, 2003; Padmavathi & Rao, 1990).

It is among only a few floras containing vitamin K. However, studies have suggested that its consumption can cause lung damage, due to its high concentrations of the alkaloid papaverine.The leaves and the top 15 cm of stem tips of the Sauropus plant have a pleasant taste, similar to fresh garden peas, and slightly nutty and are normally

eaten raw in salads or steamed, to add to stir-fry, rice and egg dishes, soups or casseroles. The leaves retain their dark green colour and firm texture on cooking and are served in restaurants as "sayur manis". The flowers and small purplish fruits of the plant have also been eaten.

Sauropus has a high level of provitamin A carotenoids, especially in freshly picked leaves, as well as high levels of vitamins B and C, protein and minerals. Nutrient content of the leaves is usually higher in more mature leaves.The crop grows rapidly in hot humid conditions but becomes relatively dormant in cooler environments. Farmers in Malaysia force the growth of stem tips by fertilisation, irrigation and the use of shade cloth. Plants are usually propagated vegetatively, since the plant grows readily from cuttings. Seed longevity is poor; seeds remain viable for only a few months (Veronica, 2006).

Sauropus became a popular ingredient of an unconfirmed weight control method in Taiwan in 1995 and several cases of poisoning were reported. The most common form of consumption with the weight control method was as an extract, with fruit juice. Rapidly progressive obstructive lung disease resulted, persisting up to forty days after the method ceased. Those consuming high levels of Sauropus appeared to be worst affected, especially those consuming the plant as the uncooked extract.

The poisoning was believed to have been associated with the alkaloid papaverine but this compound had not previously been associated with this level of toxicity. Sauropus extract has been found to have a very strong activity against *Bursaphelenchus xylophilus* (pine wood nematodes).