CHAPTER – 1

INTRODUCTION

1.1 Medicinal Plants

Since time immortal, one of the most important sources of medicine is plant flora. Compounds derived from plants i.e., phyto-chemicals have been attracting much interest of the researchers as natural alternatives to synthetic compounds. The extracts of plants are used for the treatment of various diseases and this is the basis for all Indian systems of medicine. However, alternate medicines are much developed when compared to modern system of medicine, mainly because of the lack of proper scientific knowledge in this area (Kalimathu, 2010).

The pharmacological activity of medicinal plants lies in its secondary metabolites which are comparatively smaller molecules than the primary molecules such as proteins, carbohydrates and lipids. The main advantage of plant products over the synthetic compounds in the treatment of diseases is that it will not have a deleterious effect on animals including man (Krishnakumar *et. al.*, 1997) as well as they are effective. The traditional Indian herbal medicine was used for strengthening the immune system of the body and is also known to have many essential and nutritional elements. The medicinal properties of plant are due to their secondary metabolites, which are essential oils, glycosides, vitamins, alkaloids and other active components (Sen and Batra, 2012).

According to Sofowora (1993)

"A medicinal plant is described as any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs."

According to World Health Organization (WHO) the plant that possesses therapeutic property or exerts beneficial pharmacological effect on the animal body is generally designated as Medicinal Plant (Kumar *et. al.,* 2012). It has now been established that the plants which can naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess

medicinal properties. Secondary metabolites play an important role in plant defense and other interspecies defense and are used by humans as medicines, flavorings and recreational drugs. The secondary metabolites are classified into big/small molecules, small molecules, and nano molecules (Agostini-Costa *et. al.*, 2012).

The Indian sub-continent comprises of a unique and massive diversity of flora and fauna within a relatively small geographical area due to variations in topography, altitude and climate. In spite of being a diverse country, India is known to be veritable treasure trove of medicinal plants (Phoboo *et. al.*, 2008). More than 3000 plants species are known to have medicinal properties in India (Prakasha *et. al.*, 2010). Most of the wild plants of India are rich in medicinal and aromatic properties like anti-bacterial, anti-viral, anti-helminitic, anti-cancer, sedative, laxative, cardio-tonic, diuretic and others. Such plants are important sources of bio-molecules, with application for the manufacture of pharmaceuticals and cosmeceuticals (Heinrich and Gibbons, 2001).

Humans have used medicinal plants in health care since pre-Vedic time. These are also the major sources of medicines for different types of ailments for the people living in rural India. Approximately 65% of total population of India still depends on the local herbal plants as a source of traditional medicine (Prashantkumar and Vidyasagar, 2008).

India is a land of rich biodiversity. The total number of lower and higher plants reported in India is about 45,000 species (Jain, 1992). Approximately 80 percent population of the developing countries still relies on the folk and traditional medicinal system (Gangwar and Joshi, 2009). According to World Health Organization, 80% of the populations in the world depend on traditional medical practitioners for their medicinal needs (Farnsworth, 1985). The major factors for continuous and extensive use of herbal medicines in rural India are easy availability of herbs, low cost, less toxic effects and shortage of practitioners of modern medicine (Agarwal and Paridhavi, 2012). Many formulations of plants and their products such as medicines are addressed in the form of hymns in the Vedas (Kausik and Dhiman, 1997). Whereas the scientific study of plants to determine their anti-oxidant activity is comparatively new. Numerous surveys on anti-oxidant medicinal plants had been made in many other developed and developing countries throughout the world.

As the plants provides an extraordinary reservoir for exploration of new drugs for various disease. The present study has been directed to find out the anti-oxidant potential in the extracts of six selected plants, viz. *Delonix regia, Lallemantia royleana, Phyllanthus maderaspatensis, Plantago ovata, Rosa indica* and *Solanum nigrum* that are commonly found in India. Some plant originated drugs in conventional medical practice are not pure compounds but are the extracts or plant materials that have been suitably prepared and standardized.

1.2 Free Radicals

Free radicals, due to the presence of an unpaired electron, are the electrically charged molecules (Agarwal *et. al.,* 2006). Due to the occurrence of these charged molecules they have the potential to capture electrons from other substances in order to neutralize them. Although the initial attack causes the free radical to become neutral, and another free radical is formed in the process, causing a cascade reaction to occur, until ensuing the free radicals are deactivated, thus thousands of free radical reactions can occur within seconds of the initial reaction. The anti-oxidants are capable of stabilizing, or deactivating, free radicals and in the process they attack cells and also are absolutely critical for maintaining optimal cellular and systemic health and well-being of an individual (Percival, 1998).

The free radicals biochemistry study started during the World War II (1939-1945). The two atom bombs dropped at Hiroshima and Nagasaki led to massive deaths of the entire population, and the people who survived had shortened life-span. Gerschman and Gilbert (1954) found that the lethal effects of ionizing radiation would have been due to the formation of ROS (reactive oxygen species). Since then free radicals (atoms with an unpaired electron) such as ROS and reactive nitrogen species (RNS) have gained much importance (Gilbert *et. al.,* 1981). In general, free radicals have very short half-lives of milliseconds, microseconds or nanoseconds. Some of the biologically important reactive oxygen species are shown in Table 1.1 whereas some reactive nitrogen species are listed in Table 1.2.

Reactive species	Symbol	Half life (in sec)	Reactivity / Remarks
Reactive oxygen species :			
Superoxide	$O^{2\bullet -}$	10^{-6} s	Generated in mitochondria, in cardiovascular system and others
Hydroxyl radical	$^{\bullet}OH$	10^{-9} s	Very highly reactive, generated during iron overload and such conditions in our body
Hydrogen peroxide	H_2O_2	Stable	Formed in our body by large number of reactions and yields potent species like. •OH
Peroxyl radical	$ROO^{\bullet}$	S	Reactive and formed from lipids, proteins, DNA, sugars etc. during oxidative damage
Organic hydrogenperoxide	ROOH	Stable	Reacts with transient metal ions to yield reactive species
Singlet oxygen	$^1O_2$	10^{-6} s	Highly reactive, formed during photosensitization and chemical reactions
Ozone	O_3	S	Present as an atmospheric pollutant, can react with various molecules, yielding $^1O_2$

Table No. 1.1: Some Reactive Oxygen Species with their Symbols and Half Life (Source- Devasagayam *et. al.*, 2004)

Reactive species	Symbol	Half life (in sec)	Reactivity / Remarks
Reactive nitrogen species:			
Nitric oxide	$NO^{\bullet}$	S	Neurotransmitter and blood pressure regulator, can yield potent oxidants during pathological states
Peroxynitrite	$ONOO^-$	10^{-3} s	Formed from NO and superoxide, highly reactive
Peroxynitrous acid	ONOOH	Fairly stable	Protonated form of $ONOO^-$
Nitrogen dioxide	NO_2	S	Formed during atmospheric pollution

Table No. 1.2: Some Reactive Nitrogen Species with their Symbol and Half Life (Source- Devasagayam *et. al.*, 2004).

Free radicals have been associated in the etiology of several human diseases, and in some reactive nitrogen species are shown in ageing too (Harman, 1956 and Halliwell and Gutteridge, 1997). But it should be emphasized that ROS and RNS are both produced in a human body in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and plays important roles like signaling molecules. Most of the cells can also produce superoxide ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO). The important roles of free radicals are as follows:

1. It generates ATP (universal energy currency) from ADP in the mitochondria (oxidative phosphorylation).
2. It detoxify xenobiotics by Cytochrome P450 (oxidizing enzymes)
3. It also takes part in the process of apoptosis of defective cells.
4. It kills micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes
5. Oxygenases (example LOX: lipoxygenase and COX: cyclo-oxygenases) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

In the recent years, it has become very clear that the reactive oxygen species like $O_2^{\bullet-}$ and H_2O_2 may acts second messenger. Some good observations made by some researchers had showed that reactive oxygen species may play a role in modulating the cellular function in the human body. The studies have suggested that exogenous H_2O_2 imitate the action of the insulin growth factor. The discovery of redox-sensitive transcription factors and that $NO^{\bullet}$ ($NO^{\bullet}$ is a free radical produced benzymatically) plays a physiological role in neurotransmission and vasodilatation through activation of soluble granulated cyclase supporting the concept that ROS and RNS can act as second messengers to modulate signaling pathways. This led to the investigation of the redox signaling. The studies in this area have shown an emergence of the signaling pathways and specific targets affected by both reactive oxygen species and reactive nitrogen species (Yoshikawa *et. al.*, 2000 and Devasagayam *et. al.*, 2004).

Some other important sources of free radicals are redox cycling of xenobiotics, exposure to

physicochemical agents like ionizing radiations like X-rays and γ-rays besides visible light or ultra violet rays in the presence of oxygen and an endogenous compound or a drug that act as photosensitizer. Number of damage that induced by ionizing radiations in biological systems is indirect and is usually caused by products of radiolysis of water including hydrogen radical ($^{\bullet}H$), $^{\bullet}OH$, hydrated electron (e_{aq}-), H_2O_2, peroxyl radical ($ROO^{\bullet}$), $O_2^{\bullet-}$, $^1O_2$ etc. (Von Sonntag, 1987 and Devasagayam and Kesavan, 1996). Similarly the smoke from the cigarette contains a large quantity of reactive species as reported by Devasagayam and Kamat, (2002). Cigarette tar is rich source of quinine-hydroquinone-semiquinone system which reduces O_2 to form $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$, while the smoke of the cigarette contains small oxygen and carbon-centered radicals as well as active oxidants such as $NO^{\bullet}$ and nitrogen dioxide (NO_2). Recent studies by Wentworth *et. al.*, (2003) suggested that antibodies, regardless of origin or antigenic specificity, could convert $^1O_2$ into H_2O_2 through a process that they have postulated to involve dihydrogen trioxide (H_2O_3).

During the process of ischemia-reperfusion, oxidants like $O_2^{\bullet-}$, $^{\bullet}OH$ and H_2O_2 are produced. This occurs during stroke, surgeries, transplantation, non-fatal myocardial infarction, blockage of arteries under pathological conditions, etc. In myocyte mitochondria, during ischemia in the heart, conversion of ATP to adenosine causes the generation of $O_2^{\bullet-}$, while in the blood vessels (endothelium) the pathway involved is the transition from xanthine to uric acid (Yoshikawa *et. al.*, 2000 and Devasagayam *et. al.*, 2004).

1.2.1 Molecular damage induced by free radicals

All the biological molecules present in our body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and even lead to cell death eventually causing disease in the body.

1.2.1.1 Lipids and Lipid Peroxidation

Membrane lipids present in sub-cellular organelles are highly susceptible to free radical damage. Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. During LP a large number of toxic byproducts are also formed that can have effects at a site away from the area of generation, behaving as 'second messengers'. The damage caused by LP is highly detrimental for the functioning of the cell (Devasagayam *et. al.*, 2003).

Lipid peroxidation is a free radical mediated process. The process starts with the attack by any reactive species, which can abstract a hydrogen atom from a methylene group (CH_2), and convert it into a radical ($^{\bullet}CH$). To stabilized by molecular rearrangement, this radical produces a conjugated diene, which then can react with an O_2 to give a lipid peroxyl radical ($LOO^{\bullet}$). These radicals can further abstract hydrogen atoms from other lipid molecules to form lipid hydroperoxides (LOOH) and at the same time propagate LP further. The peroxidation reaction can be stopped by various chemical reactions. The reaction of $LOO^{\bullet}$ or lipid radical ($L^{\bullet}$) with a molecule of anti-oxidants are more important one, Anti-oxidant like vitamin E or α-tocopherol (α-TOH) is used as a reaction terminator as it forms a stable tocopherol phenoxyl radical which does not involved in further chain reactions. This can be recycled by other cellular anti-oxidants such as vitamin C or (glutathione) GSH.

$$LH + OH^{\bullet} \rightarrow L^{\bullet} + H_2O$$
$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$
$$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH$$
$$LOO^{\bullet} + \alpha\text{-}TOH \rightarrow LOOH + \alpha\text{-}TO^{\bullet}$$

The process of LP, gives rise to many products of toxicological interest like malondialdehyde (MDA), 4- hydroxynonenal (4-HNE) and various 2-alkenals. Isoprostanes are unique products of lipid peroxidation of arachidonic acid and recently tests such as mass spectrometry and ELISA-assay kits are available to detect isoprostanes (Yoshikawa *et. al.*, 2000).

1.2.1.2 Carbohydrates

The free radicals like $OH^{\bullet}$ reacts with carbohydrates by randomly abstracting an atom of hydrogen from one of the carbon atoms, producing a carbon-centered radical, which leads to the breaking of chain into important molecules like hyaluronic acid. The synovial fluid present around joints, an accumulation and activation of neutrophils during inflammation produces significant amounts of oxy-radicals, which is also being implicated in rheumatoid arthritis (Devasagayam *et. al.*, 2004).

1.2.1.3 DNA

The oxidative damage caused to DNA is a result of interaction of DNA with reactive oxygen species or reactive nitrogen species. Free radicals such as $OH^{\bullet}$ and $H^{\bullet}$ react with DNA by addition to bases or abstractions of hydrogen atoms from the sugar moiety. The C_4-C_5 double bond of pyrimidine is mostly sensitive to attack by $OH^{\bullet}$, generating a spectrum of oxidative pyrimidine damage products, like thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin and others. Similarly, the interaction of hydroxyl ion with purines will generate 8-hydroxydeoxyguanosine (8-OHdG), 8- hydroxydeoxyadenosine, formamidopyrimidines and another less characterized purine oxidative product. Numerous metaboic pathways repair DNA damage (Halliwell and Aruoma, 1993).

1.2.1.4 Proteins

Oxidation of proteins by reactive oxygen species or reactive nitrogen species can generate a range of stable as well as reactive products such as protein hydro-peroxides that can generate additional radicals particularly upon interaction with transition metal ions. Most oxidized proteins that are functionally inactive are removed rapidly, while some accumulates gradually with time and thus contribute to the damage related such as ageing as well as various other diseases. Lipofuscin, an aggregate of peroxidized lipids and proteins accumulates in lysosomes of aged cells and brain cells of patients with Alzheimer's disease (Stadtman, 1992).

1.3 Anti-oxidants

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for desiring energy. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radicals. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Ivanova and Ivanov, 2000).

Cell damage caused by free radicals are the major contributor for aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Sies *et. al.*, 1992). Thus, free radicals have been implicated in the pathogenesis of at least 50 diseases (Langseth, 1993 and Halliwell, 1994). But the free radical formation is controlled naturally by various beneficial compounds known as anti-oxidants. It is when the availability of anti-oxidants is limited that this damage can become cumulative and debilitating (Percival, 1998).

Anti-oxidants are substances that neutralize free radicals or their actions (Sies, 1996). Nature has endowed each cell with sufficient protective mechanisms against many harmful effects of free radicals, like superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding. The α-Tocopherol (vitamin E) is a vital nutrient, functioning as a chain-breaking anti-oxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also a part of the normal protecting mechanism. Vitamin C has been cited as being capable of regenerating Vitamin E (Sies *et. al.*, 1992). Some other non-enzymatic anti-oxidants consist of flavonoids, carotenoids and related polyphenols, α-lipoic acid, glutathione etc.

1.4 List of Anti-oxidants

1.4.1 Carotenoids

Plants are constantly being exposed to sunlight and ultraviolet rays. Therefore they produce strong anti-oxidants to protect their cells against being damage from ultraviolet radiation and other carcinogens present in environment. Carotenoids are potent group of anti-oxidants (Paiva and Russell, 1999). Out of these the most potent and beneficial ones are Alpha-carotene, Beta-carotene, Cryptoxanthin, Lycopene, Lutein and Zeaxathin.

1.4.2 Flavonoids

The flavonoids are potent anti-oxidant phyto-chemicals (Duthrie & Crozier, 2000). They form the water-soluble colours of fruits, vegetables, grains, leaves, and bark. Many forms of flavonoids are present, and different plants contain different amount of flavonoids some of these flavonoids may have fifty times more anti-oxidant

activity as compare to vitamins C and E Similarly, flavonoids from red grapes are found to be more than thousand times more potent than vitamin E in inhibiting oxidation of human LDL cholesterol some of the common flavonoids are Catechins, Reserveratrol and Proanthocyanidins. Studies have shown that favonoids have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity (Cody, *et. al.*, 1986; Kuhnau, 1976; Havsteen, 1983 and Middleton, 1984).

1.4.3 Isoflavones

The isoflavones are commonly found in soybeans and other legumes (Vincent and Fitzpatrick, 2000). In the body, they are converted to phyto-estrogens (plant estrogens). Phyto-estrogens can inhibit the growth of hormone-dependent cancers and other types of cancers (Kirk *et. al.*, 2001). In addition, they lower total cholesterol levels and protect against heart disease. Some of the common isoflavones are Genistein and Daidzein.

1.4.4 Vitamin A

The Vitamin A is a general term that refers to fat-soluble compounds that are similar in structure and biologic activity to retinol. Vitamin A also refers to dietary precursors of retinol (Groff, *et. al.*, 1995 and Ross, 1999). The precursors of Vitamin A (retinol) are the carotenoids.

1.4.5 Ascorbic acid

The ascorbic acid or vitamin C is a monosaccharide anti-oxidant found in animals as well as in plants. One of the main enzymes required to make ascorbic acid has been lost by mutation during human evolution (Smirnoff, 2001). It is directly obtained from the diet and is also a vitamin. Inside the cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins (Meister, 1994 and Wells *et. al.*, 1990).

Ascorbic acid is well-known as a reducing agent and can reduce, and thereby neutralize, ROS such as hydrogen peroxide (Padayatty *et. al.*, 2003). In addition to its direct anti-oxidant effects, ascorbic acid is also a substrate for the anti-oxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et. al.*, 2002).

1.4.6 Tocopherols and Tocotrienols (Vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with anti-oxidant properties (Herrera and Barbas, 2001) and the α-tocopherol has been studied most because of its bioavailability and the body preferentially absorbs and metabolizes this form of vitamin E (Brigelius-Flohe and Traber, 1999).

It has been claimed that the α-tocopherol form is the most important lipid-soluble anti-oxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007).

1.4.7 Minerals

All the minerals are anti-oxidants and they are acquired through the diet as none of them are produced by the body. Minerals are also important for the proper functioning of the body and also for the assimilation of vitamins. The most powerful minerals which are known as anti-oxidants are copper, manganese, selenium and zinc (Shirwaikar *et. al.*, 2004).

Zinc is required for the activity of approximately 300 different enzymes and also in the production of proteins (McCall *et. al.*, 2000), for the formation of bones (Hyun-Ju *et. al.*, 2010), healing of wounds and sores, regulation of ribosomal, ribonucleic acid synthesis and insulin and carbohydrate metabolism.

1.4.8 Coenzyme-Q10

This anti-oxidant is also known as ubiquinone, since it is present in every living cell (Morton *et. al.*, 1957). It provides the cells with energy to effectively carry out their functions. The levels of coenzyme Q10 fall with increasing age, and therefore it have an impact on the diseases and illnesses associated with age (Ames *et. al.*, 1993 and Burke *et. al.*, 2001). Infection, stress, and poor eating habits can also affect the body's ability to produce sufficient amounts of the enzyme.

1.4.9 Glutathione

Glutathione is produced in liver from amino acids. It defends cell throughout the body, and also helps in preventing cancer, especially of the liver. It also functions as an anti-inflammatory in the treatment of allergies and arthritis. Glutathione is predominantly found in fruits and vegetables. Interaction of glutathione and vitamin C removes the free radicals from the body (Jacob, 1995).

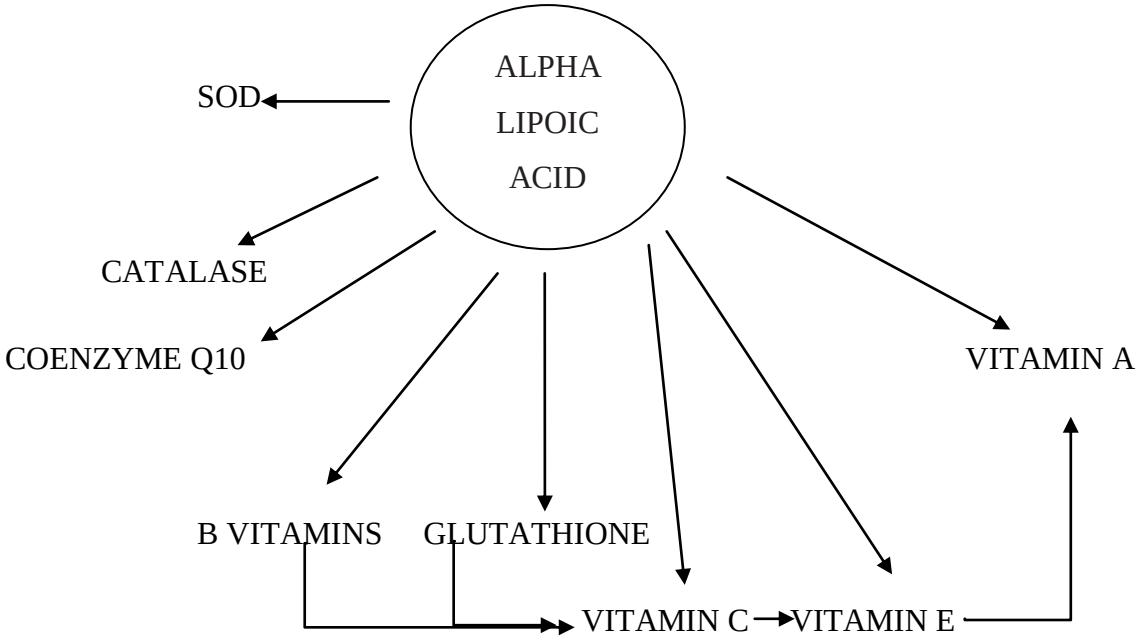

Figure No. 1: Alpha Lipoic Acid boosts the activities of other anti-oxidants

1.4.10 Lipoic acid

The lipoic acid is produced naturally by the body. It is proved to be a unique defender against free radicals, due to which it is sometimes referred to as a universal anti-oxidant. It also boosts the activities of other anti-oxidants in the body, as well as being an excellent anti-oxidant itself (Figure 1.1) (Packer and Witt, 1995 and Kagen *et. al.*, 1992). Lipoic acid also fulfils the deficiency of vitamins C or E when their levels are low (Packer and Witt, 1995).

1.4.11 Melatonin

The melatonin hormone is produced in the pineal gland of the brain during sleep and helps in maintaining the body's natural biorhythm. Exogenous melatonin helps in retardation of the aging process (Garcia *et. al.*, 2010; Ni *et. al.*, 2013 and Teran *et. al.*, 2012), especially by preventing the oxidative damage to brain cells that are associated with Alzheimer's and other conditions (Reiter *et. al.*, 2008), stroke and brain trauma (Pandi-Perumal, *et. al.*, 2013). Cluster headaches may be alleviated by melatonin, and it has the ability to enhance the immune system's ability to stop tumors from spreading. Melatonin is commonly found in tomatoes and other vegetables. Melatonin has also been found in grape products, such as dessert wines and balsamic vinegars (Vitalini *et. al.*, 2013).

1.5 Classification of Major Anti-oxidants

Anti-oxidants have been classified in different ways depending upon their role and also the colour they posses according to Gross (2006) as given below:

1.5.1 Enzymes (Body and Brown/Gray Foods)

Enzymes are protein in nature with a name ending with "ase". Usually found in cells, enzymes catalyze chemical reactions and cellular responses. Members of this class of anti-oxidants include: Superoxide dismutase, Catalases, Reductases, Peroxidases and Transferases.

1.5.2 Vitamins (Body and Brown/Gray Foods)

The three main anti-oxidant vitamins A, C and E required by humans also acts as anti-oxidants that derive from food and supplements. Vitamins A and E are fat-soluble therefore provides anti-oxidant protection in cell structures such as the outer membrane and inner nuclear organelles. Whereas vitamin C dissolves in body water and therefore they are universally distributed in the body. Vitamin C protects vitamin A and E from the damaging effect of oxygen radicals.

1.5.3 Phenolics (Blue, Red, Purple, Black - BRPB Foods)

More than 8,000 individual chemicals form phenolic group, serve plants as pigments. The phenolics are also called phenols or polyphenols. These are a water-soluble acid that not only give plants colors, but also contributes towards for its scents, tastes, and bitterness.

Among the phenolic groups, the large subclass is of flavonoids which often mentioned in current public media. Ellagic acid, quercetin, peonidin and kaempferol are examples of flavonoids that have been in the news recently.

1.5.4 Carotenoids (Orange/Yellow, Red Foods)

A group of more than 600 fat-soluble chemicals such as carotenoids (e.g., beta-carotene, lycopene, lutein and zeaxanthin are also powerful anti-oxidants. Due to their chemical structure having mostly double-bonds between carbon atoms, they are an excellent source of electrons which is required oxidative free radicals. A carotenoid molecule donates electrons to a free radical, sacrificing itself in anti-oxidant defense. Terpenes and xanthophylls are also included in this class.

1.5.5 Hormones (Body and Brown/Gray Foods)

Some recent research is identifying normal hormones which are required for cell-to-cell messaging roles in the body as having anti-oxidant functions. Presently, only a few hormones have been identified for this property such as melatonin, estradiol and insulin, but future research will likely unravel similar functions for the dozens of hormones known in human physiology.

1.5.6 Minerals (All Colors of Foods)

The minerals are adjunct elements that enhance the enzyme activity. Selenium, zinc, manganese, magnesium and copper are minerals and they are also involved in hundreds of anti-oxidant roles in the human body.

1.5.7 Glutathione (Body and Brown/Gray Foods)

Glutathione is known as the body's single most important native anti-oxidant, glutathione is a water-soluble molecule which is synthesized from food-derived amino acids. It depends on lipoic acid for its synthesis.

1.5.8 Lipid effectors (Orange/Yellow Foods)

The lipoic acid can also be named as the perfect anti-oxidant because it dissolves readily both in water and fatty layers of cells. It is small in size yet very powerful molecule. It is the only known anti-oxidant which can dissolve in both water and fat. Other lipid oriented anti-oxidants include tocopherols (like vitamin E), omega fatty acids, perillyl alcohol, phytosterols and essential oils such as limonene.

1.5.9 Saponins, steroids and stilbenes (Green, BRPB Foods)

Saponins, steroids and stilbenes are group of compounds which has established some anti-oxidant functions. Some of the very common chemicals of this group are brassinosteroid (the growth regulator of plants), resveratrol (a stilbene of red wine and dark grapes), and saponin (the waxy covering on plant leaves).

1.5.10 Sulfur-containing chemicals (Green, White Foods)

Sulfur-containing chemicals include triallyl and diallyl sulfides, organo-sulfides and sulforaphane. This group of chemicals from plants like cabbage and broccoli has been shown to have properties affecting anti-oxidant enzyme activity, inflammatory mediators and tumor growth.

1.6 Mechanism of Action of Anti-oxidants

According to Garewal (1997), anti-oxidant enzymes and anti-oxidant compounds show different mechanism of action as discussed below.

1.6.1 Anti-oxidant Enzymes

Enzymes which come under the category of anti-oxidant have the capacity to lower the free radical burden. Free radical reactions can be broken down into three stages

as initiation, propagation and termination. Anti-oxidant enzymes can affect the generation of free radicals during all these stages. The initiation phase of free radicals can be initiated by two metallo-enzymes such as super-oxide dismutase and catalase. They work by inactivating precursor molecules of free radicals, preventing the formation of ROS. Super-oxide dismutase is a Mn-containing metallo-enzyme in mitochondria and a Cu/Zn-containing metallo-enzyme in the cytoplasm. Catalase is a Fe-containing metalloenzyme in peroxisomes. It catalyses the decomposition of hydrogen peroxide, which is produced as a result of super-oxide dismutase.

Both the enzymes, super-oxide dismutase (SOD) and catalase, catalyze the reaction as seen the Table 1.4. While glutathione peroxidase which contains selenium also works as an anti-oxidant. Glutathione peroxidase is important for the decomposition of hydrogen peroxide and lipid peroxides and thereby works by interfering reaction leads to the free radical generation. Although Mn, Cu, Zn and Se are necessary components, they are only considered anti-oxidants when incorporated into their respective enzymes.

1.6.2 Anti-oxidant Compound

Vitamin E, vitamin C, and β-carotene act only by directly interfering with propagation of free radical generation. Apart from this the direct action of these nutrients, riboflavin, B vitamin, is the constituent of the enzyme glutathione reductase. Glutathione reductase is important for the regeneration of anti-oxidant defenses.

	Anti-oxidant	Role	Remarks
Enzymes	Superoxide dismutase (SOD) Mitochondrial Cytoplasmic Extracellular	Dismutates O_2^- to H_2O_2	Contains Manganese (Mn.SOD) Contains Copper & Zinc (CuZnSOD) Contains Copper (CuSOD)
	Catalase	Dismutates H_2O_2 to H_2O	Tetrameric hemoprotein present in peroxisomes
	Glutathione peroxidase (GSH.Px)	Removes H_2O_2 and lipid peroxides	Selenoproteins (contains Se^{2+}) Primarily in the cytosol also mitochondria Uses GSH
Vitamins	Alpha tocopherol	Breaks lipid peroxidation Lipid peroxide and O_2^- and OH scavenger	Fat soluble vitamin
	Beta carotene	Scavenges OH, O_2^- and peroxy radicals Prevents oxidation of vitamin A Binds to transition metals	Fat soluble vitamin
	Ascorbic acid	Directly scavenges O_2^-, OH, and H_2O_2 Neutralizes oxidants from stimulated neutrophils Contributes to regeneration of vitamin E	Water soluble vitamin

Table No. 1.3: Anti-oxidant Enzymes and Vitamins.
(Source-Deepa, 2013)

1.7 Aims and Objectives

1 Collection and preparation of different plant extracts.

2 Phyto-chemical screening of biologically most active plant extracts and fractions.

3 Evaluation of the anti-oxidant activity of the plant extracts using different assays.

4 Evaluation of the plant extract for their components using TLC and GC-MS.

Enzyme	Mineral	Reaction
Super-oxide dismutase(SOD)	CuZn,Mn, Fe(bacterial)	$2O^-_2 + 2H^+ \rightarrow O_2 + H_2O_2$
Glutathione peroxidase(GSHPx)	$Se^{(4)}$	$H_2O_2 + 2GSH \rightarrow GSSG + ROH + H_2O$
Phospholipid hydroperoxide glutathione peroxidase (PLGSHPx)	$Se^{(1)}$	$H_2O_2 + 2GSH \rightarrow GSSG + H_2O$ $ROOH + 2GSH \rightarrow GSSG + ROH + H_2O$ $PLOOH + 2GSH \rightarrow GSSG + PLOH + H_2O$
Catalase (CT)	Fe	$H_2O_2 \rightarrow H_2O + O_2$
Glutathione-S-transferase(GS-T)	None	$ROOH + 2GSH \rightarrow GSSG + ROH + H_2O$

Table No. 1.4: Mechanism of Action of Anti-oxidant Enzymes (Source-Garewal, 1997).

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