2.0 REVIEW OF LITERATURE

Consumption of medicinal herbs is tremendously increasing over a past decade as an alternative approach to improve the quality of life and maintain a good health. Medicinal plants have been used for centuries as remedies for human diseases. Extensive studies of the adverse effects of these herbal medicines and establishment of a good correlation between biomarkers and plants are essential for ensuring the efficiency and quality of herbal medicines. Plants based natural constituents can be derived from any part of the plant like stem bark, leaves, flowers, roots, fruits and seeds (Swarnalatha and Neelakanta, 2009).

The review of literature pertaining to the present study is discussed under the following headings:

2.1 Medicinal plants – A boon to mankind

2.2 Phytochemicals – Bioactive constituents

2.3 Oxidative stress / Nitrosative stress

2.4 Free radicals – The dangerous species

2.5 Free radicals induced damages/disorders

2.6 Antioxidants – The protective healers

- 2.6.1 Enzymatic antioxidants
- 2.6.2 Nonenzymatic antioxidants

2.7 Cytotoxic effect of the plant

2.8 Antibacterial activity of the plant

2.9 *Mukia maderaspatana* (L.)– A medicinal plant with multiple uses

2.1 MEDICINAL PLANTS – A boon to mankind

Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases leading to the dawn of traditional medicine (Pratchayasakul *et al.*, 2008). Many Indian plants have been used from time immemorial to treat various diseases and infections in traditional medicinal systems of India such as Siddha, Ayurvedha and Naturopathy (Muthuvelan and Raja, 2008).

A medicinal plant is any plant, which in one or more its organs contains active ingredients which can be used for therapeutic purposes or contain foundation compounds that can be used for the synthesis of useful drugs (David, 2010). Any substance naturally found in plants, animals, fungi, algae or microorganisms that is used to diagnose, treat or prevent disease and is suitable for self-care is categorized as a Natural health product (Walji *et al.*, 2010). Bioprospecting is the search of useful products derived from bioresources. The useful products may be chemical compounds, genes, micro and macro organisms and other valuable products that are useful in medicinal, industrial, agricultural and food sectors (Britto and Mahesh, 2007). Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principle natural source of medicines. The plants used as drugs are fairly innocuous and relatively free from toxic effects. The nature has provided the store house of remedies to cure all ailments of mankind. There is no doubt that plants are reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis (Sasmal *et al.*,2007).

Medicinal and aromatic plants play an important role in the healthcare of people around the world, especially in developing countries. Until the advent of modern medicine, man depended on plants for treating human and livestock diseases (Rao *et al.*, 2004). Plants have been used for medicinal purposes throughout human history and the first pharmaceuticals (that is quantified doses of medicinal

compounds as crude extracts of plant material) were derived from medicinal plants. Comparatively recently, the ability of chemists to synthesize purely artificial medicinal compounds lead to the production of many effective medicines. However as new diseases and drug resistant strains of existing pathogens continue to emerge; the potential of wholly synthesized compounds with simple structures and known modes of action is starting to diminish. As such, attention is again being focused on natural sources of lead compounds, in which exists a wealth of more complex compound structures and novel modes of action. It is the secondary metabolites of plants in biodiverse regions that invite the interest of pharmacologists seeking new lead compounds for medicines. Regions of high biodiversity contain an even greater chemodiversity and so harbour great potential for finding new compounds (McRae *et al.*, 2007).

Secondary metabolites obtained from plants are not benign molecules. Plants have evolved such chemical defenses in order to deter, stun, poison or kill threatening species. It would therefore be naive to assume that plant extracts are inevitably safe (Street *et al.*, 2008). It is proved that half of the world's best selling drugs and many potential drugs under development are derived from plants. This implies a tremendous demand for indigenous medicinal plants; therefore, these plants should be protected as a source of both food and medicine (www.inepo.com/english/uplfiles_resim/Testing_Plants_whether_they_cause_cancer .doc). Unfortunately, these locally important species are often neglected leading to the erosion of their diversity and usefulness, further restricting development options for the poorest. Research to increase the value of these species and to make them more widely available would broaden the agricultural resources and increase the livelihood options for rural communities (Ekue *et al.*, 2010).

2.2 PHYTOCHEMICALS – Bioactive constituents

Phytochemicals are a large group of plant derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Hundreds of phytochemical compounds, with several different biological functions, have been identified in plant based foods (Haneman and Cherr, 2007). In plants, phytochemicals act as a natural defense system for host plants and provide colour, aroma and flavour. More than 4000 of these compounds have been discovered to date and it is expected that scientists will discover many more. Phytochemicals are protective and disease-preventing particularly for some forms of cancer and heart diseases. The most important action of these chemicals with respect to human beings is somewhat similar in that they function as antioxidants that react with the free oxygen molecules or free radicals in our bodies (www.paho/cfni.org).

Phytochemicals are naturally occurring, non-nutritive chemicals. They appear to work alone and in combination, and perhaps in conjunction, with vitamins and other nutrients in food to prevent, halt, or lessen disease. Phytochemicals have been used as drugs for millennia. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims by US Food and Drug Administration (FDA). An important cancer drug, Taxol (paclitaxel), is a phytochemical initially extracted and purified from the Pacific yew tree (Tyagi *et al.*, 2010).

Some of the common phytochemicals are discussed in the following table: **Uses of some phytochemicals**

CLASS	USES	ACTION
Alkaloids	Raw material for the synthesis of useful drugs	Analgesic, antispasmodic, bactericidal effects
Phenols	Disinfection	Antiseptic, antitumor, anti-inflammatory, antimicrobial.
Flavonoids	In prevention of oxidative cell damage, allergies free radicals, microbes.	Antioxidant, anticarcinogens, antimicrobial, antitumor.
Saponins	Emulsifying agent	Expectorant, cough suppressant, haemolytic activity.
Essential oil	In perfumes, flavourings and medicines.	Medicating, soothing relief.
Tannins	In the production of leather and ink; in treating wounds, varicose ulcers, haemorrhoids, frostbite and burns.	Soothing relief regenerates skin, anti-inflammatory, diuretics.

Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. They are known to be synthesized by plants in response to microbial infection and have been found *invitro* to be effective against a wide array of microorganisms. Saponins are glycosides of both triterpenes and steroids that are characterized by their bitter or astringent taste, foaming property, haemolytic effect on red blood cells and cholesterol binding properties. In medicine, it is used to some extent as an expectorant and an emulsifying agent (Okingbo *et al.*, 2009).

Alkaloids comprise one of the major groups of plant constituents. Several of alkaloids were in clinical use, including reserpine (the first tranquiliser) and the dimeric indole alkaloids vinblastine and vincristine (anticancer agents) (Wang *et al.*, 2009). Sterols are amphiphilic molecules consisting of hydroxyl groups forming the hydrophilic heads and sterane skeletons with side chains forming the hydrophobic tails. Sterols found in plants are known as phytosterols and over 250 phytosterols and their related compounds have been identified from natural products. Phytosterols cannot be synthesized by humans and are thus consumed from the diet (Boukes *et al.*, 2008). These compounds protect against cardiovascular complications, decrease the risk of certain types of cancer and enhance immune functions. Plant sterols are also known to reduce serum low-density lipoproteins (LDL) cholesterol level and reduce atherosclerotic risk (Tlili *et al.*, 2010).

Terpenes are the most numerous and structurally diverse plant natural products. Terpenes, consists of isoprene, a simple hydrocarbon molecule. The term terpene usually refers to a hydrocarbon molecule while terpenoid refers to a terpene that has been modified such as by the addition of oxygen. Terpenes have shown antimicrobial activities. Plant oils, which contain terpenes, have shown increasing promine *invivo,* inhibiting multiple species of bacteria (Zwenger and Basu, 2008). Reducing sugar such as glucose are the main substrates in the respiration process to produce energy required in the metabolism (Tefera *et al.*, 2008). Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine) (Chhetri *et al.*, 2008).

Tannins or polyphenols have a number of physical and chemical properties in common, which underlie their physiological and pharmacological actions: their antioxidant and radical scavenging activities and their ability to complex with other molecules such as proteins and polysaccharides. Tannins may prevent ulcer development (Perera *et al.*, 2010).

2.3 OXIDATIVE STRESS / NITROSATIVE STRESS

Oxidative stress is an excessive shift of the oxidant-antioxidant balance towards the oxidation reaction (Andryskowski and Owczarek, 2007). Detrimental effects of exposure to high concentration of oxygen can lead to an abundance of reactive oxygen species. In situations where a body's antioxidant defences are inadequate, increased free radical formation is likely to increase the damage. This situation is generally termed as "oxidative stress" (Matsunami *et al.*, 2010). Oxidative stress reduction through the dietary intake of antioxidants has been suggested to reduce such oxidative damage (Halvorsen *et al.*, 2006). Production of reactive oxygen species and oxidative stress are associated with tissue injury and many pathological processes (Magder, 2006). New borns are more susceptible to oxidative stress due to increased production of free radicals at birth, and incompletely developed antioxidant mechanisms (Ashok *et al.*, 2008). Oxidative stress is an important contributory factor to the etiology of many cardiovascular diseases, diabetes, heart failure and hypertension (Dusting and Triggle, 2005).

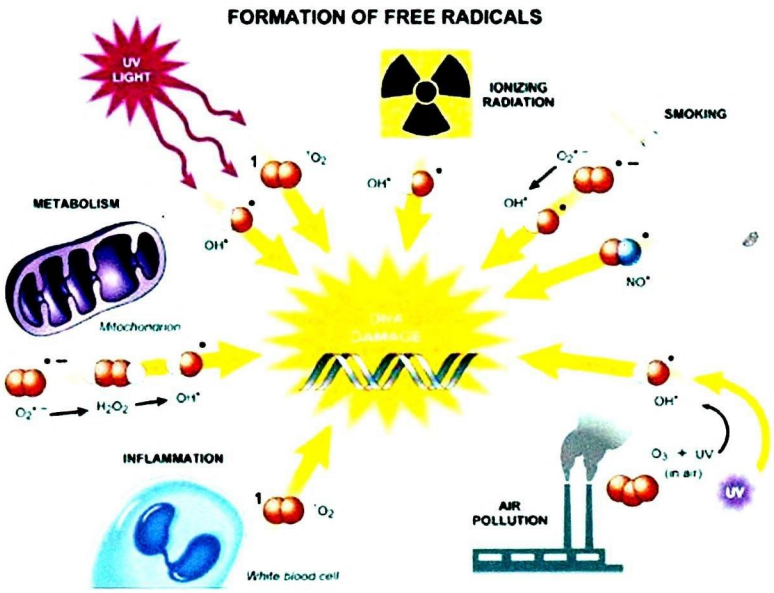
An over production of reactive nitrogen species causes nitrosative stress. This may occur when the generation of reactive nitrogen species exceeds the system's ability to neutralize and eliminate them. Nitrosative stress may lead to unwanted nitrosylation reactions that alter the structure and functions of certain proteins. At high concentrations, reactive oxygen species and reactive nitrogen species can be important mediators of damage to nucleic acids, lipids and proteins (Manjeet *et al.*, 2008).

2.4 FREE RADICALS – The dangerous species

Free radical may be defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are described as free radicals and other non-radical

reactive derivatives. ROS and RNS includes radicals such as superoxide ($O^{\cdot-}_2$), hydroxyl ($^{\cdot}OH$), peroxyl ($RO^{\cdot}_2$), hydroperoxyl ($HO^{\cdot}_2$), alkoxy ($RO^{\cdot}$), peroxyl ($ROO^{\cdot}$), nitric oxide ($NO^{\cdot}$), nitrogen dioxide ($NO^{\cdot}_2$) and lipid peroxyl ($LOO^{\cdot}$); and nonradicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen, peroxy nitrate ($ONOO^-$), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH). Nonradicals are also termed as oxidants and capable to lead free radical reactions in living organisms easily. Radicals derived from oxygen characterize as the most important class of radical species generated in living systems (Saikat *et al*, 2010).

The free radicals are generated from cellular and metabolic activites and also through exogenous sources such as human exposure to ionizing radiation, injury, oxidative drugs and pollutants (Erasto and Mbwambo *et al.,* 2009). The involvement of oxygen in metabolic processes of living organisms is coupled to its activation and formation of a number of highly reactive compounds (Gessler *et al.,* 2007). The effects of free radicals on human beings are closely related to toxicity, disease and aging (Gupta *et al.,* 2004).



Superoxide anion is a moderately reactive species capable of generating H_2O_2, which in turn can produce highly reactive hydroxyl radical ($^{\cdot}OH$) via iron-dependent catalytic reactions (Aung *et al.*, 2007). Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species and the most stable intermediate in the four-electron reduction of O_2 to water. Since H_2O_2 is uncharged, it easily passes through cell membranes by diffusion, and when inside the cells it can react with transition metal liberating hydroxyl radicals ($^{\cdot}OH$). At high concentrations, these radicals induce peroxidation of lipids and proteins, affecting cell integrity (Da Rosa *et al.*, 2008).

Hydroxyl radical is highly reactive with a half-life in aqueous solution. Ionizing radiation causes decomposition of water, resulting in formation of $^{\cdot}OH$ and hydrogen atoms. $^{\cdot}OH$ is also generated by photolytic decomposition of alkylhydroperoxides (Valko *et al.*, 2004). A high energy form of oxygen, singlet oxygen is generated in the skin upon UV-radiation and it induces hyperoxidation, oxygen cytotoxicity and decrease the antioxidant activity. Hypochlorous acid is another harmful ROS. At the sites of inflammation, the oxidation of Cl^- ions by the neutrophil enzyme myeloperoxidase results in the production of this ROS, which breaks down the heme prosthetic group and inactivates the antioxidant enzyme catalase (Hazra *et al.*, 2010). Peroxy nitrite ($ONOO^-$) is relatively stable compared to other free radical but once protonated it forms the highly reactive peroxynitrous acid ($ONO^{\cdot}OH$). Generation of excess $ONOO^-$ leads to oxidative damage and tissue injury (Hazra *et al.*, 2008). Nitric oxide could counteract oxidative damage and had protective effect against various stressfull conditions. Addition of exogenous nitric oxide with sodium nitroprusside significantly enhanced antioxidant enzymes activities and reduced ROS level, prevented lipid peroxidation and membrane damage, whereas a reversed pattern was found with the supplementation of nitric oxide scavenger (Jin *et al.*, 2010). Nitric oxide is also a key molecule responsible for acute pulmonary injury during endotoxemia (Liu *et al.*, 2009).

Lipids constitute a vital module in food and other biological systems either as storage lipids that are potential sources of energy by beta-oxidation or membrane lipids. Unsaturation in lipids makes them susceptible to oxygen attack. The ROS formed under oxidative stress has the tendency to react with double bonds of polyunsaturated fatty acids. The oxidative degradation produces lipid hydroperoxides, leading to complex changes that eventually manifest themselves in the development of food rancidity and off-flavors. The resulting products may be toxic to the cell. It has been demonstrated that the types and levels of dietary fat affect the susceptibility of lipid peroxidation and oxidative damage to cells (Kaur *et al.*, 2009). Membrane lipids succumb easily to deleterious actions of ROS (Nwanjo *et al.*, 2007). ROS can trigger a chain reaction on the cell membrane by oxidizing membrane phospholipids and generate lipid hydroperoxide within the cell membrane (Shrilatha *et al.*, 2009). ROS generated by lipid peroxidation are implicated in carcinogenesis, mutagenesis, aging, inflammation, and cardiovascular diseases (Anwar *et al.*, 2006).

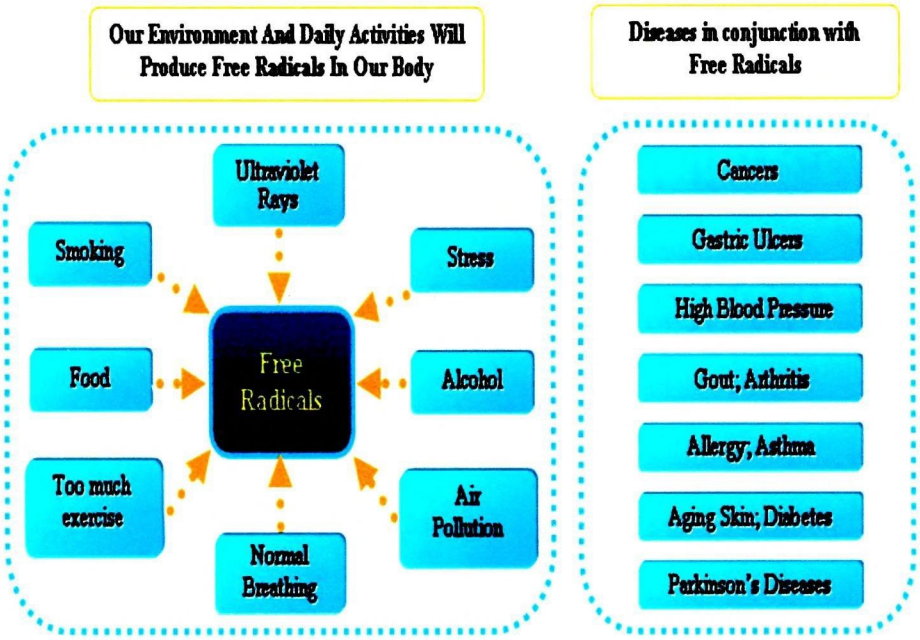
2.5 Free radicals induced damages/disorders

There is increasing evidence indicating that reactive oxygen species and free radical-mediated reactions can cause oxidative damage to biomolecules (eg., lipids, proteins and DNA), eventually contributing to aging, cancer, atherosclerosis, coronary heart ailment, diabetes, Alzheimer's diseases and other neurodegenerative disorders (Wu-Yang *et al.*, 2007). Many human diseases are caused or negatively affected by free radicals (Buricova and Reblova, 2008).

2.5.1 Macromolecules damage

Increased generation of ROS leads to an intensification of the lipid peroxidation process, one end-product of which is malondialdehyde (MDA) (Woznlak *et al.*, 2008). ROS induce membrane lipid peroxidation in sperm (Khosrowbeygi and Zarghami, 2007). The toxic lipid peroxides are known to cause

various impairments of the cell, such as membrane damage (Tavilani *et al.*, 2008). Oxidation of lipid is a major cause of food deterioration, affecting color, flavor, texture and nutritional value. Besides, it has been suggested that oxidative modification of low-density lipoproteins (LDL) may play a role in the development of atherosclerosis and the oxidative modification depends on a common initiating step – the peroxidation of polyunsaturated fatty acid components in the LDLs. The generated lipid peroxides further act on the cell/cellular components, leading to both structural and functional damage of the biomolecule as well as the cellular structure (Puttaraju *et al.*, 2006).



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Proteins are susceptible to free radical damage and may undergo structural and functional modifications (Atabek *et al.*, 2006). Oxidative damage of proteins can result in several key events that alter cellular activity including changes in protein activity, proteasomal quality control, cellular redox-balance, and interference with the cell cycle. Protein carbonylation in particular is an event caused by direct attack of ROS, metal-catalyzed oxidation, reaction with reducing sugars,

and conjugation with highly reactive carbonyl compounds produced as end-products of lipid peroxidation (Rauniyar *et al.*, 2007). Proteins are major targets for RONS because if their high overall abundance in biological systems and it has been estimated that proteins can scavenge the majority of RONS generated. Oxidative damage to proteins can occur directly by interaction of the protein with RONS or indirectly by interaction of the protein with a secondary product (resulting from interaction of radical with lipid or sugar molecule). Modification of a protein under conditions of oxidative stress can occur via peptide backbone cleavage, cross-linking, and/or modification of the side chain of virtually every aminoacid. Moreover, most protein damage is irrepairable and oxidative modification of the protein structure can lead to loss of enzymatic, contractile, or structural function in the affected proteins, thus making them increasingly susceptible to proteolytic degradation (Fisher-wellman and Bloomer, 2009).

ROS causes structural damage to essential biological molecules. DNA is one of the molecules likely to suffer oxidative damage (Cervantes-Cervantes *et al.*, 2005). DNA damage in the cells appears to be largely oxidative and is associated with a wide variety of adverse outcomes, including childhood cancer and dominant genetic diseases (Baker and Aitken, 2005). ROS-induced DNA damage can take many forms, ranging from specifically oxidized purine and pyrimidine bases, to DNA lesions such as strand breaks, sister chromatid exchanges and the formation of micronuclei (Brambilla *et al.*, 2008). Several toxic byproducts of the peroxidation can damage DNA away from the site of their generation (Rathee *et al.*, 2006). The oxidative damage to DNA may play vital role in aging and the presence of intracellular oxygen also can be responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause damage to critical cell biomolecules (Sulekha *et al.*, 2009).

When ROS are over produced, redox-active transition metal ions such as iron (II) or copper (II) can cause severe oxidative stress and thus damage tissues

and the cellular carbohydrates constituents within (Thring *et al.*, 2009). The oxidation of sugars with hydroxyl radical often releases formic acid as the main breakdown product. This may be the long-sought-after source of substrate for the enigmatic enzyme, formate dehydrogenase (Parvaiz *et al.*, 2009).

2.5.2 Aging

Aging results from exposure of cellular macromolecules to reactive oxygen species (ROS) and that accumulation of ROS-induced damage is responsible for the development of diseases associated with aging, including cancer (Curtis *et al.*, 2010). Free radical theory of aging has given much impetus to the role of ROS in the initiation and progression of the aging process (Rupesh *et al.*, 2008).

2.5.3 Cancer

Oxygen derived free radicals produced by lipid peroxidation are believed to play an important role in cancer development (Delorenze *et al.*, 2010). These free radicals are known to stimulate carcinogenesis at all three stages: initiation, promotion and progression. Higher levels of free radicals and malondialdehyde (MDA), the end products of lipid peroxidation, were reported in cancer tissues than in nondiseased organs. Hence, currently, the reduction of avoidable endogenous and exogenous sources of oxidative stress is potentially the most important means of preventing oxygen free-radical-related cancer (Senthil *et al.*, 2008).

2.5.4 Atherosclerosis

Atherosclerosis is the major cause of morbidity and mortality in the developing and developed countries. The magnitude of this problem in profound, as atherosclerosis claims more lives than all types of cancer combined and the economic costs are considerable. Atherosclerosis is characterized by the accumulation of cholesterol deposits in macrophages in large and medium sized

arteries. This deposition leads to a proliferation of certain cell types within the arterial wall that gradually impinge on the vessel lumen and obstruct the blood flow (Amran *et al.*, 2010). The etiology of atherosclerosis appears to be a multifactorial series of events, but the oxidation of lipoprotein is believed to be a primary event in the pathogenesis of atherosclerosis (Choi *et al.*, 2010). It is a complex and chronic inflammatory disease. Nitric oxide ($NO^{\cdot}$), a radical produced from L-arginine via nitric oxide synthase (NOS) and an important cellular second messenger, plays a dual role as both a beneficial molecule and a detrimental molecule in the process of inflammation. The small amount of NO produced by constitutive NOS, including endothelial NOS and neuronal NOS (nNOS), is an important regulator of physiological hemostasis, whereas the large amount of NO produced by inducible NOS (iNOS) has been closely correlated with the pathophysiology in atherosclerosis (Lee *et al.*, 2006). Antioxidative effects of flavonoids, on the oxidative modification of low-density lipoproteins might help slow the development of atherosclerosis (Azuma *et al.*, 2008).

2.5.5 Diabetes

Diabetes with an alarmingly rising incidence is a cluster of abnormal metabolic paradigm having a common feature of hyperglycemia (Singh *et al.*, 2009). Oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complication (Johansen *et al.*, 2005).

2.5.6 Neurodegenerative disorders

Neurodegenerative disorders are a heterogenous group of diseases of the nervous system, including the brain, spinal cord and peripheral nerves, which have many different etiologies (Pratap *et al.*, 2004). In many disorders of this kind,

an abnormal reaction occurs between a protein and a redox-active metal ion, for example Cu^{2+} and Fe^{3+}. This promotes the formation of ROS, which can be detrimental to the nervous system. Oxygen radicals may play an important role in the pathologies of a number of diseases of central nervous system (CNS). Oxidative stress is either a causative or an ancillary factor in the pathogenesis of major neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease (Brown, 2005).

Parkinson's disease is an age-related progressive neurodegenerative disease, which is characterized by resting tremors, rigidity, postural abnormalities, and difficulty or failure to execute willed movements, i.e., bradykinesia, akinesia and festinating gait (Sankar *et al.*, 2007). Alzheimer's disease is a progressive neurodegenerative disorder characterized by progressive cognitive impairment, deficits in acquiring memory, disordered spatiotemporal relationships and altered personality effects. Characteristic neuropathological features of Alzheimer's disease include: neuronal loss, gliosis, neurofibrillary tangles, proliferated and dystrophic neuritis, neuritic plaques and fibrils and deposits of amyloid β-peptide in the cerebral cortex. The symptoms of this disease result from degeneration and death of specific populations of synapses and neurons (Cenini *et al.*, 2008). Mitochondrial dysfunction and free radical-induced oxidative damage have been implicated in the pathogenesis of Parkinson's disease and Alzheimer's diseases, as well as other neurodegenerative disorders (Sayre *et al.*, 2005).

2.5.7 Stroke

Stroke is a major cause of death and disability in the world. ROS generated from the respiratory chain in mitochondria, ischemia-activated xanthine / hypoxanthine oxidase and lipid fatty acid metabolism play an important role in the brain ischemis-reperfusion process. Because of the high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids, relatively low antioxidant capacity, low repair activity and non replicating nature of the neuronal cells, the

brain is very susceptible to the damage caused by oxygen radical generated during ischemia- reperfusion. In the cerebral circulation system, the burst in the production of ROS damages the endothelium cell and smooth muscle cell, induce blood platelet aggregation and vascular permeability changes, and results in edema (Zhao, 2005).

2.5.8 Cataract

Cataract comprises a common group of ocular disorders manifested by lens opacities of varying size and shapes and varying in etiology and rate of progression (Sardari *et al.*, 2007). Increased lipid peroxidation due to oxidative stress has been proved to be an important factor of development of cataract (Chakraborty *et al.*, 2007).

2.5.9 Apoptosis

Apoptosis is a programmed cell death process, which is highly regulated and organized by a number of intracellular mediators controlling the development and homeostasis of multicellular organisms that result in chromatin condensation, DNA fragmentation, cytoplasmic membrane blebbing, and cell shrinkage (Lee and Lim, 2006). It has been suggested that apoptosis is triggered by oxygen- derived reactive species (ROS) (Dilsiz *et al.*, 2006).

2.5.10 Other diseases

ROS modify various cellular components and may contribute to postischemic contractile dysfunction termed myocardial stunning (Kaplan *et al.*, 2008). Besides damaging to living cells, free radicals are the major cause of food deterioration through lipid oxidation, which ultimately affects the organoleptic properties and ediblity of foods (Pandima *et al.*, 2008). ROS play a complex role in many diseases and metabolic regulation. Because viruses replicate in living cells, such metabolites influence the growth of viruses in addition to serving as a host defense mechanism. Humans infected with virus (HIV, hepatitis and

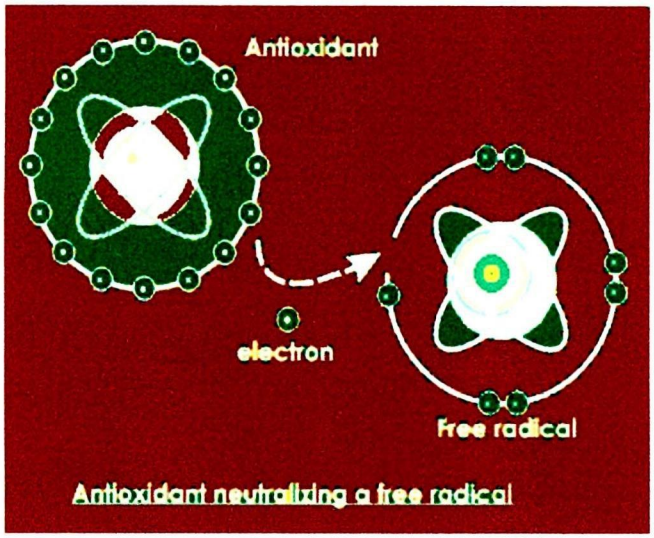
influenza) induce activation of phagocytes, which is associated with production of ROS. Chronic hepatitis B (HBV) and hepatitis C virus (HCV) infections are associated with an increased production of ROS within the liver that is responsible for the oxidation of intracellular macro molecules (Waris and Ahsan, 2006).

2.6 Antioxidants – The protective healers

The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Humans have evolved highly complex antioxidant systems (enzymatic and nonenzymatic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants can be endogenous or obtained exogenously as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase, superoxide dismutases. Non enzymatic antioxidants include vitamin E and C, thiol antioxidants, melatonin, carotenoids, natural flavonoids, and other compounds (Rahman, 2007).

Antioxidants are substances that are supplemented either endogenously or exogenously to combat the state of oxidative stress (Naik *et al.*, 2005). Antioxidants, which scavenge active oxygen species (free radicals), are found in a variety of food stuffs and are commonly referred to as scavengers. Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress. Antioxidants also play an important role in human health because the biologic defense mechanism cannot operate under severe oxygen stress (Kshirsagar and Upadhyay, 2009). Antioxidative action, one of the important physiological functions of plant chemicals, is supposed to protect living organisms (Pathirana *et al.*, 2006). Antioxidants protect the human body against free radicals that cause pathological

conditions such as ischemia, anameia, asthma, arthritis, inflammation, neurodegenerating Parkinson's diseases, mongolism, ageing process and perhaps dementias (Ara and Nur, 2009).



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Antioxidants confer protection against oxidative stress by quenching free radicals, chelating redox metals and interacting with (and regenerating) other antioxidants within the "antioxidant network". When optimal concentrations are sustained in tissues and biofluids they can function in both the aqueous and membrane domains. The most efficient enzymatic antioxidants are superoxide dismutases, catalase, and glutathione peroxidase. Non enzymatic antioxidants include vitamin C and E, carotenoids, thiols (glutathione, thoredoxin, and lipoic acid), natural flavonoids and melatonin. Few antioxidants can regenerate other antioxidants to restore the reduced intracellular state via an antioxidant network. The redox cycles of vitamins E and C have this capacity, driven by the redox potentials of the [Red / Ox] couple. Antioxidants attenuate ROS by binding to transition

metal-containing proteins, transferring or ceruloplasmin, inhibiting cellular reactions (vitamin E) and detoxifying ROS and RNS (Kannan, 2006).

2.6.1 Enzymatic antioxidants

All organisms have enzymatic mechanisms to scavenge oxidants, or to repair damage caused by free radicals. Enzymatic defences included, superoxide dismutases, catalases, selenium-dependent and independent glutathione peroxidases, peroxidase, glutathione-s-transferase (Carbone *et al.*, 2003).

2.6.1.1 Superoxide dismutases

Superoxide dismutases are ubiquitous metalloenzymes that detoxify the highly reactive superoxide anions to hydrogen peroxide and molecular oxygen (Kang *et al.*, 2008). Superoxide dismutases are the only enzymatic system decomposing superoxide radicals to hydrogen peroxide and are hypothesized to play a significant role against oxidative stress. There are three different superoxide dismutases: intracellular copper-zinc SOD (CuZnSOD), mitochondrial manganese SOD (MnSOD) and extracellular SOD (ECSOD). Superoxide dismutases not only constitute the basic superoxide consuming mechanism in oxidant-exposed cells, but they also participate in the regulation of normal cell hemostasis. Thus superoxide dismutases have multiple functions in regulating intracellular and extracellular levels of superoxide, hydrogen peroxide and nitrogen metabolites (Kinnula and Crapo, 2003).

2.6.1.2 Catalase

Catalase is an important scavenging enzyme against reactive oxygen species (ROS), as it removes hydrogen peroxide produced during metabolic processes. The enzyme is localized in the cytosol and in peroxisomes of cells (Bloch *et al.*, 2007). Catalase catalyses the decomposition of hydrogen peroxide to give water and molecular oxygen (Qujeq and Rezvani, 2007), hence has been considered an important regulator of oxidative stress. Catalase, a part of the defense system against free radical damage, is a tetrameric antioxidant enzyme with a molecular weight of 59,800 (D'souza *et al.*, 2008).

2.6.1.3 Peroxidases

Peroxidases are considered to be one of the main protective enzymes, being engaged in the removal of free radicals and activated oxygen species (Bogdanovic *et al.*, 2008). Peroxidases have different isoforms, whose number and amount could vary in plants during growth, normal and stress conditions (Katerova, 2009). Peroxidases are a widely distributed group of enzymes that can oxidize a variety of hydrogen donors at the expense of hydrogen peroxide (Mihailova *et al.*, 2009).

2.6.1.4 Glutathione peroxidase

Glutathione peroxidase is an important antioxidant defence, as this enzyme is involved in the clearance of superoxide and hydrogen peroxide as the enzyme superoxide dismutases. Glutathione peroxidase catalyses the reaction of reduced glutathione (GSH) with synthetic cummene hydroperoxide to oxidized glutathione (GSSG). In the presence of NADPH and glutathione reductase GSSG is transformed to glutathione, and NADPH is oxidized to NADP (Stukelj *et al.*, 2010). Glutathione peroxidase has the function to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide in water. Glutathione peroxidase is a selenium-containing glycoprotein. As the integrity of subcellular membranes depends heavily on glutathione peroxidase, which in turn depends on selenium, the mechanism of glutathione peroxidase is at the selenocystein site (Gallo and Martino, 2009).

2.6.1.5 Glutathione–s-transferase

The glutathione-s-transferases are a group of multifunctional proteins which play a central role in detoxification of electreophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood (Mohan and Venkataramana, 2007). Glutathione associated metabolism is a major mechanism for cellular protection against agents that generate oxidative stress, protecting cells against cytotoxic products of lipid peroxidation. Glutathione – s-

transferases are induced under conditions of oxidative stress. These are active in detoxification of numerous products, including reactive oxidant damage to DNA and lipids, such as organic epoxides, lipid hydroperoxides, and unsaturated aldehydes. Individuals lacking these enzymes may have reduced removal of lipid peroxidation products and thus may experience higher cancer protection (Dominguez *et al.*, 2007).

2.6.1.6 Polyphenol oxidase

Polyphenol oxidase is otherwise called as tyrosinase, catecholase, o-diphenol oxidase. It is an oxidative enzyme (Masum *et al.*, 2010). Polyphenol oxidase catalyze the oxygen dependent oxidation of mono and o-diquinones, highly reactive intermediates, the secondary reactions of which are believed to be responsible for the oxidative browning that occurs as a consequence of plant senescence, wounding and pathogen infection. Function of polyphenol oxidase have been proposed, including roles in the phenylpropanoid pathway, the Mehler reaction, electron cycling, oxygen regulation, flower petal coloration and plant defense (Thipyapong *et al.*, 2007).

2.6.2 Nonenzymatic antioxidants

Nonenzymatic mechanisms comprise antioxidants such as ascorbic acid, α- tocopherol, β- carotene, glutathione and flavonoids among others. These can contribute to prevent the damage caused by oxidative reactions (Sharma *et al.*, 2010)

2.6.2.1 Ascorbic acid

Ascorbic acid is the most abundant soluble antioxidant found in plant cells and is present at various concentrations in nearly all fresh food. Since humans have, through evolution, lost the ability to synthesize their own ascorbate, it must be obtained from their diet. Fresh fruits are the good source of the dietary antioxidant ascorbic acid (vitamin C). Ascorbate is often used as an indicator of the nutritional

value of food stuffs. Intracellular ascorbic acid concentration varies between species and between tissues of same species (Melino *et al.*, 2009).

2.6.2.2 α- Tocopherol

Vitamin E comprises eight naturally occurring fat-soluble vitamins of which the most predominant, essential and with the highest biological activity is α-tocopherol. It is a major antioxidant in biological systems which terminate the chain reaction of lipid peroxidation in membranes and lipoproteins (Shirpoor *et al.*, 2007). Vitamin E, a phenol, is an important chain-breaking antioxidant in lipids as it can donate hydrogen atoms to peroxyl radicals very efficiently. After the initial hydrogen atom transfer, the radical of Vitamin E or of any good chain-breaking antioxidant, must be sufficiently inert to the system and to oxygen as to not propagate the oxidative chain. Ideally, the resulting antioxidant radical will trap a second peroxyl radical (Frenette *et al.*, 2006).

2.6.2.3 Carotenoids

Carotenoids form one of the largest groups of pigments that are widely distributed in plants, and they are responsible for the yellow, orange and red coloration of tissues. They are biologically important in many organisms from bacteria and fungi to higher plants. Their prominent function is as accessory pigments during the harvesting of light and as protectants against photooxidation. Carotenoids play an important role in human nutrition, some of them exhibiting pro-vitamin A activity (Zhu *et al.*, 2002). Carotenoids are fat-soluble natural pigments with *invitro* antioxidant properties (Costantini *et al.*, 2008). These are considered to protect plants, animals, and microorganisms from the destructive effects of ROS and are known to quench superoxide anion. It has been reported that lycopene and β- carotene both scavenge superoxide effectively (Zhang *et al.*, 2007).

2.6.2.4 Glutathione

Glutathione, a tripeptide with a thiol group, is found in plants and animals in both its reduced and dimeric forms. In the reduced (monomeric) form, glutathione is a powerful endogenous antioxidant, protecting biological systems from degenerative damages associated with ageing and oxidative stress (Okonkwo and Okonkwo, 2009). Although many of these functions are associated with protection against reactive intermediates, including free radicals and electrophiles, maintenance of a suitable thiol redox balance with low-molecular weight and protein thiols are also crucial for cellular homeostasis. Glutathione functions include the glutathione redox cycle involving glutathione peroxidase and reductase, electrophilic conjugation reactions via the enzymatic activities of many glutathione transferases and a role in protein synthesis, folding, activation and inhibition (Reed, 2008).

2.6.6.5 Polyphenols

Polyphenols are commonly found in both edible and inedible plants, they have multiple applications in food, cosmetic and pharmaceutical industries. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. In addition to their roles as antioxidants these compounds exhibit a wide spectrum of medicinal properties such as antiallergic, antiinflammatory, antimicrobial, antithrombotic, cardioprotective and vasodialatory effects (Demiray *et al.*, 2009). Many dietary polyphenols are antioxidants and the possibility exists that they protect against oxidative damage by directly neutralizing reactive oxidants (Moskaug *et al.*, 2005).

2.6.6.6 Flavonoids

Flavonoids are a group of natural compounds widely found in the plant kingdom and diversified biological and pharmacological activities of these compounds have been reported during last years (Ferreira *et al.*, 2006).

Flavonoids appear to possess a variety of biological activities, including antioxidant, antiinflammatory, vasodilatory action (Yen chen *et al.*, 2005), antiviral, antitumor, hepatoprotective activities and the prevention of cardiovascular diseases. In particular, the antioxidative activity has gained the most interest, and the biological effects of flavonoids are believed to come, in large part, from their antioxidative properties (Li *et al.*, 2008). It directly neutralizes free radicals, chelating metals (Fe^{2+} and Cu^{+}) that enhance highly aggressive reactive oxygen species, inhibiting enzymes responsible for ROS production and up-regulating or protecting antioxidant defences (Puig and Castell, 2008).

2.7 Cytotoxic effect of the plant

Phytochemicals exhibiting cytotoxic or antitumor activity could lead to the production of new drugs for the treatment of cancer. Cytotoxicity studies will pave the way to the discovery of new nontoxic natural remedies and the lead molecule will be a candidate for the production of more biologically active chemicals by chemical means or enzymatic transformations (Joy and Remani, 2008). Study on cytotoxicity of plant extracts could give us an idea of the anticancer as well as toxic profile of studies plant extract. It also indicates that the plant extract, possess the potentials to kill cancer cells as well as to kill pests (Rahman *et al.*, 2010).

A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, costs little, and utilizes small amount of test material. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays once the active compounds have been isolated. It appears that BSLT is predictive of cytotoxicity and pesticidal activity. Since its introduction in 1982 (Meyer *et al.*, 1982), this *invivo* lethality test has been successively employed for bioassay- guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba*,

cis-annonacin from *Annona muricata* and ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (Pisutthanan *et al.*, 2004).

The brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24hours. The commercial availability of inexpensive brine shrimp eggs, the low cost and ease of performing the assay make brine shrimp lethality assay, a very useful bench-top method. A number of studies have demonstrated the use of the brine shrimp assay to screen plant extracts. Lethality assay has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plant extracts (Krishnaraju *et al.*, 2006).

2.8 Antibacterial activity of plants

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial properties of Indian medicinal plants have been increasingly reported (Aqil and Ahmad, 2003).

Infections are a major cause of illness and death and are common to all humans. The most frequent causal agents of infections are *Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus, Haemophilus influenza, Pseudomonas aeroginosa, Acinetobacter baumannii* and *Stenotrophomonas maltophilia.* Some additional and less frequent causative agents are *Bacillus subtilis, Bacillus cereus, Escherichia coli, Enterobacter cloacae, Listeria monocytogenes* and *Enterococcus faecalis.* Infections with these bacteria are associated with high mortality and morbidity, especially in immune-compromised patients (Bocanegra-Garcia *et al.*, 2009).

Antibiotics are an essential part for combating harmful bacterial infections *invivo*. During the last decade, infectious diseases have played a significant role in the death of millions around the world, especially in developing countries. Because of the mutagenic nature of bacterial DNA, the rapid multiplication of bacterial cells and the constant transformation of bacterial cells due to plasmid exchange and uptake, pathogenic bacteria continue to develop antimicrobial resistance, thus rendering certain antibiotics useless. An increased number of pathogens have also developed resistance to multiple antibiotics (Multiple Drug Resistance), threatening to develop complete immunity against all antimicrobial agents and therefore be untreatable. The acceptance of the plant compounds as traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants. Different antimicrobial and phytochemical constituents of medicinal plants are used for the treatment of microbial infections as possible alternatives to chemically synthetic drug to which many infectious microorganisms have become resistant (Kaushik and Goyal, 2008).

A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have been appeared in the literature (Gursoy and Tepe, 2009). Plant extracts are a very rich source of secondary metabolites with antibacterial action, and their application provides an opportunity to effectively combat antibiotic-resistant bacterial strains. It has been shown that, *invivo*, test compounds originating from plants posses antibacterial activity against a wide spectrum of human bacterial pathogens. Antibiotic resistant strains of bacteria create a significant problem in nosocomial infections (i.e. those contracted during hospital or a health care treatment (Krolieka *et al.*, 2009). In recent years antimicrobial properties of Indian medicinal plants have been increasingly reported. The traditional treatment approach is of much significance, especially in India due to the endemic presence of infective diseases which are the major causes of infant and adult mortality (Sudharameshwari and Radhika, 2007).

2.9 *Mukia maderaspatana* (L.)- A medicinal plant with multiple uses

Kingdom : Plantae

Family : Cucurbitaceae

Order : Violales

Genus : *Mukia*

Species : *maderaspatana*

Synonyms: *Cucumis maderaspatana* L.

*Mukia scabrella (*L.)f.Arn.

Melothria maderaspatana (L.) Cong.

Bryonia scabrella L.f.

Vernacular names:

Hindi : Agumaki

Tamil : Musumusukkai

Malayalam: Mukkalpeeram

Sanskrit : Trikosaki

Telugu : Noogudosa

*Mukia maderaspatana (*L.*)* is a prostrate climbing annual herb. Stem is glandular and covered with stiff hispid hairs. Leaves are simple, alternate, petioles are long, deltod, five angled, scabrid above, densely villous bellow, base cordate. Flowers are small, yellow, unisexual in axillary cymes, male flowers are fascicled

and female flowers are solitary or subfasiculate. Fruits are globose, four seeded, green when young and turns red on ripening, about 1cm in diameter. Leaves are used as an expectorant. The tender shoots and leaves are used as aperient. Roots decoction is used to control vomiting and fever. Roots are chewed to treat tooth ache. Decoction of the roots is given for flatulence. Fruit decoction is used to treat difficult and painful urination, piles and also used as brain tonic. Powdered root decoction is given to control vomiting. Seed pasties given to treat epilepsy, diarrhea and dysentery. Seed decoction is given along with paste of long pepper to treat abdominal disorders (Retnam and Martin, 2006).

Mukia maderaspatana (L.) is found throughout India ascending upto 1800m in the hills. The folklore medicine claims that it is a good diuretic, stomach ache, gentle aperient, antipyretic and antiflatulent, antiasthmatic and antibronchitis besides its use in vertigo and biliousness. It is used in Ayurvedha for various therapeutic purposes such as relief of toothache or flatulence, as an expectorant and a sudorific. Certain traditional medical practitioners also use the leaf-tea of this plant for alleviation of jaundice. Decoctions of leaves of this plant have been used by Siddha practitioners in Tamil Nadu for the treatment of hypertention. This plant leaf extract have also shown to have hepatoprotective, immunomodulatory effect and antiarthritic activity properties. Furthermore, the extract has been widely used for a long period in Siddha for the treatment of hypertension. Aqueous extract of this plant leaves provides antihypertensive effect (Raja *et al.*, 2010).