REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

The review of literature pertaining to the present study has been compiled and discussed under the following headings.

2.1. Medicinal Plant – "A Therapeutic Agent"

2.2. Phytochemical constituents in plants

Primary Constituents- "Nutritive value of Plants"

Secondary Constituents-"Secondary metabolities"

2.3. Free radicals

2.4. Types of free radicals

2.5. Oxidative stress and its effects

2.6. Common diseases of oxidative stress

2.7. Antioxidants- "a potent radical scavenger"

2.8. Antioxidant defence mechanism

2.9. Types of antioxidants

2.10. A biosafety screening of medicinal plants

2.11. Antibacterial activity of plants

2.12. *Denolix elata* –" A Miracle of World"

2.1. MEDICINAL PLANT – "A THERAPEUTIC AGENT"

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times (Kaur and Arora, 2009). Plants have been used for medicinal purposes throughout human history and the first pharmaceuticals (that is quantified doses of medicinal compounds as opposed to crude extracts of plant material) were derived from medicinal plants (McRae *et al.*, 2007).

Medicinal plants constitute one of the main sources of new pharmaceuticals and health care products. Whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described functional ingredients and nutraceuticals. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Demiary *et al.*, 2009).

Plants used for traditional medicine contains a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illness is accumulated in areas where the use of plants lies in phytochemicals that produce a definite pharmacological action on human body (Ranithaakur, *et al.*, 2009).

2.2. PHYTOCHEMICAL CONSTITUENTS IN PLANTS

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against disease or more accurately to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids, tannins and so on (Krishnaiah *et al.*, 2009).

2.2.1. PRIMARY CONSTITUENTS – "NUTRITIVE VALUE OF PLANTS"

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological

functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes (Adnan, 2010).

It has been well documented that during times of natural and manmade disasters, populations suffering from severe food shortages can become heavily reliant on wild food plants for survival. While every measure is being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast numbers of less familiar plant resources existing in the wild. Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their utilization. Most reports on some lesser known and unconventional crops indicate that they could be good sources of nutrients and many have the potential of broadening the present narrow food base of the human species (Elhassan and Yagi, 2010).

CARBOHYDRATES

Plants have thick cell walls made of carbohydrates polymers, which must be assembled outside the plasmamembrane and which constitute a significant proportion of the cell walls carbohydrates. Plants and photosynthetic microorganisms, can synthesise carbohydrates from carbondioxide and water, reducing carbondioxide at the expense of the energy and reducing power furnished bt the ATP and NADPH that are generated by the light-dependent reaction of photosynthesis (Cox and Nelson, 2008).

PROTEINS

Plants are one of the major sources of proteins. Potentially, plants provide a cheap source of industrial enzymes and biopharmaceuticals. Proteins have considerable technological importance since they affect the stability and sensory quality of plant foods (Aberoumand, 2008). Proteins are susceptible to free radical damage and may undergo structural and functional modifications (Atabek *et al.*, 2006).

CHLOROPHYLL

Photosynthesis is the most important biochemical process occurring in plants and chlorophyll is the key pigment involved in it (Samdur *et al.*, 2000). Chlorophyll, the green matter in plants, is found abundantly in nature. Through the process of photosynthesis, chlorophyll harnesses the sun's energy in order to perform various metabolic functions. Scientists' discovered the use of chlorophyll as a health-enhancing nutritional substance with many benefits (http://www.healthyyounaturally.com).

2.2.2. SECONDARY CONSTITUENTS-"SECONDARY METABOLITIES"

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances (Okigbo, *et al.*, 2009).

ALKALOIDS

Alkaloids comprise one of the major groups of plant constituents. Several of alkaloids were in clinical use, including reserpine (the first tranquillizer) and the dimeric indole alkaloids vinblastine and vincristine (anticancer agents). In this year, there are 136 new alkaloids isolated from natural resources (Wang *et al.*, 2009).

ANTHROQUINONES

Anthraquinone, also called anthracenedione or dioxoanthracene is an aromatic organic compound with formula $C_{14}H_8O_{22}$ that can be viewed as a diketone derivative of anthracene (with loss of two double bonds in the latter). The term usually refers to one specific isomer, 9, 10-anthraquinone or 9, 10-dioxoanthracene, whose ketone groups are on the central ring. Derivatives of 9, 10-anthraquinone includes many important drugs

(collectively called anthracenediones) (http://en.wikipedia.org//Anthraquinone).

CARDIAC GLYCOSIDES

The cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure. This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension (Awoyinka *et al.*, 2007).

FLAVONOIDS

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent antioxidant activity against the superoxide radical. Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of low density lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary atherosclerotic disease (Soetan and Aiyelaagbe, 2009).

PHENOLS

Phenolics are aromatic secondary plant metabolites widely spread throughout the plant kingdom and associated with colour, sensory qualities nutritional and antioxidant properties of food. The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers (Gupta and Prakash, 2009). Phenolic compounds antioxidant and cholesterol reducing activity makes them potentially protective against cancer and coronary heart diseases (Wozniak and Gowniak 2009). or 2008 ?

SAPONINS

Saponins are characterized by their surface active properties and they dissolve in water to form foamy solutions and because of surface

activity some drugs containing saponins have a very long history of usage. Saponins have been implicated as a bioactive antibacterial agent of plants containing them (Ogbonnia *et al.*, 2008).

STEROLS

Sterols are an important class of organic molecules. They are probably the most important class of the minor components and comprise a major portion of the unsaponifiable matter of most vegetable oils. Over 40 phytosterols have been identified; of these, campesterol, stigmasterol and sitosterol account for more than 95% of total phytosterol dietary intake (Tlili *et al.*, 2010). Sterols found in plants are known as phytosterols and over 250 phytosterols. Phytosterols cannot be synthesized by humans and are thus consumed from the diet (Boukes *et al.*, 2008).

TANNINS

Many human physiological activities such as stimulation of phagocytic cells, host-cell mediated tumour activity and a wide range of antiinfective actions have been assigned to tannins. One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Bansal *et al.*, 2010). Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effect. Their astringent action can help precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants (Perara *et al.*, 2010).

TERPENOIDS

The terpenoids are a large and diverse class of naturally occurring organic chemicals, derived from five-carbon isoprene units assembled in thousands of ways. They play an important role in traditional herbal remedies and under investigation for antibacterial, antineoplastic and other pharmaceutical functions. The terpenoids can be classified according to the

number of isoprene units: monoterpenoids (2 isoprene units), sesquiterpenoids (3 isoprene units), diterprenoids (4 isoprene units), sesterterpenoids (5 isoprene units), triterpenoids (6 isoprene units) and so on. The terpenoids take a large part of new natural products, the number of which is 750 (Su *et al.*, 2008).

2.3. FREE RADICALS

Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (Pourmorad *et al.*,2006).

The human body is composed of many different types of cells. Cells are composed of many different types of molecules. Molecules consist of one or more atoms of one or more elements joined by chemical bonds. Normally, bonds don't split in a way that leaves a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are formed. Free radicals are unstable and react quickly with other compounds trying to capture the needed electron to gain stability. Generally free radicals attack the nearest stable molecule, "stealing" its electron, it becomes a free radical itself, beginning a chain reaction and can be "thousands of events long". Once the process is started, it can cascade, finally resulting in the disruption of a living cell (Bhuiyan *et al.*, 2009).

2.4. TYPES OF FREE RADICALS

2.4.1. REACTIVE OXYGEN SPECIES

Free radicals are reactive oxygen species (ROS), including the hydroxyl radical ($HO^{\bullet}$), superoxide radical ($O_2^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$) and hydrogen peroxide (H_2O_2) are highly reactive, as the result of the presence of unpaired valence shell electrons. ROS form as natural by-products from normal oxygen metabolism and perform important functions in cell signaling (Heo and Jeon, 2008).

Beneficial effects of ROS in biological systems are that they serve in energy protection, phagocytosis, regulation of cell growth and intercellular signaling and synthesis of biologically important compounds at physiologic levels. On the other hand, they are highly deleterious and cytotoxic oxidants at pathologic levels which lead to cell injury and death. More than 100 diseases are related to ROS (Zou *et al.*, 2008).

Superoxide Radical (O_2•)

Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by nonenzymatic electron transfers that univalently reduce molecular oxygen. It can reduce certain iron complexes such as cytochrome (Krishnaraju *et al.*, 2009). The radical species superoxide (O_2•) and its reactive downstream products are known to cause oxidative damage to biological molecules and the presence of superoxide in the body has been linked to many different diseases (Krause *et al.*, 2010).

Hydrogen peroxide

Hydrogen peroxide has a strong oxidizing property. It can be formed *invivo* by many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds (Gulcin *et al.*, 2010). Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water (Aiyegoro and Okoh, 2010).

Hydroxyl radical

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging the biomolecules of living cells (Zhang *et al.*, 2009).

Lipid peroxidation

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Formation of free radicals may play an important role in the origin of life and biological evolution, implying their beneficial effects on organisms. Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging of organisms, and cancer promotion (Hayet *et al.*, 2008). Lipid peroxidation has been implicated in a variety of pathological processes, especially in both the ignition and promotion of artherosclerosis. So, free radical involvement in such pathological salts occurs whenever a disturbances in the pro-oxidant antioxidant balances is in favor of the former, leading to potential damage (Basuny *et al.*, 2009).

2.4.2. REACTIVE NITROGEN SPECIES

RNS include free radicals like nitric oxide (•NO) and nitrogen dioxide (•NO2-), as well as nonradicals such as peroxynitrite (ONOO-), nitrous oxide (HNO2) and alkyl peroxynitrates (RONOO). •NO is normally produced from L-arginine by endothelial nitric oxide synthase (eNOS) in the vasculature (Johansen *et al.*, 2005). An overproduction of RNS 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 (Singh *et al.*, 2008).

NITRIC OXIDE

Nitric oxide (NO) is a signaling molecule involved in many plant physiological and metabolic processes and it is acting as an intercellular and intracellular signal. NO itself is a reactive nitrogen species and its effects on different types of cells were proven to be either protective or toxic, depending on its applied concentration and location (Jin *et al.*, 2010). Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in

inflammation, cancer and other pathological conditions. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4,, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total allevation of this damage (Sanja *et al.*, 2009).

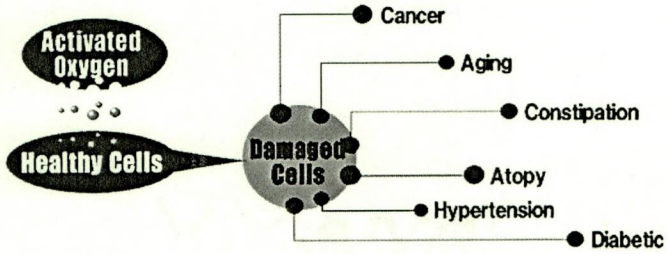
2.5. OXIDATIVE STRESS AND ITS EFFECTS

Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stressors. A stressful condition leads to the excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system (Srikumar *et al.*, 2006).

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Souri *et al.*, 2008).

2.6. COMMON DISEASES OF OXIDATIVE STRESS

Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders.



Diabetes

In diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated. Oxygen free-radical can initiate peroxidation of lipids, which inturn stimulates glycation of protein,

inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes and play a role in the long term complication of immune related diseases (Koka *et al.*, 2009).

Cancer

In carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth and finally carcinoma (Atawodi, 2005). Damage to DNA by ROS has been widely accepted as a major cause of cancer. In patients with diseases associated with a risk of cancer indicates an increased rate of oxidative DNA damage or in some instances deficient repair system such as Fanconi anemia, chronic hepatitis, cystic fibrosis and various autoimmune diseases (Waris and Ahsan, 2005).

Skin diseases

A number of skin diseases are believed to be associated with oxidative stress, including psoriasis, acne and cutaneous vasculitis. There is also evidence that ROS are involved in allergic and irritant contact dermatitis. It is consented that ROS are deleterious to wound healing process due to harmful effects on cells and tissues (Chanda and Baravalia, 2010).

Alzheimer's disease

Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease (AD). Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by progressive cognitive impairment, deficits in acquiring memory, disordered spatiotemporal relationships and altered personality effects. The symptoms of this disease result from degeneration and death of specific populations of synapses and neurons (Cenini *et al.*, 2007).

Osteoarthritis

Arthritis, a joint inflammation refers to a group of diseases that causes pain, swelling, stiffness and loss of motion in the joints.

Osteoarthritis is the most common form of arthritis and is a disease of cartilage regeneration. Osteoarthritis, also known as degenerative joint disease, is a process of progressive deterioration, of articular cartilage and formation of new bone (osteophyte) at the joint surface (Surapaneni and Vekataramana, 2009).

2.7. ANTIOXIDANTS –"A Potent Radical Scavenger"

Antioxidants are chemicals that reduce the rate of particular oxidation reactions in a specific context, where oxidation reactions are chemical reactions that involve the transfer of electrons from a substance to an oxidizing agent. Antioxidants are particularly important in the context of organic chemistry and biology; all living cells contain complex systems of antioxidant chemicals and/or enzymes to prevent chemical damage to the cells' components by oxidation. Also, they can interfere with the oxidation process by reacting with the free radicals, chelating free catalytic metals and also by acting as oxygen scavengers (Gulcin *et al.*, 2007).

Antioxidants which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Ara and Nur, 2009).

Antioxidants are a group of compounds that facilitate survival in plants and may promote the health of humans that consume a variety of plant foods (Borchardt *et al.*, 2008). Many of the biologically active substances found in plants, including phenolic compounds (flavonoids, phenolic acids), sugars, vitamins, saponins, ethereal oils, polyunsaturated fatty acids, phospholipids, enzymes, amino acids, etc., are known to possess antioxidant properties . The antioxidant activity of a medicinal plant preparation depends on the concentration and activity of individual

antioxidants entering into the composition (Arzamastsev *et al.*, 1999). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through food and /or supplements (Ebrahimzadeh *et al.*, 2010).

2.8. ANTIOXIDANT DEFENCE MECHANISM

The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals; this includes antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) that soak up all the excess reactivity that these free radicals have, turning them to harmless particles that can be get rid of (Adedayo *et al.*, 2010). Almost all organisms are protected from free radical attack by defence mechanisms such as a preventive antioxidant system that reduces the rate of free radical formation and another is a system to produce chain-breaking antioxidants that scavenge and stabilize free radicals. But, when free radical production rate exceeds the capacity of the antioxidant defence mechanisms substantial tissue injury results (Hasan *et al.*, 2009).

The natural antioxidant system is mainly classified into two categories namely *invivo* and *invitro antioxidants.* On the basis of function they can be further divided into following four categories:

First line of defence comprises of preventive antioxidants such as glutathione peroxidase, glutathione reductase, Superoxide dismutase, Catalase, selenoprotein transferrin, ferritin, lactoferrin and non-enzymatic proteins etc., which suppress the formation of free radicals. They act by quenching of [O_2] decomposition of H_2O_2 and sequestration of metal-ions.

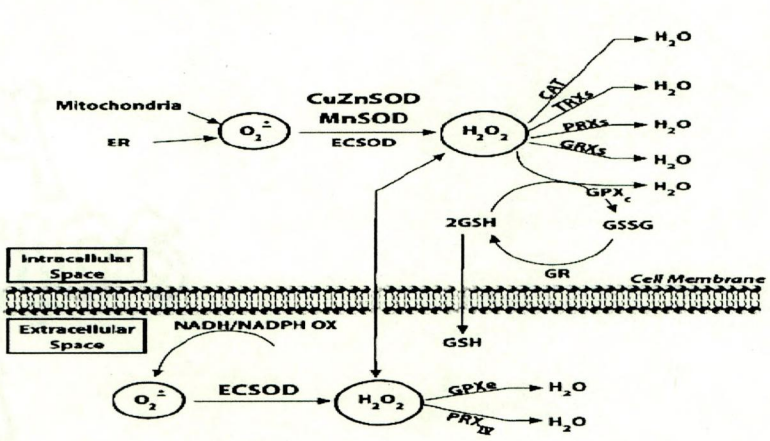
Second line of defence include the radical scavenging antioxidants mainly glutathione and antioxidant phytochemicals. They act as free radical scavengers by suppressing chain intiation or breaking chain propagation.

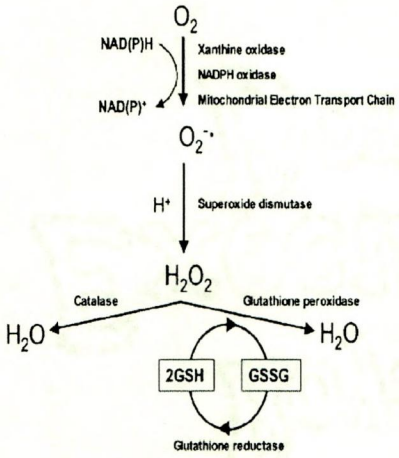
Third line of defence are complex group of enzymes required for the repair of damaged proteins, DNA, oxidized lipids and these enzymes can stop chain propagation of peroxyl lipid radicals.

Fourth line of defence is an adaptation where signal or the production and reaction of free radicals and transport of the appropriate antioxidant to the right site at the tail end of disease where immunology plays an important role (Prakash and Gupta, 2009).

2.9. TYPES OF ANTIOXIDANTS

2.9.1. ENZYMIC ANTIOXIDANTS





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 which degrades H_2O_2 into water and oxygen, is one of the major antioxidant enzymes.It is one of the first enzymes to be purified and crystallized and has gained a lot of attention in recent years because of its link to cancer, diabetes, and aging in humans and animals. Catalase has been reported to exist in multiple forms in many higher plants, such as saffron, mustard, spinach, cotton, wheat, sunflower, maize, castor bean, loblolly pine and tobacco (TayefiNasrabadi, 2008).

GLUTATHIONE PEROXIDASE

Glutathione peroxidase contains selenium as an integral component and capable of reducing lipid hydroperoxides and hydrogen peroxide. Se-GSH-Px is also involved in the reduction of fatty acid hydroperoxides generated during the production of prostaglandin, leukotrienes and related compounds via cycloxygenase and lipoxygenase pathways. Se-GSH-Px is known to be involved in the inhibition of cytoxygenase and lipoxygenase enzymes. Se-GSH-Px act as scavengers of free radicals generated in the cells/tissues of animals under varied pathological conditions (Reddy *et al.*, 2007).

GLUTATHIONE S-TRANSFERASE

Glutathione S-tranferase is a family detoxifying enzymes that catalyze the conjugation reduced glutathione with a group of compounds having electrophilic centers e.g., nitrocompounds, organophosphates and organochlorides. Since glutathione (GSH) is essential to cellular detoxification many toxic xenobiotics, monitoring this endogenous thiol during pesticide exposure is very important (Otitoju and Onwurah, 2007). The GSTs have long been demonstrated to be involved in intracellular transport of hormones, endogenous metabolites and exogenous chemicals and in the protection from oxidative damage and oxidative stress (Hou *et al.*, 2008).

PEROXIDASE

Plant peroxidase belonging to the class III secretory PODs are ubiquitous enzymes throughout the plant kingdom. These heme-containing glycoproteins using hydrogen peroxide as an electron acceptor and several substrates as electron donors are involved in a wide range of physiological processes such as lignifications, suberization, cell wall metabolism, auxin metabolism: biotic and abiotic stress tolerance and senescence (Haluskova *et al.*, 2010). Peroxidase catalyze the oxidation of large variety of substrates through the reaction with hydrogen peroxide. It typically catalyzes a reaction of the form:

$$H_2O_2 + AH_2 \text{ (electron donor)} \Longrightarrow A + 2H_2O$$

Based on this feature peroxidase is easily assayed to evaluate the degree of inactivation (Yu *et al.*, 2010).

POLYPHENOL OXIDASE

Polyphenol oxidase catalyses enzymatic browning through its action on mono and o-diphenols. Browning of dates was found to be related to enzymatic and nonenzymatic reactions which take place during development, handling and processing. PPO activity has been reported in many fruits. Previous studies have shown the major role of phenolic compounds in defense mechanisms of plant tissues in response to infections or injuries (Mustapha and Ghalem, 2007).

SUPEROXIDE DISMUTASE

Superoxide dismutase (SOD) is a metalloenzyme whose active center is occupied by copper and zinc, occasionally manganese or iron. SOD plays an important role in the protection of all aerobic life systems against oxygen toxicity and the free radicals derived from oxygen. As an enzyme, SOD has particular value as an antioxidant that can help to protect against cell destruction. It has the clear ability to neutralize superoxide, one of the most damaging free radical substances in nature (Kang *et al.*, 2008).

2.9.2. NON ENZYMIC ANTIOXIDANTS

The natural defense of the human organism against free radicals is not always sufficient mainly due to the significant exposition to free radicals from external sources in the modern world. The dietary intake of antioxidants plays an important role in the protection of the human organism againts free radicals. Many clinical and epidemiological studies show a connection between the antioxidant activity of the substances present in the diet and the prevention from such diseases as cardiovascular diseases or carcinogenesis (Buoieova and Reblova, 2008).

CAROTENOIDS

Carotenoids compounds are isoprenoid plant pigments of yellow, orange or red colour. They show high antioxidant activity and are converted

in the human body into vitamin A. Experimental studies suggest that a high consumption of vegetables and fruits rich in carotenoids protects the body against certain kinds of cancer, cataract, cardiovascular diseases and other disorders resulting from free radical activity (Gajewski *et al.*, 2010). Carotenoids are naturally occurring organic compounds known for their antioxidant activity (Galano *et al.*, 2009).

ASCORBIC ACID

Ascorbic acid is known to be a very effective free radical scavenger. It is not synthesized in humans and as such, it must be obtained from dietary sources. Even though its exact functions are not known, it is believed that ascorbic acid can neutralize reactive oxygen species such as hydrogen peroxide (Eghwrudjakpor and Allison, 2010).

α-TOCOPHEROL

Alpha-tocopherol is a lipid-soluble antioxidant associated with biological membrane of cells, especially the membrane of photosynthetic apparatus. α- tocopherol, found in green parts of plants scavenges lipid peroxy radicals through the concerted action of their antioxidants. Further, tocopherols were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts, thus protecting the structure and function of PSII (Shao *et al.*, 2008).

POLYPHENOLS

It has long been recognized that plant polyphenols are an important class of defence antioxidants (Boussaada *et al.*, 2008). The antioxidant activity of polyphenols has been reported to be important role in neutralizing free radicals and quenching oxygen or decomposing peroxides. Polyphenols of plant origin like catechins exert anticarcinogenic, antimutagenic and cardioprotctive effects which is contributed to their free radical scavenging activity (Habila *et al.*, 2010).

REDUCED GLUTATHIONE

Reduced Glutathione (GSH) is the smallest intracellular thiol (-SH) molecule. Its high electron donating capacity (high negative redox potential) combined with high intracellular concentration (mmol) generate great reducing power. This characteristic underlies its potent antioxidant action and enzyme cofactor properties and supports a complex thiol exchange system which hierarchically regulates cell activity (Chavan *et al.*, 2005).

2.10. A BIOSAFETY SCREENING OF MEDICINAL PLANTS

Bioactive compounds are often toxic to *Artemia salina* (leach) shrimp larvae. The eggs of the brine shrimp *A. salina* are readily available as fish food in pet shops. When placed in artificial seawater, the eggs hatch within 48 h, providing large numbers of larvae. This is a rapid, inexpensive, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products (Hussain *et al.*, 2010). The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties. The brine shrimp lethality assay really has proven to be a convenient system for monitoring biological activities of plant which are used as food and medicine in our community and has also provided some biochemical basis for the ethnomedicinal uses of plants (James and Jacob, 2010). Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor activities (Sharker and Shahid, 2010).

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Brine shrimp naupuli have been previously utilized in various bioassay systems. Among these applications have been the analyses of pesticidal residues,

mycotoxins, stream pollutants, anesthetics, dinoflagelate toxins, morphine like compounds, carcinogenicity of phorbol esters and toxicants in marine environment. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay. The variation in BSLA results may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or μg/mL) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds (Peteros and Uy, 2010). Good Cytotoxic effects of crude extracts indicate that it can be selected for further cell line assay, because there is a correlation between cytotoxicity activity against brine shrimp naupuli using extracts (Ripa *et al.*, 2010).

2.11. ANTIBACTERIAL ACTIVITY OF PLANTS

A survey of world health organization (WHO) indicates that about 70-80% of the world population in the developing countries depend on herbal sources as their primary health care system. Phytoconstituents such as flavonoids, alkaloids, tannins and triterpenoids are rich source of many medicinal plants challenges the modern medicine and stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms. The less availability and unaffordable cost of new generation antibiotics initiated to look for alternative phytomedicine to discover plant derived constituents with claimed antimicrobial activity. The extractable bioactive compounds in medicinal plants are a significant alternative approach to synthetic antibiotics, which could be used as valuables in human disease management (Senthilkumar *et al.*, 2010).

Plant cells fundamentally are chemical factories and many possess a rich supply of therapeutically useful constituents. Thus, plants around us can be investigated for the purpose of identifying those that may be potent against infectious organisms and hence useful in treating ailments caused by microorganisms (Akinpelu *et al.*, 2009). Higher plants have been shown to be a potential source for the new antimicrobial agents. The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases and about 20% of the plants or their extracts in the world have been submitted to biological or pharmacological tests (Gursoy and Tepe, 2009).

INFECTIOUS DISEASES

A disease which is caused by viruses, bacteria, fungi, protozoa and helminthes after being transmitted from one host or reservoirs to the other host, is known as infectious diseases. An infectious disease is any change in the state of normal health in which part or the whole body of an individual does not function properly due to the presence of an infectious agent or its products (Dubey and Maheswari, 2005). Virtually any microorganism can cause disease under right set of condition. This is because an infectious disease is as much the result of the failure of human defense system as it is the result of the special properties of pathogenic microorganisms (Atlas, 1995).

Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli, Vibrio cholerae, Areomonas spp., Shigella spp., Salmonella spp., Pseudomonas spp., Klebsiella spp., Campylobacter spp.*, and *Staphylococcus aureus* are most common. However, antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms (Acharyya *et al.*, 2009).

DISEASES CAUSED BY BACTERIA

Diarrhea occurs worldwide and causes 3.2% of all deaths. Diarrhea is most commonly caused by gastrointestinal infections, which kill around 1.8

million people globally each year, mostly children in developing countries. The pathogenesis of infectious diarrhea has been extensively studied. It is caused by a variety of enteric pathogens including bacteria such as enterotoxigenic *Escherichia coli, salmonella, Shigella flexeneri, Vibro cholera* and *Campylobacter jejuni.* Mechanism by which these organisms disrupt intestinal function to cause malabsortion or diarrhea are microbial attachement and localized effacement of the epithelium, production of toxins and direct epithelial cell invasion (Brijesh *et al.*, 2006).

Urinary tract infections (UTI) are most common form of bacterial infections, affecting people throughout their lifespan. The pathogenesis of complicated and uncomplicated UTI is complex and influenced by many host biological behavioural factors and properties of the infecting uropathogens. Leading etiological agents of UTI's include *Escherichia coli, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Proteus mirabilis* (Sharma *et al.*, 2009).

ANTIBIOTICS

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life, since their introduction. However over the past few decades these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug-resistant bacteria. Systemic studies among various pharmacological compounds have revealed that any drug may have the possibility to possess diverse function and thus may have useful activity in completely different spheres of medicines. It is essential to investigate newer drugs with lesser resistances (Sarkar *et al.*, 2007). The clinically useful antibiotics now in use have major setbacks. Apart from the narrow spectrum of antimicrobial activity many of them have been found to be neurotoxic, nephrotoxic, ototoxic or hypertensive and few others cause severe damage to the liver

and cause bonemarrow depression and importantly, infectious pathogens have developed resistance to all known antibiotics (Aiyegoro and Okoh, 2009). Thus there has been a continuing search for new and more potent antibiotics (Heisig, 2001).

MULTIPLE DRUG RESISTANT MICROORGANISMS

Antimicrobial resistance is a global concern (Blondeau *et al.*, 2000). Antimicrobial drug resistance in human bacterial pathogens is a continuing worldwide issue and as a consequence, effective treatment and control of such organisms remains an important challenge. Bacterial resistance has appeared for every major class of antibiotic (McCarrell *et al.*, 2008).

Resistance is spreading rapidly, particularly in hospitals, where many different bacterial strains can come into contact with each other and where antibiotics are heavily used. The catch is that, the more antibiotics used, the more the resistance to its spreads which forces us to try other antibiotics. Multiple drug resistant organisms are becoming common causes for infections in acute and long-term care units. The use of antimicrobial drugs to control infectious diseases must be among the greatest achievement of medicine in this century. The evolution of antibiotic-resistance pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources (Chellaram and Edward, 2009).With the increase of antibiotic resistance, development of new natural plant species with antibacterial activity has been in urgent need (Yang *et al.*, 2008). The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan, 2006).

2.12. *Denolix elata* – "A MIRACLE OF WORLD"

Delonix elata is a deciduous tree about 2.5-15 m tall, with a spreading, rather rounded crown, crooked poor stem form and drooping branches. Bark smooth, shining, sometimes flaking. Leaves 3-6 or more, bipinnate; pinnae usually 4-6 pairs; leaflets 10-14 pairs, oblong or

oblanceolate-oblong, 0.6-1.2 cm long. Flowers in terminal corymbs, stalks pubescent, lowest flowers stalks longest. *Denolix elata* is widely cultivated in the tropics for example it was introduced into the Botanical Garden of Calcutta in 1792 and 1799 and fine plantations of it are in Saurashtra: *Delonix elata* is a multipurpose tree commonly found planted or cultivated (http://www.worldagroforestry.org).Tree 2.5–15 m. high, with rounded-spreading crown; bark rather smooth, buff or grey (http://plants.jstor.org).

The less colourful cousin of Flamboyant or Gul Mohr, *Delonix elata* is a medicinal tree. It is often seen planted as an avenue tree. The tree does well in sandy soils. Its leaves serve as a traditional medicine for rheumatic problems. The Tamil name of the tree is `Vadanarayan'. The medical usefulness of the tree is acknowledged by people living in the villages who take a decoction of the leaves to get relief from rheumatic problems like pain and stiffness of the joints, especially the knees. The tree yields a convenient, not expensive and easily available medicine. More of these trees should be planted by roads and in large gardens. Elderly ladies of Tamilnadu who believed in traditional medicines have an effective cure for young girls suffering from dysmenorrhoea (pain and discomfort during menstruation) (http://www.hindu.com).

The antiarthritic effect of oral administration of ethanolic extract of *Delonix elata* on Freund's adjuvant induced arthritis has been studied in Wistar albino rats. The loss of body weight during the arthritic condition was corrected on treatment with ethanolic extract of *Delonix elata* at 250 and 500 mg.kg-1body weights. The swelling of the paws during the secondary lesions was also markedly reduced on treatment with ethanolic extract of *Delonix elata* and this results was confirmed using radiographic analysis and the changes in the density of Hind Limb Bone Mass (HLBM) was measured using photodensitometer and aluminium step wedge. The HLBM was significantly reduced on treatment with ethanolic extract (250 and 500 mg.kg-1body weight) of *Delonix elata* and standard drug

Indomethacin (10 mg.kg-1) (Kilimozhi, 2009). The seed oil of *Delonix elata* contains palmitic (18.8%) and stearic (14.6%) acids as the major component fatty acids among the saturated acids, with a small amount of myristic acid (0.4%) (Daulatabad *et al.*, 1987). *Delonix alata* (L.) parts such as barks and leaves are boiled together and the decoction is given to cattle for the treatment of babesiosis (Minja, 1999).