2

REVIEW OF LITERATURE

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Sidha. Only few of them were scientifically explored. In India several plants have been inferred for various studies for their medicinal value. Numerous studies have shown that traditional medicine comprises medicinal plants, minerals and organic matter. Medicinal plants are natural sources yielding valuable herbal products, which are often used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different to chemical constituents isolated from their crude extracts. Of particular importance, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects in various degenerative diseases, including cancer, neurological and cardiovascular diseases (Sudhakar *et al*., 2006 and Rather *et al.,* 2010).

The Review of literature pertaining to the study **"Antitumorigenic Effect in DLA Tumor Induced Mice and Antimicrobial Potential of *Ficus racemosa* and its Characterization by Spectral and *in silico* Studies"** is presented under the following headings:

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2.1. Free radicals

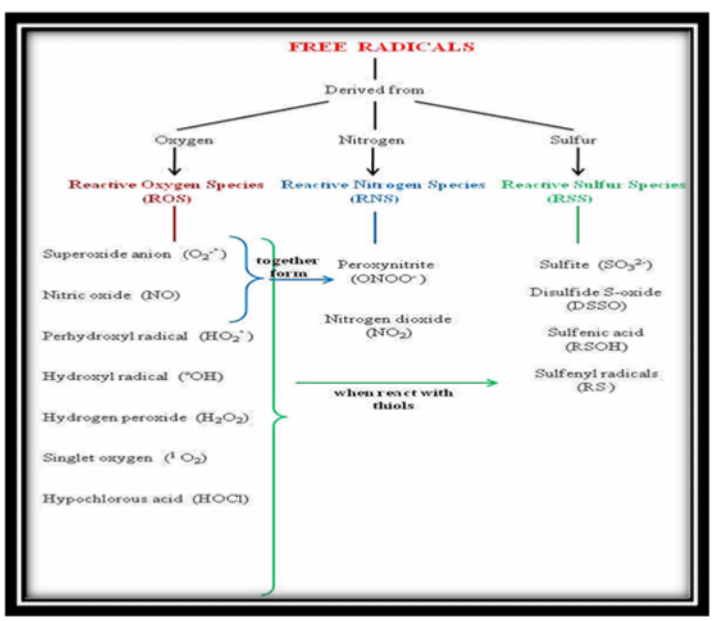
A free radical can be defined as any atom or molecule possessing one or more unpaired electrons. A major source of free radicals in biological systems is molecular oxygen. They are formed when oxygen interacts with certain molecules. Once formed, these highly reactive radicals can start a chain reaction hence they have significant biological importance. They are generally unstable and very reactive. The biologically relevant free radicals derived from oxygen are the superoxide anion (O_2^-), the perhydroxyl radical (protonated superoxide and HO_2), the hydroxyl radical ($HO^\cdot$) and free radical nitric oxide (Cuzzocrea *et al.,* 2001).

Free radicals are highly reactive compounds, which are mostly generated during cellular respiration and normal metabolism. Possession of unpaired electrons in their outer shell causes them to be more reactive than their corresponding non-radicals, because they act as electron acceptors and essentially “steal” electrons from other molecules and thereby modify their chemical structures, action which is referred to as oxidation. Free radicals are liberated from a variety of sources, including inflammatory cells, dysfunctional mitochondria and excitotoxic mechanisms that are stimulated by increased glutamate and aspartate concentrations (Eghwrudjakpor and Aillison, 2010 and Shekhawat *et al.,* 2010).

Free radicals can be produced from enzymic and non enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The non-enzymatic process can also occur during oxidative phosphorylation in the mitochondria (Droge, 2002 and Valko *et al.,* 2007).

Figure 1

Types of free radicals



(Mathew *et al.,* 2011)

Free radicals are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation,

inflammation, mental stress, excessive exercise, ischemia, infection, cancer and aging. Exogenous free radicals result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe and As), certain drugs (cyclosporine, tacrolimus, gentamycin and bleomycin), industrial solvents, cooking (smoked meat, used oil and fat), radiation (Young and Woodside, 2001; Valko *et al.,* 2005 and Parthasarathy *et al.,* 1999). After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals. Free radicals may be oxygen derived (ROS) reactive nitrogen species (RNS) and (Figure 1) Reaction Sulfur Species (RSS) may arise from common exogenous and endogenous processes (Cooke *et al.,* 2003).

2.1.1. Reactive oxygen species

The generation of Reactive Oxygen Species (ROS) is a normal process that occurs during the oxidation - reduction of the respiratory chain as well as in other body functions. In case of prolonged exercise, the increased oxygen consumption generates an increase in ROS, representing a challenge for the limited capacity of antioxidant systems in different tissues (Kormanovski *et al.,* 2011).

Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide are highly reactive and potentially damaging transient chemical species. These species play a dual role as both toxic and beneficial compounds (Matute *et al.,* 2009). These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damages the cell structures, DNA, lipids and proteins (Valko *et al*., 2006).

Molecular oxygen is mainly utilized for oxidative phosphorylation many other reactions as a substrate for metabolic activities being catalyzed by oxygenases, oxidases and hydroxylases enzymes. The ability to utilize oxygen for metabolization of fats, protein and carbohydrates for energy is at a cost of

generating partially reduced oxygen species including free radicals (Singh *et al.,* 2009).

At low or moderate concentrations, ROS exert beneficial effects on biochemical responses such as signal transduction, immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including the damage of macromolecules such as lipids, proteins and DNA (Pham-Huy *et al.,* 2008: Uttara and Mishra, 2008 and Khanavi *et al.,* 2009). Interaction of these reactive species with nucleic acids may lead to a wide variety of nucleobase products, deoxyribose products, strand breaks and DNA crosslinks. Many of the modifications are substrates for DNA repair. However, a consequence of unrepaired damage is, potentially, mutations, which can lead to cancer (Cooke *et al.,* 2003).

2.1.1.1. Hydroxyl radical

The hydroxyl radical is important in radiobiological damage and is more reactive towards cellular constituents than superoxide radicals. When superoxide and hydrogen peroxide react together they produce hydroxyl radicals (Coyle and Puttfarcken, 1993). Hydroxyl radicals are produced from hydrogen peroxide in metal-catalyzed redox reaction such as the Fenton reaction (Naik *et al.,* 2006b).

$$Fe^{3+} + {}^{\bullet}O_2^{-} \longrightarrow Fe2^{+} + O_2.$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{-} + OH^{\bullet}$$
$$Fe^{3+} + HO^{-} \longrightarrow Fe^{2+} + OH^{\bullet}$$

(Lipinski, 2011).

Hydroxyl radical is the most reactive oxygen species (ROS). It has the shortest half-life compared with others and is considered to be responsible for much of the biological damage in free radical pathology (Jelili *et al.,* 2010). The hydroxyl radical in the cells can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing hydroxyl radical is very important for the protection of living systems (Luo *et al.,* 2010 and Chanda *et al.,* 2011).

2.1.1.2. Superoxide anions

Superoxide anion is normally formed during the first step of cellular oxidation reaction and they magnify their effect by producing other kinds of cell damaging free radical and oxidizing agents (Liu and Ng, 2000). Superoxide anion is the first generated reactive oxygen species (ROS) after oxygen enters living cells. It was once considered to be highly deleterious to cell functions and aging. Superoxide signaling has been shown in many physiological responses such as transcriptional regulation protein activation, bioenergy output, cell proliferation and apoptosis (Wu *et al.,* 2010).

The superoxide free radical anion is formed when the transfer of a single electron to its outer shell reduces oxygen. Superoxide anion radical is generated via several cellular oxidase systems such as NADPH oxidase, Xanthine oxidase and peroxidases. Once formed, it participates in several reactions (Pham-Huy *et al.,* 2008), yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical ($OH^{\cdot}$), peroxyl nitrite ($ONOO^{-}$) and hypochlorous acid (HOCl). Among ROS, the superoxide anion ($O_2^{\bullet -}$) plays a pivotal role in inflammation particularly in patients with inflammatory joint disease (Afonso *et al.,* 2007).

The major source of superoxide *in vivo* is the electron leakage that results from the electron transfer chain of the mitochondria. Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA in living systems (Wu and Cederbaum, 2004 and Luo *et al.,* 2010).

They have also been shown to be a key contributor in the secondary events following brain injury and the formation of the oxygen radical. Superoxide anion is believed to be one of the final events of several metabolic pathways in the cascade which leads to delayed neuronal death after traumatic or ischemic brain injury (Eghwrudjakpor and Aillison, 2010).

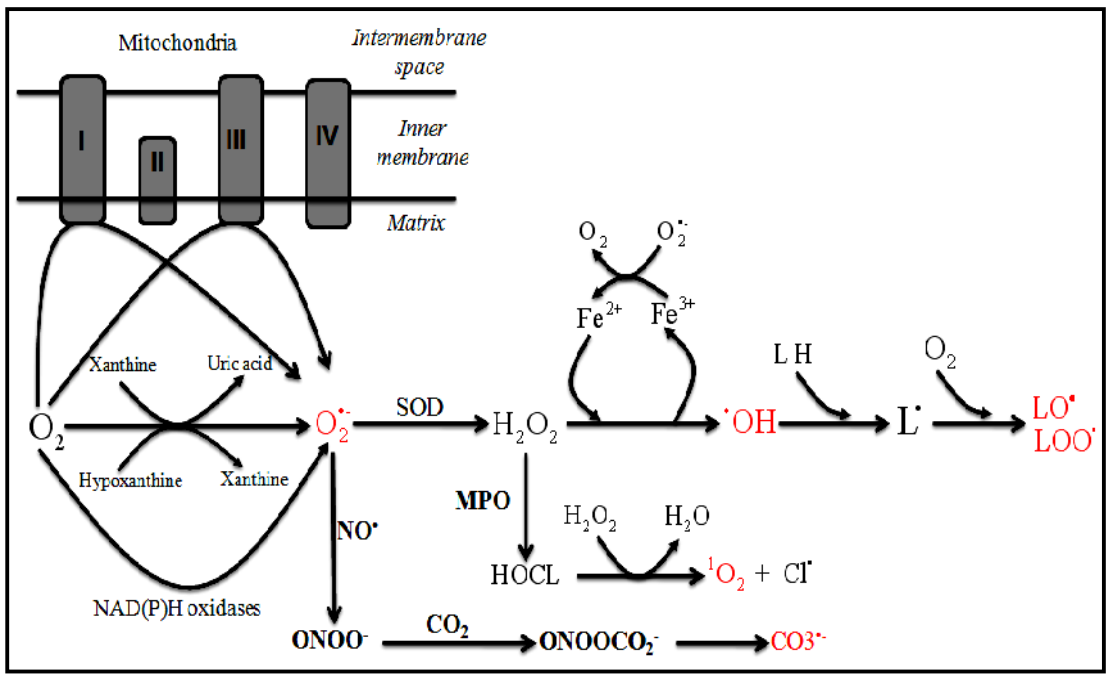
A major portion of the biological consumption of molecular oxygen occurs during reduction to water via oxidative phosphorylation in mitochondria. However a small portion of the total oxygen consumed is reduced in a specific pathway

yielding superoxide and hydroxyl free radical all of which can be potentially damaging the respiring cells (Vuillaume, 1987). Superoxide anion radical is known as an initial radical and plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide or singlet oxygen in living systems (Luo *et al.,* 2010).

The superoxide anion radical ($O_2•^-$) is generated via several cellular oxidase systems such as NADPH oxidase, xanthine oxidase and peroxidases. The Figure 2 shows the production of free radicals. Once formed, it participates in several reactions yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical (OH•), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) (Valko *et al.,* 2004; Bahorun *et al.,* 2006 and Genestra, 2007).

Figure 2

Production of free radicals



(Kunwar and Priyadarsini, 2011)

2.1.1.3. Hydrogen peroxide

Non-radicals containing two electrons per orbital, which is a stable configuration in a molecule, include singlet oxygen, hydrogen peroxide, hypochlorous acid and nitric oxide (Saikat *et al*., 2010).

Hydrogen peroxide is not a radical because it has no unpaired electron, and has limited reactivity and permeability to biological membrane unlike the charged O_2^- (Halliwell and Gutteridge, 1984). Hydrogen peroxide is not a free radical but falls in the category of reactive oxygen species (Halliwell *et al.,* 2000). It is an oxidizing agent that is not particularly reactive but its main significance lies in that it is the main source of hydroxyl radicals in the presence of transition metal ions. Hydrogen peroxide can be generated from the two-electron reduction of oxygen. In biological systems hydrogen peroxide is generated by the production of superoxide. Two superoxide molecules can react together to form hydrogen peroxide and oxygen (Winston and Di Giulio, 1991).

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

The ability of H_2O_2 to initiate lipid peroxidation is dependent on its ability to generate hydroxyl radical through the Fenton reaction. Mitochondrial derived oxygen is dismutated to hydrogen peroxide by manganese superoxide dismutase. Hydrogen peroxide itself is not very reactive but it can sometimes be toxic to the cells, it may give rise to hydrogen radicals in the cells. Superoxide converts superoxide anion radical produced in the body to hydrogen peroxide and catalase decomposition of hydrogen peroxide to water and oxygen (Noyan *et al.,* 2005). Harber Weiss reaction explains that both H_2O_2 and superoxide radical are required in the presence of metal catalyst for the formation of hydroxyl radical, the oxygen species largely responsible for the damage of macromolecules (Amanda *et al*., 2007).

Hydrogen peroxide is commonly taken as an indicator of oxidative stress because it is induced by active oxygen species and also influencing the level of lipid peroxidation. However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and /or peroxidase (POD) to water and oxygen (Ahmadizadeh *et al.,* 2011).

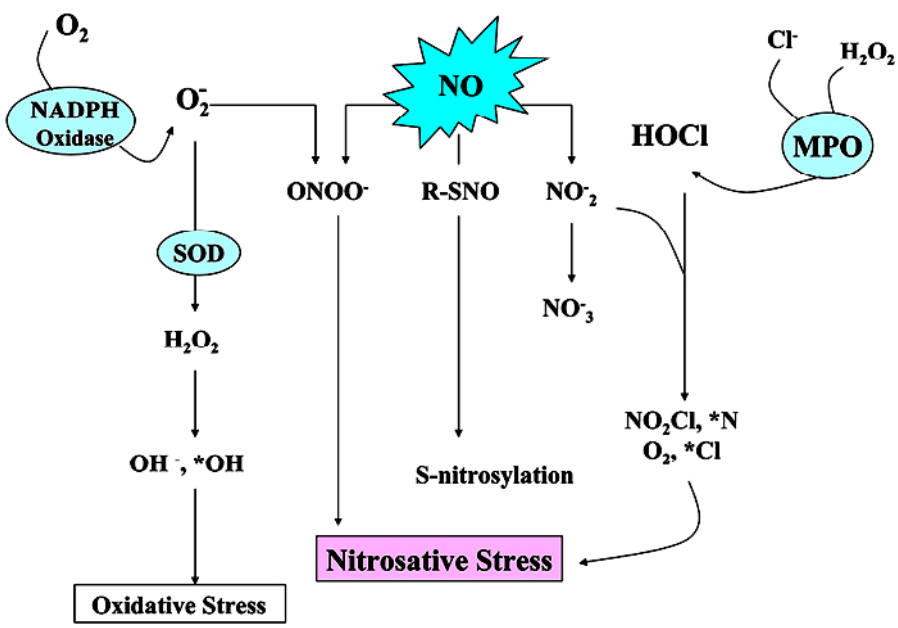
2.1.2. Reactive nitrogen species (RNS)

The RNS possess very short half-life and exhibits high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids leading to

cell death. The reactive nitrogen species are a family of antimicrobial molecules derived from nitric oxide (NO) and superoxide ($O_2^{\cdot-}$) produced via the enzymatic activity of inducible nitric oxide synthase 2 (NOS_2) and NADPH oxidase respectively. NOS_2 is expressed primarily in macrophages after induction by cytokines and microbial products, notably interferon-gamma (IFN-γ) and lipopolysaccharide (Iovine *et al*., 2008). The Figure 3 shows the overview of generation of reactive nitrogen species. RNS act together with reactive oxygen species (ROS) to damage cells, causing nitrosative stress. Therefore, these two species are often collectively referred to as ROS/RNS (Naskar *et al*., 2011).

Figure 3

Generation of reactive nitrogen species



(Eleuteri *et al.,* 2009)

2.1.2.1 Nitric oxide

Nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Agarwal and Prabakaran, 2005). The toxicity of NO increases

greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite (Pourmorad *et al.,* 2006).

Nitric oxide is an intermediary molecule in various biological pathways. In vertebrates, nitric oxide is synthesized through the reaction of L- arginine with oxygen by the catalysis of nitric oxide synthase enzyme. The production of nitric oxide is necessary for the nonspecific host defense that helps to kill intracellular pathogens and tumors (Batcioglu *et al.,* 2006).

Saha *et al.* (2010a) stated that NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Overproduction of NO can mediate toxic effects such as DNA fragmentation, cell damage and neuronal cell death. During infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects like tumor growth. The peroxynitrite produced during the reaction of NO with O_2^- is probably responsible for genetic damage (Roberfroid and Calderon, 2008).

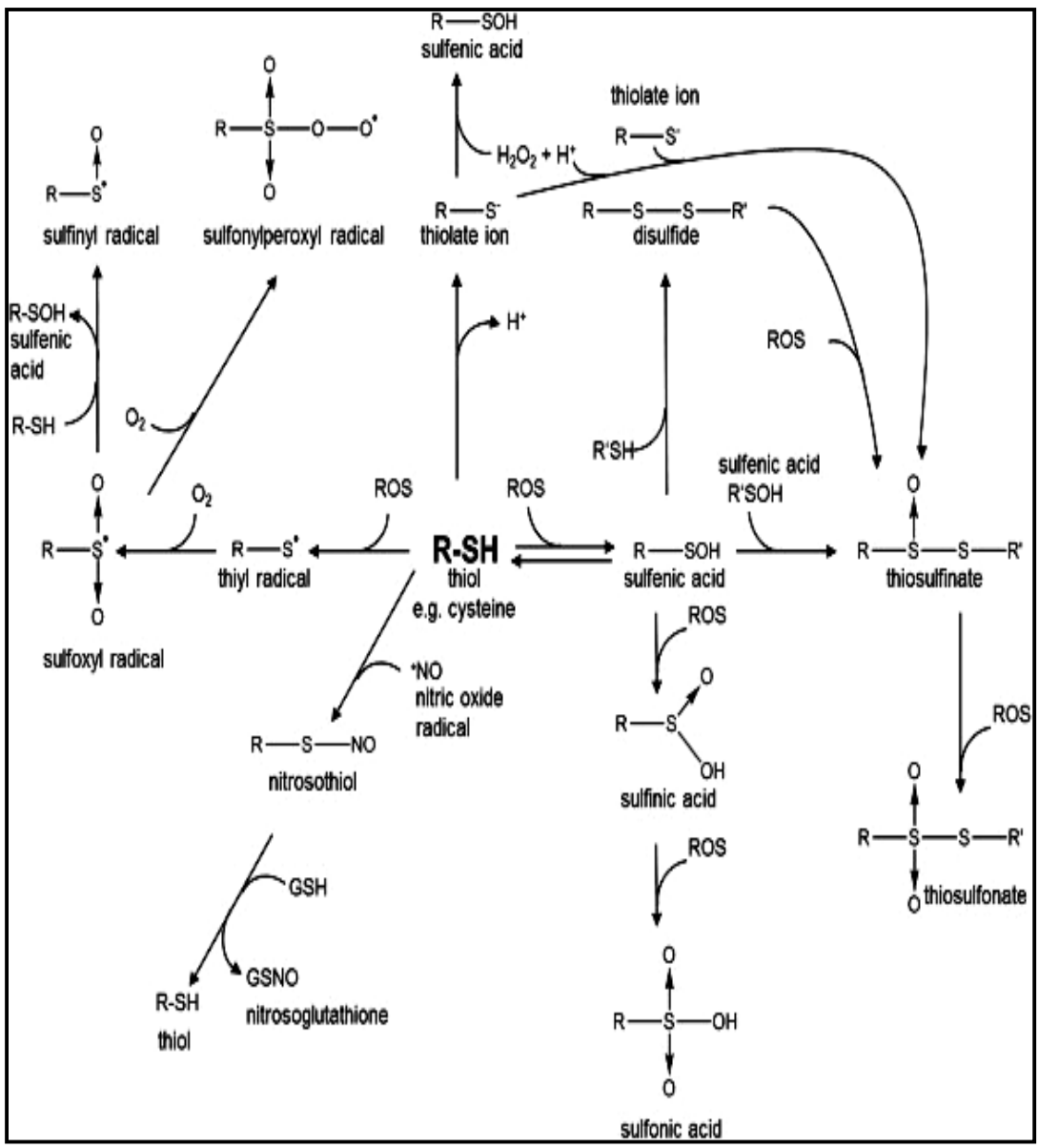
Nitric oxide is also implicated for inflammation, cancer and other pathological conditions. Nitric oxide (Reactive Nitrogen Species) formed during their reaction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4, NO_3 and NO_2 is very reactive and these are responsible for altering the structural and functional behavior of many cellular components (Hassan and Hassan, 2010 and Sasikala *et al.,* 2011).

2.1.3. Reactive Sulfur Species (RSS)

Several naturally occurring S-contatining molecules, cysteine, methionine, glutathione and Fe-S clusters are themselves RSS and because they are physiologically active they make up part of the intrinsic plant defence repertoire against herbivore and pathogen attack. Furthermore, RSS can also be used as redox-active pharmacological tools to study cell metabolism (Gruhlke and Slusarenko, 2012). The Figure 4 shows the generation of various reactive sulfur species.

Figure 4

Generation of reactive sulfur species



(Gruhlke and Slusarenko, 2012)

2.1.4. Synthetic free radicals

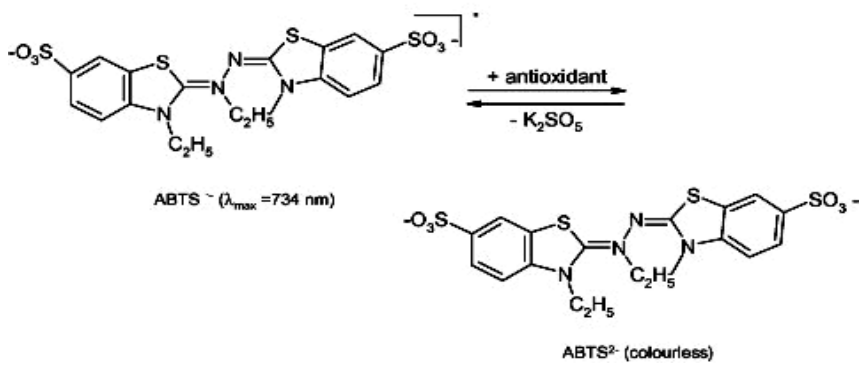
Synthetic free radicals have been extensively used for screening and also used to evaluate the antioxidant capacity of complex mixture and individual components (Henriquez *et al.,* 2002).

2.1.4.1. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)

The ABTS a more drastic radical chemically produced often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids from the reaction of ABTS with potassium persulfate overnight in water (Figure 5).

Figure 5

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS)



(Zulueta *et al.,* 2009)

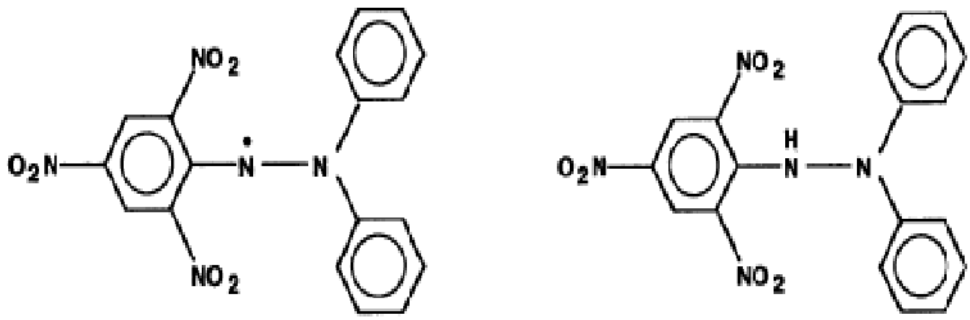
The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity (Huang *et al.,* 2011). The decolorization of ABTS radical refects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants (Karthika *et al.,* 2012).

2.1.4.2. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH)

The DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. DPPH is a stable free radical that shows maximum absorption at 517nm in methanol. The effect of antioxidants on DPPH radical scanvenging was conceived to be due to their proton-donating ability (Figure 6). In DPPH test, the antioxidants were able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. The antioxidative activity of a substance can be expressed as its ability to scavenging the DPPH free radical (Luo *et al*., 2010).

Figure 6

Diphenylpicrylhydrazyl radical (DPPH)



a) Diphenylpicrylhydrazyl (free radical)

b) Diphenylpicrylhydrazine (non radical)

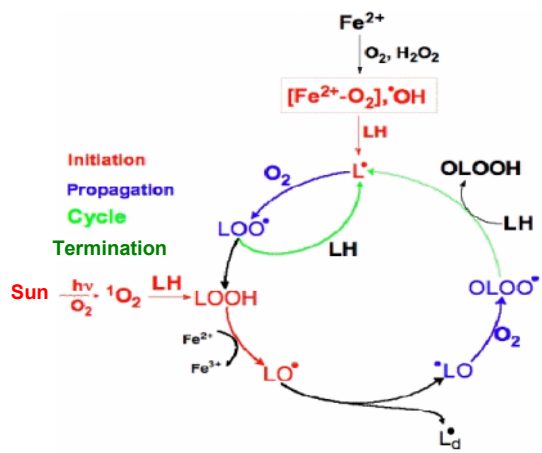
(Molyneux, 2004)

Lipid peroxidation

Lipid peroxidation (LPO) is an autocatalytic process, which is a common cause of cell death. Decomposition of lipid peroxides initiates the chain reactions that produce reactive carbonyl compounds. The by-products of lipid peroxidation are the toxic compounds MDA and LH whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability (Umamaheswari *et al.,* 2011).

Figure 7

Mechanism of Lipid peroxidation



http://www.photobiology.info/Buttner.html.

Lipid peroxidation is initiated by free radical attack on cell membrane PUFA which generates large amount of toxic radical products that are implicated in tumor initiation and promotion of colorectal cancer (Bhagat *et al.,* 2011).

Lipid peroxidation is initiated by the attack on a fatty acid or fatty acyl side chain of any chemical species (Figure 7). Especially the group of polyunsaturated fatty acids (PUFAs) is highly susceptible to reactions with free radicals. Peroxidation of fatty acids in lipids may lead to a radical chain reaction (Rajeshwari and Andallu, 2011).

2.2. Oxidative stress

Oxidative stress has been generating much interest primarily because of its accepted role as a major contributor to the etiology of both normal senescence and severe pathologies with serious public health implications such as neurogenerative diseases, diabetes, obesity, cancer and atherosclerosis. The triggering factors for oxidative stress may be diverse, ranging from genetic or environmental factors to pure stochastic events such as metabolic fluctuations. Oxidative stress can results from diminished levels of antioxidants but can also result from increased production of reactive species. Reactive species behave as true second messengers that control important cellular functions (Matute *et al.,* 2009 and Durackova, 2010). Oxidative stress is a condition in which the cellular production of reactive oxygen species exceeds the physiological capacity of the antioxidant defense system to render ROS inactivates. Oxidative stress may be increased in diabetic patients (Sankar *et al.,* 2010 and Rajeshwari and Andallu, 2011).

Oxidative stress increase causes continuous increase in the concentration of lipid peroxidation products and decrease in the level of enzymatic and nonenzymatic antioxidants.Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. In other words, oxidative stress results from an imbalance between formation and neutralization of ROS/RNS. Hydroxyl radical and peroxy nitrite in excess can damage cell membranes and lipoproteins by a process called lipoperoxidation. Oxidative stress

defines as a pervasive condition of increased and /or inadequate removal of ROS (Ahmad *et al.,* 2008). Oxidative stress significantly impacts multiple cellular pathways that can lead to the initiation and progression of varied disorders throughout the body (Maiese *et al.,* 2010).

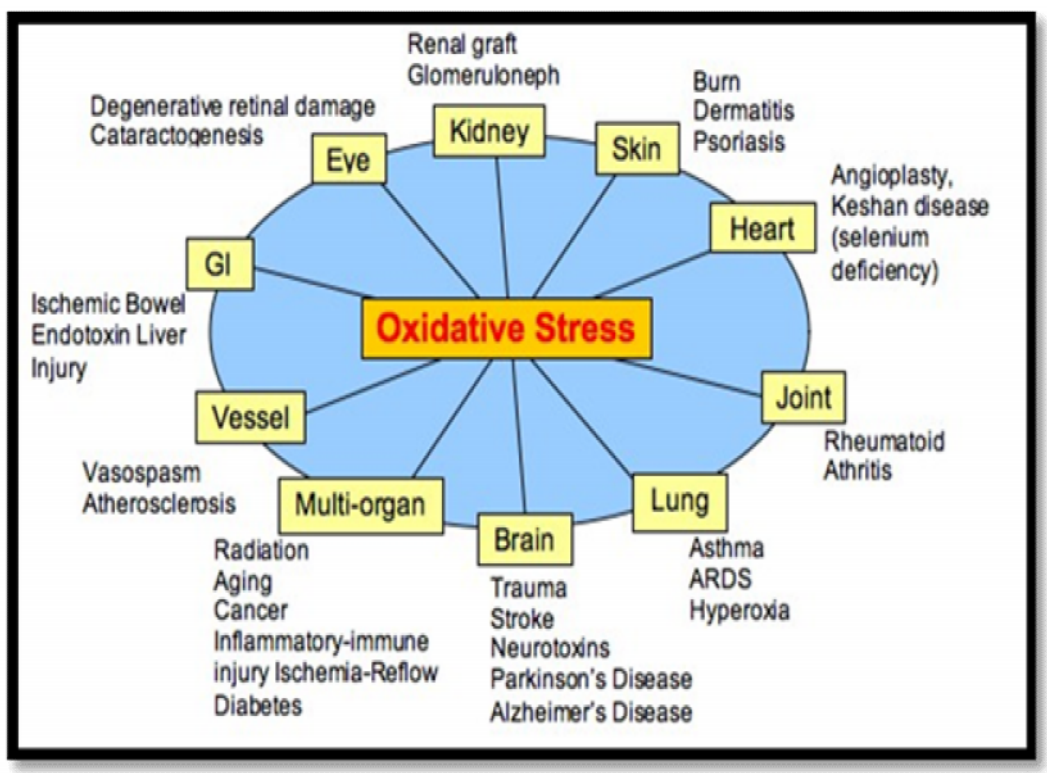
Oxidative Stress Induced Diseases

Cancer

Cancer is one of the most prominent human diseases that have stimulated scientific and commercial interest in the discovery of new anticancer agents from natural sources (Sowemimo *et al.,* 2009). The development of cancer in humans is a complex process including cellular and molecular changes mediated by diverse endogenous and exogenous stimuli. It is well established that oxidative DNA damage is responsible for cancer development (Figure 8).

Figure 8

Oxidative stress in human



(http://www.enzoprofessional.com/default.aspx?Page=2365)

Cancer initiation and promotion are associated with chromosomal defects and oncogene activation induced by free radicals. A common form of damage is the formation of hydroxyl bases of DNA, which are considered as an important event in chemical carcinogenesis (Halliwell and Gutteridge, 2007). This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Oxidative DNA damage also produces a multiplicity of modifications in the DNA structure including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites (Brambilla *et al*., 2008). Cancer is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues) and sometimes metastasis (spread to other locations in the body via lymph or blood) (Jagatheesh *et al.,* 2010).

2.3. Antioxidants

Antioxidants may be defined as radical scavengers that protect the human body against free radicals. Previous epidemiological studies have shown that the intake of natural antioxidant is associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging (Mustafa and Thunibat, 2008). The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated *in situ* (endogenous antioxidants) or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals to protect the cells against their toxic effects and to contribute to disease prevention (Pham-Huy *et al.,* 2008).

Antioxidants can be of synthetic origin and a great number of secondary metabolites are isolated from plants, such as various phenolic compounds. Antioxidants work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function. Several herbs and plant spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme and turmeric. The majority of the active antioxidant compounds are flavanoids, lignans, catechins and isocatechins. In addition to the

above compounds found in natural foods, vitamins C and E, β-carotene and tocopherol are known to possess antioxidant potential (Ara and Nur, 2009). Many human diseases are caused or negatively affected by free radicals. Antioxidants either exogenous or endogenous whether synthetic or natural can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (Santhi and Annapoorani, 2009).

Some medicinal plants such as Ginkgo (*Gingo biloba* L., *Ginkgoaceae*) and Green tea (*Camellia sinesis* L. (Kuntze), *Theaceae*) are very important regarding the content of natural antioxidant substances and are widely used in folk medicine and pharmacy (Kratchanova *et al.,* 2010 and Stankovic *et al*., 2010). Antioxidants interact with and stabilize free radicals and may prevent some of the damages created by free radicals. Propagation and initiation of free radicals chain reaction can be delayed or minimized by the donation of hydrogen from the antioxidants (Hamad *et al.,* 2010).

2.3.1. First line defense antioxidants

Antioxidants can be classifieds into three main types: first line defense antioxidants, second line defense antioxidants and third line defense antioxidants. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR) and some minerals like Se, Mn, Cu and Zn comes under first line defense antioxidants. Reduced glutathione (GSH), Vitamin C, Vitamin E, uric acid, carotenoids, albumin, bilurubin, Vitamin A and flavonoids come under second line defense antioxidants (Gupta and Sharma, 2006). Lipase, proteases, DNA repair enzymes, transferases and methionine sulphoxide reductase come under third line defense antioxidants (Irshad and Chaudhuri, 2002).

The first line of defense is the preventive antioxidants, which suppress formation of free radical (enzymes such as catalase, superoxide dismutase and glutathione peroxidase). The first lines of defense against hydrogen peroxide mediated injury are antioxidant enzymes like CAT, SOD and GPx (Bukan *et al.,* 2003 and Burlakova *et al.,* 2010).

2.3.1.1. Catalase

Catalase, the heme-containing enzyme found in aerobic eukaryotes is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, glyoxylate cycle (Photo respiration) and purine catabolism. It finishes the detoxification reaction started by SOD (Rahman, 2007).

$$H_2O_2 \longrightarrow 2H_2O + O_2$$

The catalase (CAT) catalyses dismutation of H_2O_2 (catalytic mode) or use H_2O_2 to oxidize substrates such as methanol, ethanol, formaldehyde, formite or nitrite (Lenzen, 2008). Catalase and peroxidases are important enzymes present in the intracellular spaces, where they can regulate the level of H_2O_2, catalase is a haem protein with an extremely high turnover rate (Singh *et al*., 2009).

2.3.1.2. Glutathione peroxidase

Glutathione peroxidase is a group of enzymes consisting of several compounds such as glutathione peroxidase, glutathione reductase and the co-factor glutathione and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) the most abundant of which contain selenium. These enzymes, like catalase, play a vital role in H_2O_2 catabolism and the detoxification of endogenous metabolic peroxides and hydroperoxide, which catalyzes GSH. They also reduce organic peroxides to alcohols, providing another route for eliminating toxic oxidants (Gallo and Martino, 2009 and Manjusha *et al.,* 2011).

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$$ (Finaud *et al.,* 2006)

2.3.1.3. Glutathione reductase

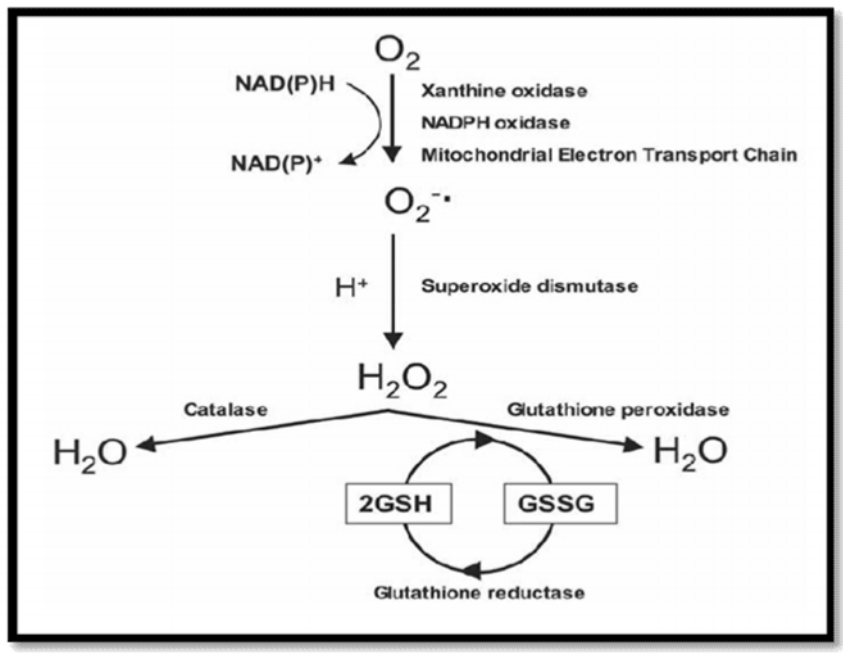
Glutathione reductase also known as GSR or GR is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl from GSH, which is an important cellular antioxidant. Glutathione reductase plays important role in protecting haemoglobin red cell enzymes and biological cell membrances against oxidative damage by increasing the level of reduced glutathione in the process of aerobic glycolysis (Manjusha *et al.,* 2011). Glutathione (GSH) plays a central role in coordinating cellular oxidant defense processes and is present in high

concentration in all cells (Qiao *et al.,* 2010). The Figure 9 and 10 shows the action of enzymic antioxidants.

$$GSSG + NADPH + H^{+} \xrightarrow{GRD} 2GSH + NADP^{+}$$ (Casao *et al*., 2010)

Figure 9

Action of enzymic antioxidants



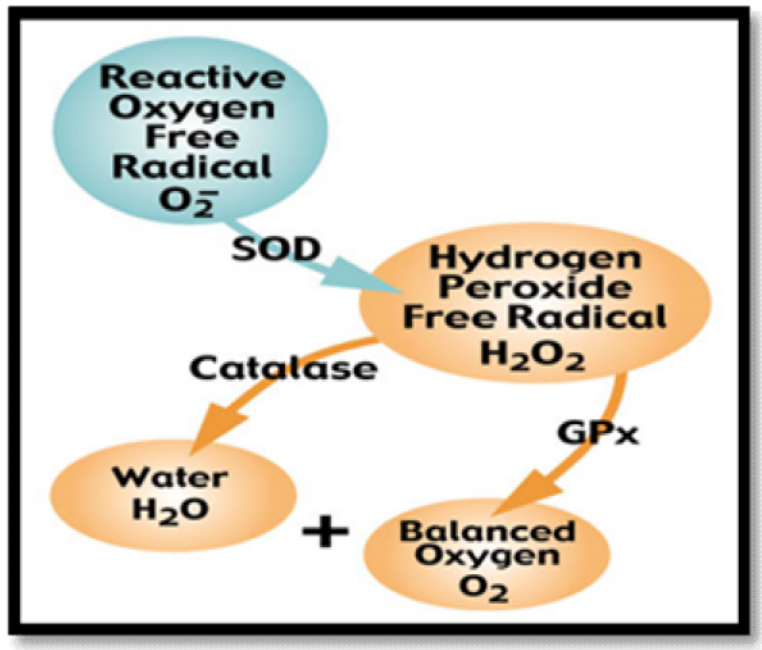
(Bukan *et al.,* 2003 and Pandey and Rizvi, 2010)

2.3.1.4. Superoxide dismutase

Superoxidase is an enzyme that repairs cells and reduce the damage done to them by superoxide, the most common free radical in the body. It protects oxygen metabolizing cells against harmful effects of superoxide free radicals. It is an endogenously produced enzyme present both in prokaryotes and eukaryotes is a group of metallo enzymes with various prosthetic groups. Cu-Zinc SOD in the cytoplasm with two subunits and sensitivity to cyanide and hydrogen peroxide. Mn SOD in the mitochondrial matrix and in prokaryotes and is insensitive to cyanide. Fe SOD usually found in prokaryotes and in the chloroplasts of some plants (Singh *et al.,* 2009).

Figure 10

Superoxide dismutase (SOD)



(http://www.glisodininfo.com/images/GliSODin%2520Chart2.jpg&imgrefur)

2.3.2. Second line defense antioxidants

The antioxidant enzymes are complemented by small molecule second line defense antioxidants. The small molecule antioxidants are present extracellularly, intracellularly and include Vitamin A, E, C, glutathione, uric acid, carotenoids, albumin and bilurubin (Gupta and Sharma, 2006).

2.3.2.1. Vitamin A

Vitamin A is a fat-soluble vitamin, which is essential for growth maintenance and differentiation of epithelial cells. β-carotene, present in cell membranes, is converted into Vitamin A when the body needs it (Finaud *et al.,* 2006). Vitamin A breaks the chain of lipid peroxidation to cell membrane and prevents the formation of lipid peroxide (Lira and Dimenstein, 2010).

$$\beta\text{-Carotene (CAR)} + LOO^{\circ} \longrightarrow LOO - CAR^{\circ}$$

$$LOO - CAR^{\circ} + LOO^{\circ} \longrightarrow LOO - CAR - OOL$$

2.3.2.2. Vitamin C

Ascorbic acid (Vitamin C) consists of a 6- carbon lactone ring with 2, 3 - enediol moiety and shows antioxidant activity due to enediol group. It is a leading natural antioxidant that can scavenge ROS and has anticarcinogenic effects. The antioxidant mechanism of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and removal of molecular oxygen. Scavenging aqueous radicals and regenerating alpha-tocopherol from the tocopheroxyl radical are also one of its well-known antioxidant properties. It is an excellent electron donor because of low standard one electron reduction potential (Prakash and Gupta, 2009).

Vitamin C (AA) is important water-soluble compound that fulfill several roles in living system and its important sources include citrus fruits (such as oranges and sweet lime). It is a chain breaking antioxidant and is found both intracellularly and extracellularly. It prevents lipid peroxidation due to peroxyl radicals and thereby neutralizes the reactive oxygen species such as H_2O_2 (Fusco *et al*., 2007 and Romieu *et al*., 2008).

$$AH + Fe^{3+} / Cu^{2+} \longrightarrow A + Fe^{2+} / Cu^{+}$$

$$Fe^{3+} / Cu^{+} + O_2 \longrightarrow O_2 + Fe^{3+} / Cu^{2+}$$

$$Fe^{3+} / Cu^{+} + H_2O_2 \longrightarrow OH + OH + Fe^{3+} / Cu^{2}$$

(Lutsenko *et al.,* 2002)

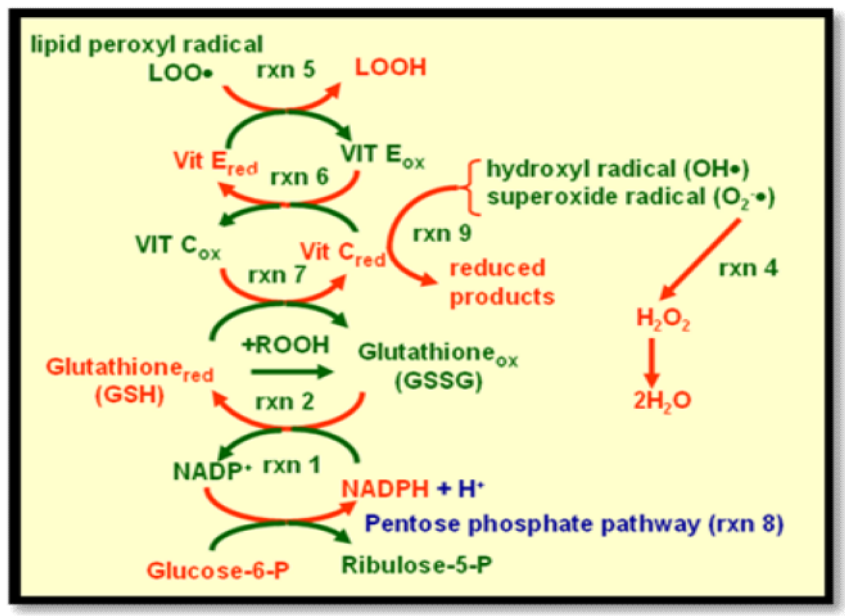
2.3.2.3. Vitamin E

Vitamin E refers to a family of eight molecules having a chromanol ring (chroman ring with an alcoholic hydroxyl group) and a 12-carbon aliphatic side chain containing two methyl groups in the middle and two more methyl groups at the end. Tocopherols and tocotrienols are non-polar constituents of biological membranes that exist in nature in lipid phase. Tocopherols consist of a chromane ring and a long saturated phytyl chain. Tocopherols commonly known as tocolsare 2- methyl 1-2-(4', 8'-trdecyl) chromane -6-ols, when 3 double bonds are present at positions 3,7 and 11 of the side chain I tocols they are called tocotrienols. The alpha and Kama and omega - tocols and tocotrienols differ in the number and

position of methyl groups attached to the 5, 7 and 8 position of the ring structure (Prakash and Gupta, 2009). α-Tocopherols are lipophilic antioxidants synthesized by all plants. α-Tocopherols interact with the polyunsaturated acyl groups of lipids, stabilize membranes, and scavenge and quench various reactive oxygen species (ROS) and lipid soluble byproducts of oxidative stress (Shao *et al.,* 2008). The Figure 11 shows the non enzymic antioxidants defense system.

Figure 11

Non enzymic antioxidant defense system



(http:// www.biochem.arizona.edu/classes/bioc460/summer/.../pentose.ppt)

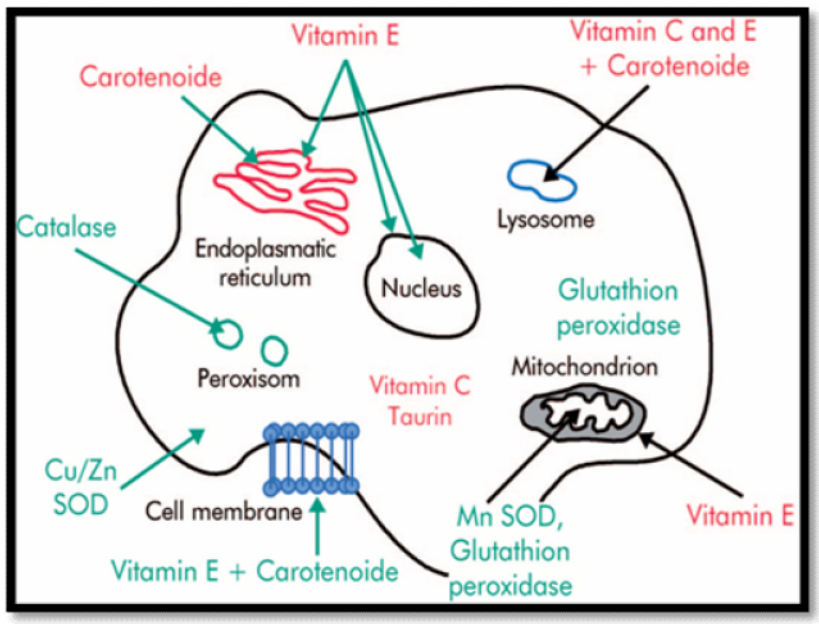
2.3.2.4. Reduced glutathione

Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and inturn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. Reduced glutathione, which is a substrate for glutathione peroxidase, neutralizes hydroxyl radicals and singlet oxygen since it is present in higher

concentration in the cells, it protects cell from free radical attack (Ahmad *et al.*, 2010). The Figure 12 shows the antioxidants defense system.

Figure 12

Antioxidant defense system



(www.royal-canin.de/uploads/pics/mono_3gif_01.gif)

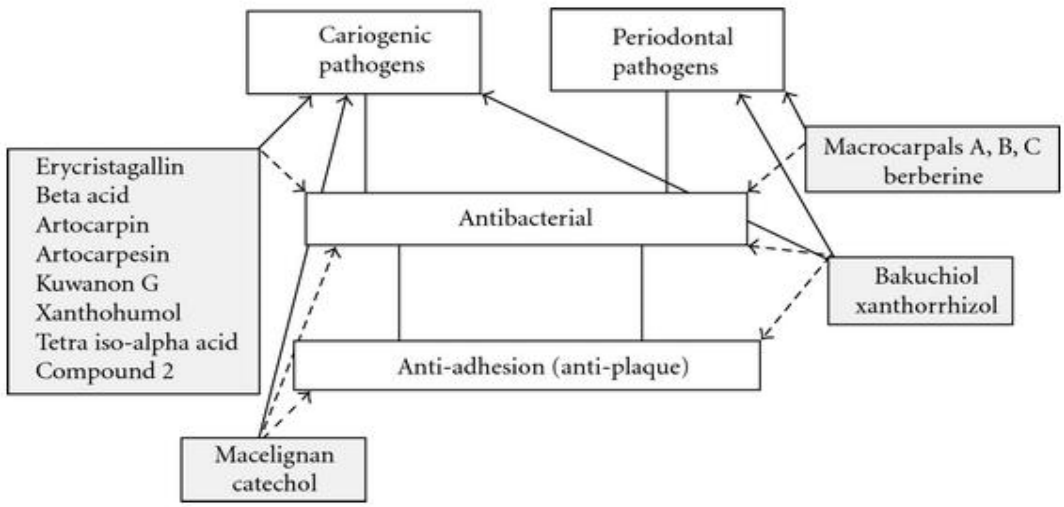
Phytochemical as antitumorigenic and antimicrobial agents

Some phytochemicals produced by plants have antimicrobial activity allowing these plants to be studied and used for the development of new antimicrobial drugs (Nascimento *et al.,* 2000). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity; it also facilitates pharmacology studies leading to the synthesis of pure and potent compounds with decreased toxicity (Manna and Abalaka, 2000). Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective, antimicrobial and antioxidant activity (DeFeudis *et al*., 2003). Figure 13 shows Potential application of phytochemicals in the prevention and treatment of oral diseases caused by cariogenic and periodontal microbial pathogens, where the likely uses of phytochemicals are

indicated with respect to their target pathogens (solid arrows) and biological activities (dashed arrows).

Figure 13

Phytochemicals in disases



(DeFeudis *et al*., 2003)

Knowledge of the chemical constituents of plants is very important, not only for the discovery of drugs and other therapeutics agents, but also in disclosing new sources of such economic materials as tannins, oils, gums and precursors for the synthesis of complex chemical substances. Additionally, knowledge of the chemical constituents of plants helps in discovery of the true relevance of folkloric medicines. Chemical constituents may be therapeutically active or inactive. The ones that are active are called active constituents and the inactive ones are known as inert chemical constituents (Mojab *et al.,* 2003).

In order to promote the use of medicinal plants as potential sources of antimicrobial compounds (Nair and Chanda, 2006; Deepa *et al*., 2011 and Dhanamani *et al.,* 2011). It is pertinent to thoroughly investigate their composition activity and thus validate their use. The effectiveness of phytochemicals in the treatment of various diseases may lie in their antioxidant effects (Akinmoladun *et al.,* 2007).

In fact, many phytochemicals, including polyphenols, are rapidly degraded and metabolized in the human body. Moreover, genetic variation in pathways affecting absorption, metabolism and distribution of these natural substances, could influence exposure at the tissue level, thus modifying disease risk in individuals. Nevertheless, this wide group of natural molecules represents a promising class as anticancer and antimicrobial drugs, since their multiple targets in cancer and microbial cells, with limited toxic effect on normal cells (Ross, 2007).

Phytochemicals are a potential alternative source of safer chemicals which are not only anticancerous but are also antioxidants, antidiabetic, antimutagenic and of other physiological benefits. These bioactive secondary metabolites show various degree of their individual antiproliferetive effect and induction of apoptosis in various types of human cancers in various cytotoxic pathways (Barh, 2008). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.,* 2008).

Phytochemicals can prove their therapeutic efficacy in mono-treatments or in association with classical chemotherapeutic drugs. In the latter case, a double positive effect can be expected: first, phytochemicals can synergize with cytotoxic drugs, increasing their efficacy and lowering the toxic side effects on normal cells; second, combined treatment can delay resistance onset (Manach *et al.,* 2009).

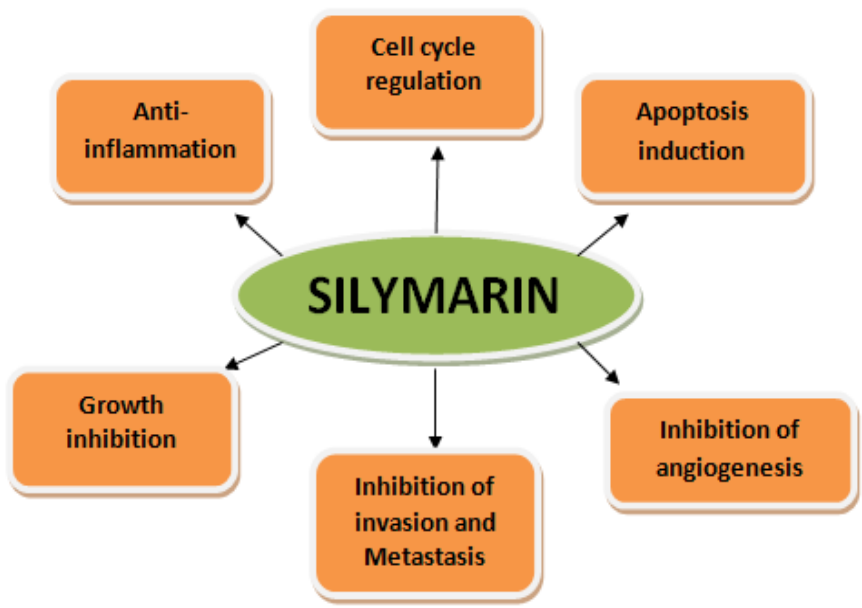
The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. The most important of these chemically active or bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Manjamalai *et al*., 2010).

Silymarin, a standardized extract obtained from seeds of *Silybum marianum* (*Asteraceae or Compositae*) is widely used in treatment of liver diseases of varying

origins and cancer (El-Samaligy *et al.,* 2006 and Dixit *et al.,* 2007). It consists of a mixture of three bioflavonoids found in the fruit, seeds and leaves of this plant namely Silybin, Silydianin and Silychristine (Khan *et al.,* 2006). Seeds of *S. marianum* have been used to treat liver and gall bladder disorders, including hepatitis, cirrhosis and jaundice and to protect the liver against poisoning from chemicals, environmental toxins, snake bites, insect stings, mushroom poisoning and alcohols (Ball and Kowdley, 2005 and Kren and Walterova, 2005). It also protects liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation and preventing liver glutathione depletion (Skottova *et al.,* 2003). The Figure 14 shows in the mechanism of action of silymarin.

Figure 14

Mechanism of action of silymarin



(Ramasamy and Agarwal, 2008)

Silymarin is an important bioactive principle having anticancer, antiinflammatory, antioxidant and immunomodulatory effects (Okawa *et al.,* 2001; Lebedev *et al.,* 2001; Fraschini *et al.,* 2002; Kohno *et al.,* 2002; Tyagi *et al.,* 2002; Yanaida *et al.,* 2002 ; Johnson *et al.,* 2003; Soto *et al.,* 2004 and Katiyar, 2005). It is also useful to treat alcoholic DNA damage; in addition to alcoholic liver injury

(Saravanan and Pugalendi, 2005).The dietary silymarin exerts a chemopreventive effect on 4-Nitroquinoline 1-oxide-induced rat tongue carcinogenesis, when fed during the promotion phase. This cancer protective effect of silymarin might relate to the control of carcinogen-induced hyper-cell proliferation and/or alteration of the amino acid metabolic pathway (Sobolova *et al.,* 2006).

2.4. Antitumor activity of medicinal plants

Cancer is the second leading cause of death in the Western world. Cancer is one of the life threatening diseases with more than 200 different types. A tumor, or mass of cells, formed the abnormal cells may remain within the tissue in which it originated (a condition called *in situ* cancer) or it may begin to invade nearby tissues (a condition called invasive cancer). An invasive tumor is said to be malignant and cells shed into the blood or lymph from a malignant tumor are likely to establish new tumors (metastases) throughout the body (Madhusudan and Middleton, 2005).

Numerous drugs and compounds have been reported to have antitumor effects on different organ cancer such as lung, liver, breast and ovarian (Llovet *et al*., 2003). Isolation and identification of some potent antitumor compounds from plants has encouraged scientists to screen different parts of plant species against cancer cell lines (Emami *et al*., 2005). Shylesh *et al.* (2005) have shown the induction of cell-specific apoptosis and protection from DLA challenge in mice by an active fraction from *Emillia sonchifolia.* The result showed that the hexane extracts was found to be most active and *in vitro* cytotoxicity to DLA and thymocytes. The aqueous extract from the roots of *Glycyrrhiza glabra* inhibits the *in vivo* and *in vitro* proliferation of ELA tumor cells and may be used as a potential supplemental source for cancer therapy (Sheela *et al*., 2006).

Qin *et al*. (2006) reported that vaccination with pSLC-3P-Fc (DNA vaccine) by gene gun inoculation induced a strong antitumor response in a mouse tumor model, which significantly inhibited tumor growth and prolonged the survival time of the tumor-bearing mice. Derivatisation of diospyrin, a bisnaphthoquinonoid isolated from *Diospyros montana* Roxb., led to the modification of its inhibitory

activity towards a murine tumor model, ELA and two human cancer cell lines: (A375) malignant skin melanoma and (Hep2) epidermoid laryngeal carcinoma (Sarma *et al*., 2007).

The effects of the anticancer drug irinotecan combined with methanolic extract of propolis, a water-soluble derivative of propolis, quercetin and naringin on the growth of ELA tumor and the life span of tumor bearing Swiss albino mice may be beneficial in maximizing antitumor activity and minimizing post-chemotherapeutic reactions to the cytostatic drug (Benkovic *et al*., 2007). Similar antitumor properties of *Zanthoxylum rhoifolium* Lam leaves was investigated *in vitro* and *in vivo* using the ELA tumor model (Silva *et al*., 2007). The extract of *Tinospora cordifolia* (Guduchi) against ELA tumor mice resulted in growth inhibition and induction of apoptosis in a dose-dependent manner (Thippeswamy and Salimath, 2007).

Rajkapoor *et al.* (2007) have evaluated the antitumor and cytotoxic activity of methanol extract of *Phyllanthus polyphyllus* (MPP) on ELA tumor mice and human cancer cell lines. Oral administration of MPP increased the survival time and significantly reduced the solid tumor volume in a dose dependent manner. Haematological parameters, protein and packed cellular volume, which were altered by tumor inoculation, were restored. MPP significantly increased the level of superoxide dismutase and catalase. In a cytotoxic study against human cancer cell lines, MPP found to have antitumor and cytotoxic activity on ELA and human cancer cell lines.

The methanol extract of *Careya arborea* bark (MECA) was tested for antioxidant and hepatoprotective activity in ELA tumor-bearing mice. Tumor control animals inoculated with ELA showed a significant alteration in the levels of antioxidant and hepatoprotective parameters (Senthilkumar *et al.,* 2008). Siva kumar *et al.* (2008) have investigated antitumor and antioxidants activities of *Triumfetta rhomboidea* against DLA bearing Swiss albino mice. Treatment with plant has decreased the tumor volume and viable cell count thereby increasing the life span of DLA bearing mice. Hematological profile and liver biochemical

parameter has also been studied. This studies show that methanol extract of plant has significant antitumor and antioxidant activity *in vivo.*

Rajeshkumar *et al.* (2008) has evaluated aqueous extract of *Phyllanthus amarus* as a potent anticarcinogenic activity against 20-methylchalnthrene induced sarcoma development and increased the survival of tumor harboring mice. The extract administration (p.o) was also found to prolong the life span of DLA and ELA bearing mice and reduced the volume of transplanted solid tumors. The extract inhibited aniline hydroxylase, a P- 450 enzyme. Harikumar *et al.* (2009) have shown that *Phyllanthus amarus* inhibits cell growth and induced apoptosis in DLA cells through activation of caspase-3 and downregultion of bcl-2. This study provides some insights in to the possible mechanism by which *Phyllanthus amrus* brings about apoptosis and growth inhibition in DLA cells.

Kuzhuvelil *et al.* (2009) reported, *Phyllanthus amarus* extract could significantly inhibit the solid and ascites tumor development in mice induced by DLA tumor cells. In the present study, the apoptotic effect of *P.amarus* against DLA cells in culture was evaluated. *P.amarus* produced significant reduction in cell viability as determined by the MTT assay. It also induces the formation of apoptotic bodies with characteristic features like plasma membrane invagination, elongation, fragmentation, and chromatin condensation.

Antitumor activity of *Mylabris cichorii* extracts against murine Ascites Dalton's Lymphoma was studied by Prasad *et al.* (2010). *Terminalia arjuna, Dillenia indica* and *Oroxylum indicum* were screened for anticancer efficacy against Dalton's lymphoma (Brahma *et al.,* 2011). Antiproliferative and antioxidant activity of *Aegle marmelos* (*Linn*.) leaves in Dalton's Lymphoma Ascites transplanted mice was reported by Chockalingam *et al.* (2012). Tumors threaten an individual's life when their growth disrupts the tissues and organs needed for survival (Jena *et al.,* 2012). Sangameswaran *et al.* (2012) reported the antitumor activity against ELA bearing Swiss albino mice by the acetone and ethanol extracts from the leaves of *Sida Veronicaefolia.*

2.5. Antibacterial activity of medicinal plants

The volume of antibiotic prescribed is the major factor in increasing rates of bacterial resistance rather than compliance with antibiotics (Pechere, 2001). The rise in the failure of chemotherapeutic agent and antibiotics resistance exhibited by pathogenic microbial infectious agents has led to screening of several medicinal plants as for their potential antimicrobial activity (Natarajan *et al.,* 2003).

"Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability to withstand the effects of antibiotics". The wide spread use of antibiotic both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria (Goossens *et al.,* 2005 and Soulsby, 2005). Antimicrobial potential of different medicinal plant is being extensively studied all over the world (Arora and Kaur, 2007). Because of mutagenic nature of bacterial DNA, the rapid multiplication of bacterial cells and the constant transmission of bacterial cells due to plasmid exchange, pathogenic bacteria continue to develop antimicrobial resistance (Kaushik and Goyal, 2008).

Pharmacological industries have produced a number of new antibiotics in the last three decades, inspite of the increased resistance to these drugs by microorganism. In general bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. The world is now looking towards India for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants and abundance of traditional knowledge such as Unani, Siddha and Ayurveda (Chitravadivu *et al*., 2009a).

The frequency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developed countries. There is an urgent need to control microbial resistance by improved antibiotic usage and reduction of hospital cross infection. According to WHO, plants are a source of compounds that have the ability to combat disease of compounds of antimicrobial, antiviral and antifungal activities. In addition medicinal plants have been used for human ailments and diseases because they contain component of

therapeutic value. Also they are less toxic to humans and environmentally friendly due to less pollutant produced in production and have minimal health hazards (Abeysinghe *et al.,* 2010).

The four main types of clinical syndrome that caused by *E.coli* are urinary tract infection, diarrhoea, pyogenic infections and septicemia. Common pathogenic bacteria and the types of bacterial diseases they cause include: *Escherichia coli* and *Salmonella* that cause food poisoning, *Staphylococcus aureus* causes a variety of infections in the body, including boils, cellulitis, abscesses, wound infections and food poisoning (http://www.localhealth.com/article/bacterial-diseases) and *Shigella* causes diarrhea and fever (Uyigue and Anukam, 2011). *Klebsiella pneumoniae* is a serious disease causative with high incidence of fatality. It frequently causes urinary infection. *Staphylococcus* commonly causes localized suppurative lesions in human beings (Ananthanarayan and Paniker, 2003).

Proteus vulgaris is a rod-shaped, Gram negative bacterium that inhabits the intestinal tracts of humans and animals (http://en.wikipedia.org/wiki/ Proteus_vulgaris) and inculde in urinary tract and wound infection. *Pseudomonas aeruginosa* is a Gram negative, rod-shaped, asporogenous and monoflagellated bacterium that has an incredible nutritional versatility. It is a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage and hospitals. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections and bacteremia, wound infection, eye inections, pseudomonal infections are complicated and can be life threatening (http://emedicine.medscape.com/article/226748-overview).

Microorganisms vary greatly in their pathogenicity, the pathogenicity islands are fairly large segments of the genome of pathogenic stress that are absent in non-pathogenic strains (Pommerville, 2006). Infectious diseases are the important causes of morbidity and mortality among humans and it account for about half of the death in tropical countries (Khosrani and Behzadi, 2006). Infectious diseases have been a life-threatening problem for humans before antibiotic era (Seyyednejad and Motamedi, 2010).

Shigella flexneri is a human intestinal pathogen, causing dysentery by invading the epithelium of the colon and is responsible, worldwide, for an estimated 165 million episodes of shigellosis and 1.5 million deaths per year. The bacterium is commonly found in water polluted with human faeces. It is transmitted in contaminated food or water and through contact between people. Upon infection, humans develop severe abdominal cramps, fever and frequent passage of bloody stools (Uyigue and Anukam, 2011). Shigellosis is not only a significant cause of infant mortality in developing nations but maintains endemic levels of infection worldwide (http://www.ebi.ac.uk/2can/genomes/bacteria/Shigella_flexneri.html).

Klebsiella pneumoniae is among the most common Gram negative bacteria. It is a common hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumonia and intra abdominal infections. (http://www.phagetherapycenter.com/pii/PatientServlet?command=static_klebsiella).

These organisms have the ability to survive in harsh condition due to their multiple environmental habitats (Ahameethunisa and Hopper, 2010). Herbal treatment would promise a greater viable solution for effective treatment of diseases caused by bacteria (Khan *et al.,* 2007 and Rahman and Hossain, 2010).

2.6. *In silico* docking and drug design for tumors

Bioinformatics has, out of necessity, become a key aspect of drug discovery in the genomic revolution, contributing to both target discovery and target validation. The pharmaceutical industry has embraced genomics as a source of drug targets and as a corollary, has recognized that bioinformatics is crucial to exploiting the data produced on a genome-wide scale (Searls, 2000).

Computer-aided drug design (CADD) is a widely used term that represents computational tools and resources for the storage, management analysis and modeling of compounds. It includes development of digital repositories for the study of chemical interaction relationships, computer programs for designing compounds with interesting physicochemical characteristics, as well as tools for systematic assessment of potential lead candidates before they are synthesized

and tested. Over the years, new technologies such as comparative modeling based on natural structural homologues have emerged and began to be exploited in lead design. These, together with advances in combinatorial chemistry, high throughput screening technologies and computational infrastructures, have rapidly bridged the gap between theoretical modeling and medicinal chemistry. CADD now plays a critical role in the search for new molecular entities (Klebe, 2006).

Current focus includes improved design and management of data sources, creation of computer programs to generate huge libraries of pharmacologically interesting compounds, development of new algorithms to assess the potency and selectivity of lead candidates and design of predictive tools to identify potential ADME/Tox liabilities (Song *et al.,* 2009).

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Computer - Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug - receptor interactions. One of those methods is called docking. The site of drug action, which is ultimately responsible for the pharmaceutical effect is a receptor. Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions (Virupakshaiah *et al.,* 2007).

Molecular modeling technologies have mainly been developed during the past decades, due to the development of fast computers and are today essential tools in drug development used for protein structure determination, sequence analysis, protein folding, homology modeling, docking studies and pharmacophore determination (Shoti and Leach, 2007).

Structure-based (direct) drug design is generally performed using a known 3D structure of a specific biological target (Nadendla, 2004).

Ligand-based (indirect) drug design to correlate physicochemical properties of compounds with their pharmacological activity and the calculated mathematical relationship can predict the activity of novel compounds (Osman, 2000).

Molecular docking is commonly used in the field of drug design to predict the binding of small molecules to biological protein targets. This method gives the possibility to study an active site in detail and can be used for hit identification, virtual screening, binding mode determination and lead optimization. Generally, the docking methodology is used to fit a compound into an artificial model or to a known three-dimensional binding site, which can be utilized to explore ligand conformation, orientation and feasible molecular interactions such as hydrogen bonding and hydrophobic interactions. Thus, molecular docking is a powerful tool for the design of ligands toward a specific protein target (Lakowicz, 2006).

'Docking program' is used to place computer-generated representations of a small molecule into a target structure in a variety of positions, conformations and orientations. Each such docking mode is called a 'pose'. In order to identify the energetically most favorable pose, each pose is evaluated ('scored') based on its complementarity to the target in terms of shape and properties such as electrostatics. A good score for a given molecule indicates that it is potentially a good binder (Kroemer, 2007).

Docking explores the ways in which two molecules, such as drugs and enzyme receptors fit together and dock to each other well. The molecules binding to a receptor inhibit its function and thus act as drug. Complexes were identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations (Babu *et al.,* 2008).

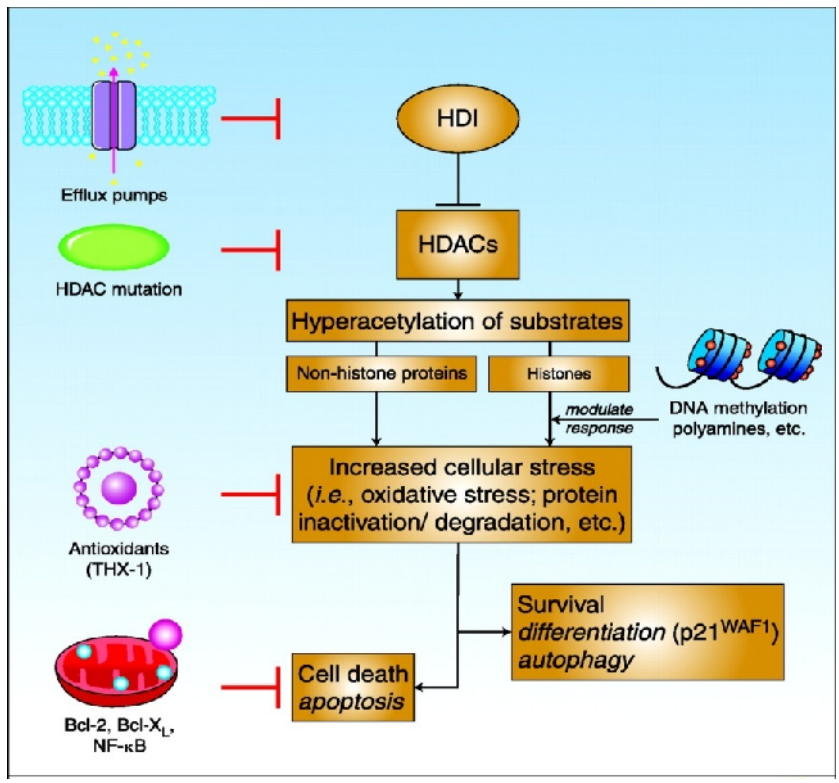
2.6.1. Histone deacetylase

For the last four decades, a number of potential approaches have been proposed for the treatment of cancer. One of the recent targets is Histone deacetylase (HDAC) (Saha *et al.,* 2010b). It has been widely recognized in recent years that HDACs are promising targets for therapeutic interventions intended to reverse aberrant epigenetic states associated with cancer (Pandolfi, 2001 and Baylin and Ohm, 2006). HDAC is an enzyme that removes an acetyl group from histones, which allows them to bind DNA and inhibit gene transcription (Elaut *et al.,* 2007 and Santini *et al.,* 2007). Acetylation and deacetylation of chromatin

histone protein by HDAC alters chromatin structure and dynamically affects transcriptional regulation (Liu *et al.,* 2006b). The Figure 15 shows the mechanism of action of histone deacetylase inhibitors.

Figure 15

Mechanism of action of Histone deacetylase inhibitors



(http://englishclass.jp/reading/topic/Histone_deacetylase)

Histone deacetylase inhibitors (HDACIs) are emerging as a new class of anticancer agents. HDACIs have shown activity against diverse cancer types and notable effects on tumor cell proliferation, programmed cell death, differentiation and angiogenesis *in vitro* and *in vivo.* Currently, there are more than a dozen of phase I and II clinical trials involving the use of HDACIs in patients with haematological and solid malignancies (Marks *et al.,* 2004).

In preclinical studies several classes of HDACIs have been found to have potent anticancer activities, with remarkable tumor specificity, and some have demonstrated promising therapeutic potential in early-phase clinical trials for haematological malignancies such as cutaneous T-cell lymphoma,

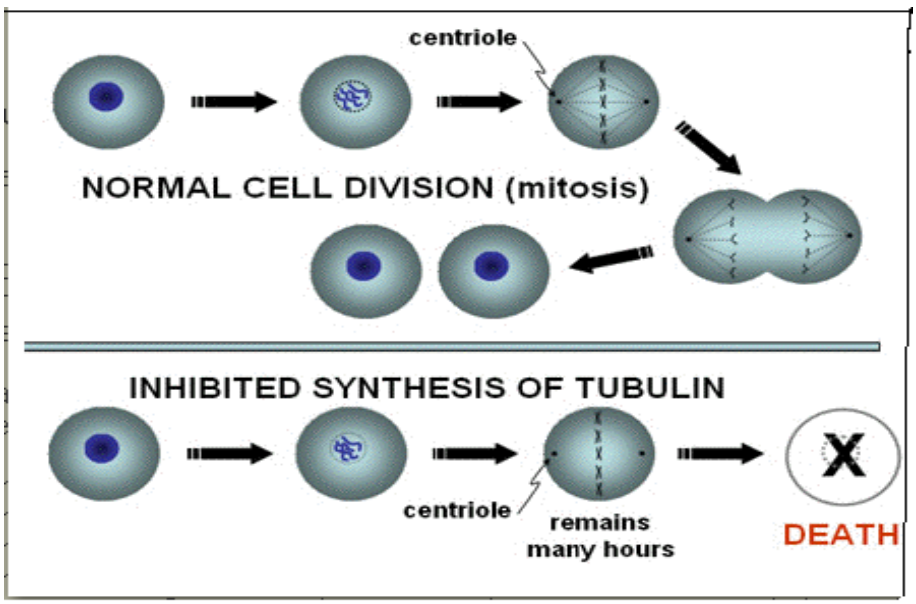
myelodysplastic syndromes and diffuse B-cell lymphoma (Lindemann *et al.,* 2004; Jabbour and Giles, 2005 and Marks and Jiang, 2005).

2.6.2. Tubulin

Microtubules the key components of the cytoskeleton are long, filamentous, tube-shaped protein polymers that are essential in all eukaryotic cells. They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signalling, and in cell division and mitosis. Their importance in mitosis and cell division makes microtubules an important target for anticancer drugs (Jordan and Wilson, 2004). The Figure 16 shows the tubulin mediated inhibition of mitosis.

Figure 16

Tubulin mediated inhibition of mitosis



(http://www.photobiology.info/Christensen.html).

Microtubule inhibitors disrupt microtubule dynamics of tubulin polymerization and depolymerization, which results in the inhibition of chromosome segregation in mitosis and consequently the inhibition of cell division (Mulligan *et al.,* 2006). In normal mitosis, the chromosomes are pulled by microtubules, formed from tubulin, towards the two centrioles, marking the location where the nuclei of the two daughter cells will be formed. After that, the cell

membrane is pinched off and the chromosomes decondense in the newly formed cells. If the chromosomes are not separated by the "ropes" formed from tubulin, the normal process of mitosis will not be completed and cell death may result. An inhibition of the tubulin function leads to an arrest of the cells in mitosis and no further cell division as long as the chromosomes are not transported to the two poles. A large number of cells have been shown to die while they are arrested in this phase of cell division. A mitotic arrest is the mechanism behind the function of mitotic inhibitors commonly used in cancer therapy (http://www.photobiology.info/Christensen.html).

2.7. *In silico* docking and drug design for microbial infections

The emergence of multidrug-resistant bacteria has challenged researchers to develop novel therapies for the prevention and treatment of infectious diseases (Anderssona *et al.,* 2001). Antibacterial drug discovery has experienced a paradigm shift from phenotypic screening for antibacterial activity to rational inhibition of preselected targets. The pharmaceutical industry embraced genomic information as the basis for a rational, target-directed antibacterial drug discovery strategy to complement the classic empirical approach. Central to the paradigm shift was the belief that the bacterial genomes harbor a variety of so-far-unexploited targets with the potential for potent and selective antibiotics against a broad spectrum of bacterial pathogens (Payne *et al.,* 2004).

2.7.1. Deacetoxy C synthase

Deacetoxy / deacetylcephalosporin C synthase (acDAOC/DACS) from *Acremonium chrysogenum* is a bifunctional enzyme that catalyzes both the ring-expansion of penicillin N to deacetoxycephalosporin C and the hydroxylation of the latter to deacetylcephalosporin C (Wu *et al.,* 2011). Deacetoxycephalosporin C synthase (DAOCS) from *Streptomyces clavuligerus* catalyses the oxidative ring expansion of the penicillin nucleus into the nucleus of cephalosporins. The reaction requires dioxygen and 2-oxoglutarate as co-substrates to create a reactive iron-oxygen intermediate from a ferrous iron in the active site (Oster *et al.,* 2004)

2.7.2. Pantothenate Kinase

Coenzyme A (CoA) is a key component of cellular metabolism and is essential for bacterial viability since disruption of the CoA biosynthetic pathway is lethal. CoA synthesis begins with the phosphorylation of pantothenate (Vitamin B5) by pantothenate kinase (Dunster *et al.,* 2002). Pantothenate kinase, which triggers the first step in the production coenzyme A (CoA) a molecule that is indispensable to all forms of life, CoA plays a pivotal role in the cells' ability to extract energy from fatty acids and carbohydrates; bacteria need CoA to make their cell walls. The job of pantothenate kinase is to grab a molecule of pantothenic acid (Vitamin B5) and another molecule that contains a chemical group called "phosphate." The enzyme then removes the phosphate group from that molecule and sticks it onto pantothenic acid. In humans, certain mutations in this enzyme block its ability to put the phosphate group onto pantothenic acid. That diminishes the production of CoA and causes the pantothenate kinase associated neurodegenerative disease (Hong *et al.,* 2006).

The pantothenate kinase is a key rate-determining enzyme of this pathway and become a prime target for its inhibition. By doing so, we are not only inhibiting this CoA biosynthetic pathway, but also inhibiting the microbial growth. It should therefore be possible to develop selective small molecule inhibitors for the pantothenate kinases that are expressed by the pathogenic microorganisms of interest (Leonardi *et al.,* 2005b).

2.8. Medicinal plant selected for the study

Ficus racemosa Linn., syn. (Moraceae), is a moderate to large-sized large deciduous tree distributed all over India and Ceylon found throughout the year, grows in evergreen forest, moist localities, along the sides of ravines and banks of streams. The bark is used for treatment of dysentery. The sap of this plant is a popular remedy for mumps and other inflammatory enlargements; the milky juice of this plant is popular among traditional healers as an anti-inflammatory remedy. In Sri Lankan indigenous system of medicine, it is used in the treatment of skeletal fracture. The hypoglycaemic and anti-diarrhoeal activity of *Ficus racemosa* leaves

the vernacular names such as Sanskrit: Udumbara; Bengali: Jagnadumar; Gujrati: Umbro; Hindi: Gular Kanada: Attimara; Malayalam: Atti; Marathi: Umbar; Oriya: Jajnadimbri Tamil: Atti; Telgu: Atti (Harer Sunil and Harer Priyanka, 2010).

The root used for hydrophobia, powerful tonic and healing wounds, cuts and sores. The leaves used for bronchitis, bilious affection and antihypertensive and in inflammation, lymphadenitis, in sprains and fibrositis. The latex used for checking glandular enlargements, piles and diarrhea, aphrodisiac and haemorrhoids. The bark used for antidiabetic, refrigerant, as wash for wounds, highly efficacious in threatened abortions, uropathy, to cattle in rinder-pest diseases, acrid, galactagogute, good for the gravid uterus, asthma, piles and lactating mothers to increase the secretion of milk. The fruits used for acrid, astringent to bowels, tonic, styptic, allays thirst, leucorrhoea, diarrhoea, dyspepsia, menorrhagia, astringent, stomachic, refrigerant, carminative, curing dry cough, laryngitis, diseases of the kidney and spleen, leprosy and nose bleeding and for expelling intestinal worms (http://en.wikipedia.org/wiki/Ficus_racemosa).

In the present study, various parts of *Ficus racemosa* were subjected to screen antioxidant, antitumorigenic and antimicrobial activity, phytochemical analysis and the identified structures were subjected to an *in silico* validation. The layout of the study and the methodology adopted are presented in the next chapter.