Chapter - II

Review of Literature
2. REVIEW OF LITERATURE

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted in the modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise-use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community (Patwardhan, et al., 1992). The Greek physician Galen (AD 129–200) devised the first pharmacopoeia describing the appearance, properties and use of many plants of his time. The foundations of the modern pharmaceutical industry were laid when techniques were developed to produce synthetic replacements for many of the medicines that had been derived from the natural products and their phyto-chemistry studies actually began with the work of Sertturner, who first isolated morphine from opium plant. This, in turn, was obtained from opium poppy (Papaver somniferum) by processes that have been used for over ancient days. Many such developments followed for instance, Quinine from cinchona tree had its origin in the royal households of the South American Incas. Before the first European explorers arrived, the native people of the Americas had developed complex medical systems replete with diagnosis and treatment of physical as well as spiritual illnesses. Indigenous people derived and identified many medicines and poisons from thousands of plants. A review of some plants that originated from Central and South America indicates that most of them either had potentially toxic characters or were from food sources. The following are a few examples (King et al., 1992). In the early 1500s, Indian fever bark was one of the first medicinal plants to find appreciative consumers in Europe. Taken from the cinchona tree (Cinchona officinalis), the bark was used as an infusion by native people of the Andes and Amazon highlands to treat fevers. Jesuit missionaries brought the bark back to Europe. By the early sixteenth century, this
medicine was known as ‘Jesuit fever bark’, quite a transformation. The name coca 
(Erythroxylum coca) comes from an Aymara word meaning ‘tree’. In Andean cultures, the 
leaves of the coca tree have been primarily chewed to obtain perceived benefits. From 
ancient times, indigenous people have added alkaline materials such as crushed seashells 
or burnt plant ashes to the leaves in order to accentuate the pharmacologically active 
moiety of coca. In 1860, a German chemist Carl Koler isolated cocaine, the chemical 
responsible for the biological activity. He found that cocaine could act as a local 
aesthetic in eye surgery. As the years passed, scientists observed that cocaine paralyzed 
nerve endings responsible for transmitting pain. As a local anaesthetic, it revolutionized 
several surgical and dental procedures. Pot curare arrowhead poison used in the East 
Amazon is predominately from the species Strychnos guianensis. Tube curare in the West 
Amazon is from Chondrodendron tomentosum; curare in modern medicine is made from 
this and named as tubocurarine. The jaborandi tree (Pilocarpus jaborandi) secretes 
alkaloid- rich oil. Several substances are extracted from this aromatic oil, including the 
alkaloid pilocarpine, a weapon against the blinding disease, glaucoma. American Indians 
on the island of Guadeloupe used pineapple (Ananas comosus) poultices to reduce 
inflammation in wounds and other skin injuries, to aid digestion and to cure stomachache. 
In 1891, an enzyme that broke down proteins (bromelain) was isolated from the fresh juice 
of pineapple and was found to break down blood clots. Other pharmaceuticals that have 
their origin in botanicals include atropine, hyoscine, digoxin, colchicine and emetine. 
Reserpine, an anti-hypertensive alkaloid (Rauwolfia serpentina) became available as a 
result of work carried out by Ciba-Geigy in India. It is pertinent to note that most of these 
early discoveries are mainly based on traditional medicines; many products could act as 
poisons in toxic doses (Patwardhan et al., 2004). 

In India, Karnataka state is endowed with rich natural resources especially along 
the Western Ghat ranges. The Western Ghats range arises abruptly in the west from the 
Arabian Sea coast and descends gradually towards the dry Deccan plains in the east. The 
total area of the Western Ghats is estimated to be about 20,000 Sq.Kms. The good climatic 
conditions and altitudinal gradients have resulted in the development of a variety of forests 
from evergreen to semi evergreen, moist de
It is one of the richest biodiversity centres and is considered as one among the eighteenth hot spot of the world (Pascal et al., 1982).

The plant selected for this investigation is *Erythrina mysorensis* and its stem bark fig.1 and 2 respectively, which is native to India and Malaysia and commonly known as Haalivana. The plant is a showy, ornamental tree with straight trunk which is usually armed with prickles when young. It is highly valued ornamental tree described as one of the gems of the floral world. It has proven for fodder production and as a sturdy component of windbreaks. The plant species are useful for soil enrichment. *Erythrina mysorensis* is a large tree commonly reaching 15 to 20 m height in 20 to 25 years. On favourable sites, the stem can reach a diameter at breast height (dbh) of 50 to 60 cm. The smooth bark is streaked with vertical lines of green, buffy grey and white. The leaves are trifoliate. The leaflets are commonly light green, heart shaped, 7 to 12 cm wide and 12 to 18 cm long. The trees are deciduous, typically losing their leaves before flowering except under very humid conditions.
**Toxanomy of Plant**

**Kingdom** : Plantae

**Phylum** : Spermatophyta

**Sub-phylum** : Angiospermae

**Class** : Magnoliopsida

**Order** : Fabales

**Family** : Fabaceae

**Synonym** : E. indica Lam.

**Common Names:** Indian coral tree (Eng.); Harivana, Haalivaana (Kan.); Hongaarae, Pongaarae (Tul.).

**Habit** : Tree

**Habitat** : Planted in hedges and as a support for pepper and betel vines

**Distribution** : Udupi, Shimoga

**Traditional uses:** The bark and leaves are used in many traditional medicines, including *paribhadra*, an Indian preparation said to destroy pathogenic parasites and relieve joint pain. Juice from the leaves is mixed with honey and ingested to kill tapeworm, roundworm and threadworm (Hegde, 1993). Women take this juice to stimulate lactation, to correct and regulate menstrual cycle. It is also commonly mixed with castor oil to treat dysentery. A warm poultice of the leaves is applied externally to relieve rheumatic joints pain. The bark is also used as a laxative, diuretic and expectorant in human beings. The morphological characteristics of the plant have been described in “Material and Methods” section. The decoction of the stem bark of this tree is traditionally used by Bamoun population (Western Cameroon tribe) against liver disorders such as hepatitis and jaundice (Moundipa *et al.*, 2002). In Mali, *Erythrina senegalensis* is used against several diseases and symptoms among which the main reported are malaria, jaundice, infections, gastrointestinal disorders, amenorrhea, dysmenorrhoea, sterility, onchocercosis, body pain, abdominal pain and headache (Togola *et al.*, 2008).
Medicinal properties of Genus *Erythrina*

*Erythrina mysorensis* have a reputation for its medicinal properties in India, China and also in South East Asia. According to Hartwell (1967–1971) seeds are used in folk remedies for cancer. Annam reported to have the same medicinal attributes as *Erythrina indica*, whose bark is used for fever, hepatosis, malaria, rheumatism, toothache, also for boils and fractures. Perry (1980) cites many more uses for *Erythrina indica* bark, is used for poulticing fresh wounds in Malasia, boiled roots are taken internally or externally for beri-beri, grated wood used for hematuria. List and Horhammer, (1969–1979) reported that the root of *Erythrina indica* is used for rheumatism, bark and leaves serve as a vermifuge.

Australian Aboriginees (2003) used parts of *E. vespertilio* as a sedative, similarly to the use of some South American species of *Erythrina* that are employed for better and deeper sleep and to reduce anxiety. The roots and bark of many species of *Erythrina* exude a deep orange dye, which can be used for fabrics and artefacts. The wood is also used to make artefacts, especially of a spiritual nature. It is very soft and easy to carve. The powdered seeds have been employed as aphrodisiac and deliriant, but dosing is very unreliable and dangerous and should never exceed half a seed. It is also believed that they are used to fortify agave wines and maize beer. The bright orange to purplish seeds are also used to manufacture artefacts and jewelery. The leaves are sometimes eaten although they cause a mild sedation. Similarly the flowers are prepared as a tea to drink before bedtime to ensure a deep sleep. 'Mulungu bark' which is derived from *E. crista-galli* is a potent sedative and muscle relaxant.

**Pharmacology**

List and Horhammer, 1969–1979 states that all *Erythrina* species contain 'Erythrina alkaloids', which include erythrane, erythroidine, coralline and others. These alkaloids are found in the whole plant, but especially in the seeds. In small doses they cause sedation, skeletal muscle relaxation and if taken before bedtime a long and deep sleep. In higher doses (more than a quarter seed) the effects become exponentially stronger and unpredictable. An initial deep sedation is followed by erratic and manic behaviour ranging from excessive laughter to uncontrollable sexual desires and deep depression. The seeds
are usually too potent for accurate dosing, but dried flowers or dried leaves are suitable for teas or smoking mixes.

Saraswathy et al., (2008) have investigated the ethanolic extract of the stem bark of *Erythrina indica* was screened for its *in vitro* antioxidant activity by Ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods and found that the ethanolic extract of the stem bark of *Erythrina indica* possess significant antioxidant activity.

Nkengfack (2001) study isolated five compounds from the methanol extract of stem bark of *E*: epilupeol, 6-hydroxygenistein, 3s, 28- dihydroxyolean-12-ene, epilupeol, and stigmasterol. Different partitionates showed mild to moderate antimicrobial activity and varying degrees of cytotoxicity.

**Chemical constituents of Genus Erythrina**

Leung et al., 1972 reported that per 100 g, the leaves are to contain 60 calories, 81.5 g H2O, 4.6 g protein, 0.8 g fat, 11.7 g total carbohydrate, 4.1 g fiber, 1.4 g ash, 57 mg Ca, 40 mg P, 1.8 mg Fe, 2,300 µg β-carotene equivalent, 0.24 mg thiamine, 0.17 mg riboflavin, 4.7 mg niacin, and 3 mg ascorbic acid. Similarly, Duke (1981) also reported that per 100 g, the leaves contain (ZMB): 325 calories, 24.9 g protein, 4.3 g fat, 63.3 g total carbohydrate, 22.2 g fiber, 7.6 g ash, 308 mg Ca, 222 mg P, 5.2 mg Fe, 0.91 mg thiamine, 0.52 mg riboflavin, 6.54 mg niacin, and 78 mg ascorbic acid. List and Horhammer, (1969–1979) reported that seeds contain the alkaloid erythraline, erylodine, erysonine, eryspine, erysothiopine, eryso-thiovine, crysovine, erythraline, erythramine, erythratine, and hypaphorine. The similarity in alkaloid and amino acid patterns in *E. fusca* and *E. glauca* were considered by Krukoff (1972) in rendering these species synonymous.

Phytochemical investigation of the hexane and CH2Cl2 extracts of *Erythrina stricta* roots and *E. subumbrans* stems led to the isolation of six pterocarps, one flavanone, one isoflavone, two alkaloids, five triterpenes, six steroids and alkyl trans-ferulates. The structures of all known compounds were determined on the basis of spectroscopic evidence. Sophoradiol, a mixture of stigmast-4-en-3-one and stigmasta-4,22-dien-3-one, lupeol, cycloeucalenol, a mixture of 3β-hydroxystigmast-5-en-7-one and 3β-hydroxystigmast-5,22-dien-7-one and melilotigenin C were first isolated from the genus *Erythrina* (Rukachaisiriikul 2007).
Innok (2009) reported that three new isomeric flavanones, fuscaflavanones A1 (1), A2 (2) and B, together with six known flavanones, lupinifolin (4), lonchocarpol A, a mixture of lonchocarpols C1 and C2, and a mixture of lonchocarpols D1 and D2, five pterocarps, sandwicensin, phaseollidin, erythrabissin I, and a mixture of dolichins A and B, one chalcone, isobavachalcone, and one isoflavone, wighteone, were isolated from the bark of *Erythrina fusca* LOUR. Their structures were elucidated on the basis of spectroscopic data. Some isolates were tested for antiplasmodial and cytotoxic activities and it was found that 5 and 9 exhibited moderate antiplasmodial activity against *Plasmodium falciparum*. For cytotoxicity, compounds 1, 4, 5, 9 and 12 showed moderate to weak activity against KB, BC and NCI-H187 cells, whereas 2 exhibited only weak activity against KB cells.

Nkengfack (2001) carried out bioassay-directed fractionation of the CH$_2$Cl$_2$-MeOH (1:1) extract of the stem bark of *Erythrina indica*, has resulted in the isolation of two new isoflavone derivatives named indicanines D and E together with 11 known compounds including: six isoflavones (genistein, wighteone, alpinumisoflavone, dimethylalpinumisoflavone, 8-prenyl erythrinin C, and erysenegalensein E), one cinnamate (erythrinassinate B), two pentacyclic triterpenes (oleanolic acid and erythrodiol), and two phytosterols (stigmasterol and its 3-O-b-d-glucopyranoside). The structures of the new compounds were elucidated by means of spectroscopic analysis. The *in vitro* cytocidal activity against KB cells of some of the isolated compounds is also reported.

Majinda (2005) showed that the genus *Erythrina* is very rich in secondary metabolites particularly of the flavonoids class. A literature survey of non-alkaloidal secondary metabolites from *Erythrina* showed the presence of flavanones, flavonols, chalcones, cinnamoylphenols, stilbenoids, isoflavones, isoflavans, isoflavonones, pterocarps, isoflav-3-enes, 3-phenoxychromones, coumestans, 3-phenyl-coumarins, lignans, cinnamate esters, simple phenolics, triterpenes, sesquiterpenes, long-chain carboxylic acids, and long-chain alcohols. The documented bioactivities of some of the isolated metabolites range from antimicrobial, anti-inflammatory, inhibition of platelet aggregation, tyrosinase inhibition, phospholipase A$_2$ (PLA$_2$) inhibitors, cyclooxygenase inhibitors, antioxidant, inhibitors of NA$^+$/H$^+$ exchange system, phospholipase C inhibitors,
behavioural depression, muscle relaxation, β-adrenergic inhibition, diuretic, anticancer, cytotoxic, DNA-repair properties, oestrogenic or progestrogenic activities, antitrypanosomal, antiplasmodial, and anti-HIV activities.

Several compounds such as 2,3 dihydroauriculatin (Taylor et al., 1986), Erybraedin A (Wanjala et al., 2002), isoflavonoids 6-8 diprenylgenistein (Oh et al., 1999) isolated from this plant have shown antimicrobial activities on various microbial species. Senegalensein also isolated from the stem bark of ES (Fomum et al., 1987) exhibited HIV inhibitory activity (Meragelman et al., 2001). Alpumisoflavone isolated from the methanol extract of the same plant material (Oh et al., 1999) is found to prevent the establishment of schistosomiasis infection when applied to mouse skin. Erythrisenegalone and senegalensein, two prenylated-flavonones, both isolated from the stem bark of ES (Fomum et al., 1985; Fomum et al., 1987) have shown anti-tumour activity. Many pharmacological works have demonstrated the antimicrobial (Koné et al., 2004; Magassouba et al., 2007), antiplasmodial, analgesic and anti-inflammatory (Saidu et al., 2000) and the hepatoprotective and antioxidant (Donfack et al., 2008) effects of extracts from the stem bark of Erythrina senegalensis DC.

Cui (2008) isolated four new chalcones 1 - 4, named abyssionones A - D, from the stem bark of the plant Erythrina abyssinica and their structures were elucidated on the basis of spectroscopic analyses. The compounds 1,3, and 4 were found to exhibit moderate cytotoxic activity against the human colorectal cancer cell line (Caco2) with IC50 values of 13.3, 15.1, and 11.1 µM, respectively.

The methodology and the various parameters examined in the present study to evaluate the different pharmacological activities were based on the previous research works. In the light of earlier literature, the review in the subsequent section will emphasize on the available reports of physiological, biochemical and pathological aspects of the systems under study.

Based on the traditional claims the following pharmacological parameters have been selected and investigations were conducted to evaluate the therapeutic potency of stem bark of Erythrina mysorensis Gamb., to confirm the ethno-medical claim. The
available literature on the scientific studies of the said plant clearly suggests the need for both phytochemical screening and pharmacological evaluation. The following activities have been selected for the present investigation.

- Antioxidant activity
- Anti-inflammatory activity
- Wound healing activity
- Anti-microbial activity
- Anti-tumor activity
- Anti-epileptic activity
- Anti-anxiety activity
- Anthelmintic activity

2.A. Antioxidant activity:

2.A.i. Free Radicals

Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds. The delicate balance between their two antagonistic effects is clearly an important aspect of life. At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, and deleterious process that can damage all cell structures (Halliwell et al., 2007, Bahorun et al., 2006). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as “free radical scavengers” by preventing and repairing damages caused by ROS and RNS, and thereby can enhance the immune defense and lower the risk of cancer and degenerative diseases (Valko et al., 2006, Parthasarathy et al., 2006).
1999). The theory of oxygen-free radicals has been known about fifty years ago (Valko et al., 2007). However, only within the last two decades, has there been an explosive discovery of their roles in the development of diseases, and also of the health protective effects of antioxidants.

2.A.ii. Free Radical Formation

Atoms are most stable in the ground state Karlsson (1997). An atom is considered to be "ground" when every electron in the outermost shell has a complimentary electron that spins in the opposite direction. By definition a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron(s). Goldfarb, (1999) suggests that, any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with anti parallel spins from cellular structures or molecules. Thus the chain reaction continues and can be "thousand of events long". The electron transport chain (ETC), which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen acts as the terminal electron acceptor within the ETC. and that anywhere from 2 to 5% of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape (Siodin and Westing, 1990). Dekkers (1996), states that during exercise oxygen consumption increases 10 to 20 fold to 35-70 ml/kg/min. In turn, electron escape from the ETC is further enhanced. Thus, when calculated, .6 to 3.5 ml/kg/min of the total oxygen intake during exercise have the ability to form free radicals.
2.A.iii. Generation of free radicals and oxidants

Formation of ROS and RNS can occur in the cells by two ways: enzymatic and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the respiratory chain, the phagocytosis, the prostaglandin synthesis and the cytochrome P450 system (Pacher et al., 2007). For example, the superoxide anion radical \( (O_2 \cdot^-) \) is generated via several cellular oxidase systems such as NADPH oxidase, xanthine oxidase, peroxidases. Once formed, it participates in several reactions yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical (\( \text{OH}^+ \)), peroxynitrite \( (\text{ONOO}^-) \), hypochlorous acid (\( \text{HOCl} \)), etc. \( \text{H}_2\text{O}_2 \) (a non radical) is produced by the action of several oxidase enzymes, including amino acid oxidase and xanthine oxidase. The last one catalyses the oxidation of hypoxanthine to xanthine, and of xanthine to uric acid. Hydroxyl radical (\( \text{OH}^+ \)), the most reactive free radical \textit{in vivo}, is formed by the reaction of \( O_2 \cdot^- \) with \( \text{H}_2\text{O}_2 \) in the presence of Fe\(^{2+} \) or Cu\(^{+} \) (catalyst). This reaction is known as the Fenton reaction. Hypochlorous acid (\( \text{HOCl} \)) is produced by the neutrophil-derived enzyme, myeloperoxidase, which oxidizes chloride ions in the presence of \( \text{H}_2\text{O}_2 \). Nitric oxide radical (\( \text{NO}^- \)) is formed in biological tissues from the oxidation of L-arginine to citrulline by nitric oxide synthase (Genestra, 2007). Free radicals can be produced from 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 (i.e. aerobic respiration) in the mitochondria (Valko et al., 2007, Droge, 2002). ROS and RNS 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, aging. Exogenous ROS/RNS result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation. (Frei, 1997, Genestra, 2007). After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals.

Antioxidants are substances that reduce the rate of oxidation. Antioxidant protects against tissue damage by giving up their electrons more easily than the tissue, hence by
donating electrons to substance that have oxidized and acts as reducing agent. Antioxidants counteract the toxicity of free radicals by preventing oxidative damage (Evans and Gopinathan, 1995). Antioxidants are a group of vitamins, enzymes and minerals that may reduce the risk of some forms of cancer, cardiovascular and neurodegenerative diseases. Our body manufactures some antioxidants, which include folate, vitamin C and E. The minerals such as selenium, and certain substances called "phytochemicals" found in fruits and vegetables. Oxygen is part of the air we breathe and is vital to life. When oxygen is metabolized or used in the body, cells form by-products called free radicals. These free radicals are also created when our body is exposed to various environmental factors such as tobacco smoke and radiation. If these formed free radicals are not controlled it cause damage to cell wall, certain cell structures and genetic materials within the cells. Such damage causes various health problems, aging etc. Antioxidants protect the cells against these injuries by neutralizing the free radicals.

2.A.iv. Properties of free radicals:

Free radicals with an unpaired electron have the following properties:

- Highly reactive, extremely short life span measured in micrograms.
- Self-perpetuating and diverse chemical reactivity.
- Low chemical specificity.
- Can be generated both in-vivo and in-vitro. (Sinclair et al., 1991)

2.A.v. Beneficial activities of free radicals and oxidants

At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and can act as weapons for the host defense system. Indeed, phagocytes (neutrophils, macrophages, monocytes) release free radicals to destroy invading pathogenic microbes as part of the body’s defense mechanism against disease (Droge, 2002). The importance of ROS production by the immune system is clearly exemplified by patients with granulomatous disease. These patients have defective membrane-bound NADPH oxidase system which makes them unable to produce the superoxide anion radical (O_2^-·), thereby resulting in multiple and persistent infection (Droge, 2002). Other beneficial effects of ROS and RNS involve their physiological roles
in the function of a number of cellular signaling systems (Pacher et al., 2007). Their production by non phagocytic NADPH oxidase isoforms plays a key role in the regulation of intracellular signaling cascades in various types of non phagocytic cells including fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes, and thyroid tissue. For example, nitric oxide (NO) is an intercellular messenger for modulating blood flow, thrombosis, and neural activity. NO is also important for nonspecific host defense, and for killing intracellular pathogens and tumors. Another beneficial activity of free radicals is the induction of a mitogenic response (Pacher et al., 2007). In brief, ROS/RNS at low or moderate levels are vital to human health.

2.A.vi. Deleterious activities of free radicals and oxidants and Pathogenesis

When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) (Droge, 2002). Oxidative lipoproteins by a process called lipid peroxidation. This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Lipid peroxidation occurs by a radical chain reaction, i.e. once started; it spreads rapidly and affects a great number of lipid molecules. Proteins may also be damaged by ROS/RNS, leading to structural changes and loss of enzyme activity (Frei, Halliwell, 2007). Oxidative damage to DNA leads to the formation of different oxidative DNA lesions which can cause mutations. The body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants (Willcox et al., 2004, Pacher et al., 2007). If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases as well as the aging process and some acute pathologies (trauma, stroke). 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. For example, hydroxyl radical and peroxynitrite in excess can damage cell membranes and several workers have reported that antioxidants prevent, slow the progress of and can even reverse heart disease. Antioxidants have positive influences on the cholesterol in our blood stream by increasing the levels of HDL and decreasing the levels of LDL. They also prevent the deposition of cholesterol
from being deposited on the walls of the coronary arteries. They also improve the flow of blood through already diseased coronary arteries by dilating the arteries to provide better flow to the heart muscles and also minimizes blood clotting in these diseased narrow blood vessels.

Many medical studies from the last few years revealed that antioxidants prevent the body against the higher risks of cancer, including cancers of the mouth, throat, lung, oesophagus, stomach, prostate and colon. They also enhance the body’s defense mechanisms against various germs, particularly viruses. (Jacob, 1999; Susan, 2000).

2.A.vii. Therapeutic use of Antioxidants

The body makes use of a great quantity of antioxidants and free scavengers for different needs. Vitamin C and E are some of the important naturally occurring antioxidants. Water-soluble antioxidants like Vit C are best at protecting the tissues that contain water-soluble compounds such as proteins. Fat-soluble antioxidants like Vit E are best at protecting fat based tissues such as membranes of all cells (Evans and Gopinathan, 1995). Some of the reported plant extracts which exhibited the antioxidant properties are discussed below.

Shyura et al., (2005) have been examined antioxidant activities of twenty-six medicinal herbal extracts that have been popularly used as folk medicines in Taiwan. The results of scavenging DPPH radical activity show that, among the 26 tested medicinal plants, Ludwigia octovalvis, Vitis thunbergii, Rubus parvifolius, Lindernia anagallis, and Zanthoxylum nitidum exhibited strong activities and their IC50 values for DPPH radicals were 4.6, 24, 27, 36, 50 μg/mL, respectively. As for the superoxide anion scavenging activity (IC50, μg/mL), the top five most significant activities were observed in plant extracts of Ludwigia octovalvis (26 μg/mL), Vitis thunbergii (58 μg/mL), Prunella vulgaris (113 μg/mL), Saurauia oldhamii (124 μg/mL), and Rubus parvifolius (151 μg/mL). The IC50 values for DPPH and superoxide anion of catechin (positive control) were 2.5 and 7.2 μg/mL, respectively. It was also observed in the present study that, at 1 mg/mL, Ludwigia octovalvis and Bombax malabaricum exhibited significant protection on
Aqil et al., (2006) screened methanolic crude extracts of 12 traditionally used Indian medicinal plants for their antioxidant and free radical scavenging properties using α-tocopherol and butylated hydroxy toluene (BHT) as standard antioxidants. Antioxidant activity was measured by ferric thiocyanate (FTC) assay and compared with the thiobarbituric acid (TBA) method. Free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radicals. The overall antioxidant activity of Lawsonia inermis was the strongest, followed in descending order by Ocimum sanctum, Cichorium intybus, Piper cubeba, Punica granatum, Allium sativum, Delonix regia, Terminalia chebula, Terminalia bellerica, Mangifera indica, Camellia sinensis, and Trigonella foenum-graecum. Seven plants, namely Terminalia chebula, Mangifera indica, Terminalia bellerica, Punica granatum, Ocimum sanctum, Cichorium intybus, and Camellia sinensis, showed strong free radical scavenging activity with the DPPH method. Phytochemical analysis of plant extracts indicated the presence of major phytochemicals, including phenolics, alkaloids, glycosides, flavonoids, and tannins. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

In this study, Antioxidant activity of Cyperus rotundus rhizomes extract (CRRE) was evaluated in a series of in vitro assay involving free radicals and reactive oxygen species and IC50 values were determined (Nagulendran et al., 2007). CRRE exhibited its scavenging effect in concentration dependent manner on superoxide anion radicals, hydroxyl radicals, nitric oxide radical, hydrogen peroxide, and property of metal chelating and reducing power. The results obtained in the present study indicate that C. rotundus rhizomes extract can be a potential source of natural antioxidant.

Matkowski et al., (2008) tested five medicinal plants from the subfamily Lamioideae of the Lamiaceae for antioxidant activity and screened for polyphenols content. Aerial parts of Ballota nigra, Lamium maculatum, Leonurus cardiaca, Marrubium vulgare, and Galeopsis tetrahit were extracted with methanol (MeOH) and subsequently...
partitioned by liquid-liquid extraction between petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH). All 25 extracts and subfractions were assayed for DPPH and HO• scavenging and phosphomolybdenum reduction. Total polyphenols and free hydroxycinnamic acids were determined by spectrophotometric assays. Predictably, all species possess remarkable antioxidant capacity, but the relative differences between species and fractions depended on the method of testing. However, only polar fractions from L. cardiaca and B. nigra are the most potent.

Khalaf et al., (2008) have screened the methanolic crude extracts of some commonly used medicinal plants for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of green tea (Camellia sinensis Linn.) was the strongest, followed in descending order by black tea (Camellia sinensis Linn.), Eugenia caryophyllus (Spreng.) Bullock and Harrison, Piper cubeba Linn, Zingiber officinale Roscoe and Piper nigrum Linn. Trigonella foenum graecum Linn. and Elettaria cardamomum (Linn.) The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity.

Kumar et al., (2008) carried out the study to determine the antioxidant activity of selected medicinal plants namely Albizia amara, Achyranthes aspera, Cassia fistula, Cassia auriculata and Datura stramonium by inhibition of lipid peroxidation technique. The highest inhibition of lipid peroxidation activity was observed in A. amara (96%) followed by C. fistula (89%) and C. auriculata (89%). Our study indicates that the antioxidant activity of A. amara could be harnessed as a drug formulation.

Extracts of three medicinal plants (Tilia argentea, Crataegi folium leaves and Polygonum bistorta roots) used in Turkish phytotheraphy were screened for their phenolic profiles and antioxidant properties by Demiray et al., (2009). The antioxidant activity of the extracts was determined by ABTS•+ radical cation scavenging activity. The predominant phenolic compounds detected in different extracts of the plants were catechin, protocatechuic and chlorogenic acids. The highest phenolic contents were obtained by
using 70% acetone as aqueous solvent, whereas the lowest phenolic contents were obtained by water extraction due to Folin Ciocalteu results. The results indicate that acetone extracts of *Tilia argentea* had the highest antioxidant capacity as free ABTS radical scavengers. The lowest phenolic contents and antioxidant capacities were obtained from *Polygonum bistorta* root extracts.

*Naphade et al.,* (2009) evaluated antioxidant activity of aqueous, chloroform and methanol extract of the aerial parts of plant of *Tricholepis glaberrima DC* by the FTC and TBA methods. Methanolic extract showed higher antioxidant activity than the chloroform and aqueous extract. The results obtained in their study indicate that the plants of *Tricholepis glaberrima DC* are a potential source of natural antioxidants.

*Krishnaraju et al.,* (2009) assessed antioxidant activity of the crude extracts of bark of *A. polystachya* using NBT, DPPH, ABTS and FRAP assays. The potent fraction (AP-110/82C) was tested for *in vivo* efficacy. The methanol, aqueous methanol and water extracts exhibited potent antioxidant activity compared to known antioxidants. Due to its natural origin and potent free-radical scavenging ability, *A. polystachya* could be used as a potential preventive intervention for free radical-mediated diseases.

*Sakat and Juvekar* (2010) have designed to investigate the antioxidant activity of aqueous and methanol extracts of *Erythrina indica* Lam leaves by *in vitro* methods viz. 1, 1-Diphenyl-2-Picrylhydrazyl, nitric oxide radical scavenging activity, and inhibition of lipid peroxidation by thiobarbituric acid reactive substances (TBARS) method on isolated rat liver tissues. Results showed that the aqueous and methanol extracts exhibited significant DPPH radicals scavenging activity with an IC$_{50}$ value $342.59 \pm 19.59$, $283.24 \pm 12.28$ µg/mL respectively. Nitric oxide radicals were significantly scavenged by the aqueous and methanol extracts (IC$_{50} = 250.12 \pm 10.66$; $328.29 \pm 3.74$ µg/mL). Lipid peroxidation induced by the Fe$^{2+}$ was inhibited by the aqueous extract with low IC$_{50}$ value ($97.29 \pm 2.05$ µg/mL) as compared to methanol extract (IC$_{50} = 283.74 \pm 5.70$ µg/mL). Both the extracts were exhibited similar quantities of total phenolics. Total flavonoids were found to be in higher quantities than total flavonols in aqueous extract as compared to
methanol extract. From the results, it is concluded that the aqueous and methanol extracts of *E. indica* leaves possess significant antioxidant activity that may be due to the presence of flavonoids and related polyphenolic compounds.

*Erythrina indica* Lam (Family: Fabaceae) has good ethno pharmacological value in Indian folk medicine. The present study was undertaken to evaluate two different extracts of *Erythrina indica* bark for antioxidant and anti-inflammatory activity by simple, reliable, and less time consuming methods by Ajay Kumar *et al.*, (2010). Standard methanolic and ethyl acetate extracts of *Erythrina indica* was tested *in vitro* for its anti-inflammatory activity and antioxidant activity using anti denaturation and reducing power methods respectively. Methanolic extract possess significant anti-inflammatory and antioxidant effects where as, ethyl acetate extract possess moderate anti-inflammatory and antioxidant activities.

Debnath *et al.*, (2010) evaluated the anti-oxidant activity of the hydroalcoholic extract of the bark of *Erythrina fusca* Lour (HAEEF) using different *in vivo* experimental models. Animals are used for the demonstration of an injury by exogenous agents of epileptic seizure on the brain with its physiological significance. Epileptic seizure challenged animals treated with HAEEF at doses of 250 mg/kg and 500 mg/kg showed antioxidant activity. Thus, their results suggests that the hydro-alcoholic extract of *Erythrina fusca* Lour bark possess antioxidant effect against the animal models of epilepsy.

2.B. Anti-inflammatory activity

2.B.i. Inflammation

Inflammation is defined classically as a protective and defense reactions by the body, in response to some physical or chemical injury. During inflammatory conditions various pathological changes are take place. The production of active inflammatory mediators is triggered by microbial products or by host proteins, such as proteins of the complement, Kinins and coagulation systems that are themselves are activated by microbes and damaged tissues. Inflammation is typically accompanied by pain, swelling, redness, and heat due to above factors. A closer look at the area of inflammation reveals that the
small blood vessels are dilated, which brings in more blood and warmth, and other fluid, which causes swelling of the area.

2. B. ii. Classification of Inflammation

Inflammation may broadly classify into three categories:

1. Acute inflammation.
2. Chronic inflammation.
3. Miscellaneous kinds of inflammation.

This third category may include allergic and dermatological disorders.

1. Acute inflammation

When a tissue injury is caused by a single event such as mechanical trauma, a thermal or chemical burn or a single exposure to non-replicating antigen the protective phenomena results in inflammation and repairative process proceeds smoothly from injury to recovery. Thus whole inflammatory process at least in acute inflammation exemplifies a beneficial homeostatic mechanism trying to restore the affected tissue to its normal healthy state.

2. Chronic Inflammation

There are many diseases which are distinguished by signs and symptoms characteristic of response to chronic inflammatory process of unknown etiology. Some of the rheumatic disorders are characterized by a lack of detectable anti-globulin (rheumatid factor) and antinuclear antibodies in the serum. These are often loosely called as collagen disease which is suggestive of involvement of structure and / or metabolism of collagen in the diseased process. Several classifications have been suggested (Kulonen, 1971). The main members include rheumatic fever, rheumatoid arthritis, ankylosing spondylitis and osteoarthritis, but many other disorders exhibiting chronic inflammatory changes such as periarteritis nodosa, scleroderma and systemic lupus erythematosus are frequently included in general classification (Goodman and Gillman 1970). However they have included these disorders in miscellaneous group.
There are some other types of chronic inflammations caused by self-replicating parasites like bacteria, virus or neoplasm. Such inflammation may become much more complex because of persisting injurious agents or their degraded products. When noxious agents cannot be destroyed or early eliminated, the inflammatory responses try to isolate them from the rest of the organism by forming granuloma (e.g., in pulmonary tuberculosis and silicosis or gumma as in syphilis). The ultimate response of this type is classified as Gohn complexes which contain viable but imprisoned tubercle bacilli functionally isolated from the host. Gout is characterized by acute and chronic inflammatory response to the deposition of microcrystals of sodium urate in the joints and tissues. Now the etiology of gouty arthritis is totally well understood.

3. Miscellaneous kinds of inflammation.

This group of disorders is not essentially inflammatory but its components are inflammatory origin. Many of the dermatological conditions consist of acute, subacute and chronic inflammatory reactions to various known and unknown prime causes. All these disorders mainly involve skin and can readily be assessed. Some examples of these skin diseases are pemphigus, pemphigoid and discoid lupus. These, however, apparently seem to be immunologic but they hardly respond to immunosuppressive therapy (Ehringer and Mackay, 1969). Contact dermatitis on the other hand is a manifestation of delayed hypersensitivity. Encephalomyelitis involving spinal cord and motor incoordination. Rejection of the transplanted organ is clearly initiated by normally protective immunological reactions but it is mediated by the familiar sequelae of inflammatory stimulus leading to cardinal signs of inflammation and, above all, the loss of function.

Lysosomal Enzymes- It is postulated that lysosomal components such as hydrolytic enzymes or cationic proteins play important roles in the initiation of inflammation, tissue injury and connective tissue breakdown (Weissman and his co-workers, 1964, 1969, Shen, 1967, Janoff and Zweifach, 1964). Anderson (1970) found higher levels of catalytic enzymes in inflamed tissue or serum of arthritic rats as compared to normal animals. In rat adjuvant arthritis, it has been stressed that the potential destructive capacity of connective tissue is acid hydrolases and is liberated within the endogenous cellular elements of connective tissue or derived from migrating leukocytes (Anderson, 1970). However
Paulous and Whitehouse (1973) have stressed that the presence of potentially destructive enzymes in serum is not the sole factor in injury, because Collin and Lewis, (1971) have found no correlation between maximum enzyme activity and presence of tissue damage. This is further supported by the fact that, in rheumatoid arthritis the catabolic activity is not the sole problem, but there is also the articular damage which may be due to adopted leukocytes in synovial tissue. Mediators of inflammation shown in fig.3.

Studies revealed the presence, within human polymorphonuclear leukocyte lysosomes, of enzymatic activity capable of degrading the non-collagenous proteoglycon matrix of hyaline cartilage at neutral pH (Ignarro et al., 1973). Rapid breakdown of the sulfated mucopolysaccharide constituents of cartilage was enhanced in a neutral pH or balanced salt solution by lysosome granule lysates derived from human, but not from rabbit or guinea pig, polymorphonuclear leukocytes. Thus, human leukocytes are remarkably different from other species with regard to the capacity of their lysosomal enzymes to degrade intact cartilage under conditions of neutral pH and balanced ionic movements. The neutral protease activity of human leukocyte lysosomes to degrade
hemoglobin was demonstrated (Ignarro, 1973). The presence of elevated lysosomal contents namely neutral proteases, acid hydrolases, chemotactic factors, kinin generating factors, vascular permeability factors, pyrogens in the synovial fluid of arthritic patients has been clearly documented (Ignarro, 1974).

Lysosomal enzymes secretion occurs as a result of interaction between the leukocyte of macrophage plasma membrane of and immunologic reactant or stimulus endocytosis of particulate reactants is not a prerequisite for enzyme secretion, however, secretion occurs in the absence of phagocytosable particles (Henson, 1971; Hawkins, d 1972; Oronsky et al., 1973; and Ignarro 1974). This immunologically-provoked selective secretion of lysosome granule contents from neutrophils appears to result from leakage into the extracellular, environment of primary lysosomes contents at precisely the time when newly-formed heterophagic cacuoles are still open to the extracellular compartment while merging at their surfaces with the lysosomes. All this important data indicate that there may be some kind of regulation by autonomic neurohormones, glucocorticoids, prostaglandins and cyclic nucleotides in immunologically provided secretion of lysosomal mediators of inflammation by human neutrophils. They found that Aspirin (high doses), phenylbutazone and indomethacin inhibit the increase of acid phosphatase in liver and inflamed tissue during various types of experimental inflammation in rats (Naik, 1973). There are also conflicting reports regarding action of anti-inflammatory agents on these lysosomal enzymes (Weissman, 1968). However, they feel that these catabolic enzymes, which are degraded from connective tissue or cellular elements make a common pathway for inflammatory process. Hence one should consider these enzymes for the evaluation of anti-inflammatory agents.

There are some reports that α -globulin like macromolecules are present in pregnancy serum or adjuvant arthritic rats and are capable of stabilizing isolated liver lysosomes (Hempel et al., 1970). Thus one may presume that these substances may be endogenous natural anti inflammatory substances. The future research on the effect of lysosomes stabilization, enzyme deficiency or inhibition may probably lead to find out factors involved in chronic inflammatory process.
B. Prostaglandins (PGS)

Rabwell and Pharris (1972) have claimed that prostaglandins of E series are involved in cellular injury and inflammation. Most of the non-steroidal anti-inflammatory agents are active inhibitors of its production from its precursor, arachidonic acid. These prostaglandins E₁, E₂ often induce the increase of vascular permeability in animals and flare response in human beings (Horton, 1963, Crunkhorn and Wills, 1971, Kaley and Weiner, 1971, 1971) PGE type has been identified in the inflammatory exudates of carrageenan induced inflammation in the rat (Wills, 1969). Prostaglandins occur relatively late in inflammatory process and are often associated with migration of leukocytes into inflamed site (Willoughby, 1971). In rabbits it has been shown that during phagocytosis, prostaglandins are released from leukocyte lysosome (Higgs and Youlten, 1972) and also during endocytosis (Anderson, 1971).

Aspinall and Cammarata (1969) and Zurier and Quasgliata (1971) have reported that PGE₂, elicits potent antiarthritus effects in a model of adjuvant polyarthritis in the rat, but prostaglandin was not anti-inflammatory in acute inflammatory model PGE₂, PGA₁, and PGA₂ inhibited whereas PGF₂-α increased the immunologic release of betaglucoronidase from human leukocytes (Zuier et al., 1973).

Further PGE₂, PGA₁, PGA₂ and PGF₂-α were reported to depress phagolytosis by polymorphonuclear leukocytes (Cox and Karmovsky, 1973). Aspirin, indomethacin and salicylates have been shown by a number of workers to inhibit synthesis or release of PGE₂ and PGF₂-α from arachidonic acid from variety of tissue (Vane, 1971; Smith and Wills, 1971; Ferreira et al., 1971; Smith and Lands, 1971). The inhibition of prostaglandin synthetase by anti-inflammatory agents depends on its order of potency in carrageenan induced inflammation (Tomlinson et al., 1972). Though non-steroidal anti-inflammatory drugs suppress prostaglandin syntheses, recently it has been shown that PGE₁ and PGE₂ at high doses suppress local inflammation of arthritis and carrageenan induced inflammation (Aspinall et al., 1969, Glenn et al., 1972) with some side and toxic effects like hyperplasia prostavation and diarrhea which may contribute to its anti-inflammatory effect.
Denko (1974) has shown that the involvement of prostaglandins in urate crystal inflammation. He explained that the prolonged inflammation of urate crystals is due to the constant formation or release of prostaglandins from antecedent phospholipids in the membrane. These released prostaglandins may occur with membranolysis. He concludes that urate crystal inflammation may be a membrane disease.

In many cases the mechanisms by which prostaglandins elicit some of their actions are thought to involve cyclic AMP (Kahn and Lands, 1973). Similarly, with regard to their potential anti-inflammatory effects certain prostaglandins have been reported to stimulate the synthesis and elevate the levels of cyclic AMP in human leukocyte (Scott, 1970; Bourne and Melmon, 1971, Bourne et al., 197).

In addition PGE 1, PGE2 and PGF2-α were reported to stimulate adenylate cyclase activity in human mixed leukocytes (Poigar et al., 1973). Endogenous cyclic AMP and cyclic GMP might mediate the opposing effects of prostaglandins on lysosomal enzyme secretion from neutrophils. The prostaglandins, especially the biphasic actions of PGF 2 on neutrophil function argue in favour of a modulatory function for these tissue hormones in the inflammatory process. Glucocorticoids reduce the rapid accumulation of cyclic GMP provoked by immune reactants but have no effects on levels of cyclic AMP.

2.B.iii. Different methods for evaluating anti-inflammatory agents

1. **Acute Inflammation**
   1. Carrageenan-induced Paw Edema in Rats (Winter CA et al., 1962)
   2. Histamine Induced Paw Edema in Rats (Amann R et al., 1995)
   3. Acetic Acid-Induced Vascular Permeability (Whittle BA 1964)
   4. Xylene Induced Ear Edema (Thickness and weight parameter) (Junping K et al., 2005)
   5. Arachidonic Acid-Induced Ear Edema (Romay et al., 1998)
   6. Phorbol Myristate Acetate-Induced Ear Edema in Mice (Griswold DE et al., 1998)
   7. Oxazolone-induced Ear Edema in Mice (Evans PD et al., 1971).
Review of Literature

2. Sub-Acute Model
1. Carrageenan Induced Granuloma Pouch Model (Selye, 1953)
2. Formalin-induced Paw Edema (Turner R, 1971)
3. Chronic Model
4. Cotton Pellet-Induced Granuloma in Rats (Goldstein et al., 1976)
5. The Glass Rod Granuloma (Vogel H, 1996)

The above mentioned models have given broad spectrum for the evaluation of the anti-inflammatory activity. In different models, the inflammation has produced by different inducers by releasing inflammatory mediators. Each is having different mechanism of action for producing inflammation either by increased in vascular permeability, the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair. Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used such as brewer’s yeast, formaldehyde, dextran, egg albumin, kaolin, aerosil, sulfated polysaccharides like carrageenan or naphthoylheparamine. For producing edema histamine, xylene, arachidonic acid, phorbol myristate acetate, oxozolone, croton oil and formalin are also used.

For evaluating the most effective and widely used model for inflammation is carrageenan induced paw edema, Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, Chondrus crispus. It is used as an endemogen was introduced by Winter et al., (1962). Carrageenan initially releases histamine and serotonin followed by release of prostaglandins, protease and lysosomes producing edema.

Many plants have been used in the treatment of inflammation namely; Acacia burkei (Fabaceae), Acacia sieberiana (Fabaceae), Achyranthes aspera (Amaranthaceae), Acokanthera oppositifolia (Amaranthaceae), Alepidea amatymbica (Apiaceae), Apenia cordifolia (Mesembryanthemaceae), Asclepias drummiana (Asclepiadaceae), Athrixia phyllicoides (Asteraceae), Barbarea ovata (Asteraceae), Berchemia zeyheri (Rhamnaceae), Berkheya speciosa (Asteraceae), Bidens pilosa (Asteraceae), Boophane disticha
(Amaryllidaceae), Bowiea volubilis (Lilaceae), Brachylaena elliptica (Asteraceae), Bridelia micrantha (Euphorbiaceae), Bulbine alooides (Lilaceae), Bulbine latifolia (Lilaceae), Capparis tomentosa (Capparaceae), Cavacoa ayrea (Euphorbiaceae), Cenchrus ciliaris (Poaceae), Cephalaria zeyheriana (Dipsaceae), Chaetacme aristata (Ulmaceae), Clausena anisata (Rutaceae) (Watts and Breyer-Brandwijk, 1962; Hutchings et al., 1996).

Srinivas et al., (2000) studied the anti-inflammatory effect of Heliotropium indicum, and Leucas aspera on carrageenin induced hind paw oedema and cotton pellet granuloma in rats. The animals were treated with H. indicum and L. aspera and the standard drugs viz., acetylsalicylic acid and phenylbutazone. H. indicum and L. aspera produced significant anti-inflammatory effect in both acute and subacute models of inflammation. In acute inflammation, L. aspera was more effective than acetylsalicylic acid. However, in subacute inflammation, these two drugs were found to be less effective than phenylbutazone. H. indicum and L. aspera possess anti-inflammatory effects in both acute and subacute inflammation.

Mujumdar et al., (2000) studied the Anti-inflammatory activity of Curcuma amada rhizome extract in albino rats. Rhizomes of Curcuma amada were extracted and subjected to spectroscopic studies. The extract was screened for Anti-inflammatory activity in albino rats using acute carrageenan paw oedema and chronic granuloma pouch model. The extract of Curcuma amada rhizomes showed Anti-inflammatory activity in acute and chronic administration in albino rats.

Aguilar et al., (2002) assessed in vivo the anti-inflammatory activity of two Cat's claw bark extracts, by comparing a spray-dried hydroalcoholic extract against an aqueous freeze-dried extract, to determine which extract was more effective. They used the carrageenan-induced paw edema model in mice. The results showed that the anti-inflammatory activity was significantly higher using the hydroalcoholic compared with the aqueous extract (PB 0.05).

Njamen et al., (2003) have isolated erycristagallin, a pterocarpene isolated from Erythrina mildbraedii, which was tested in vitro for its antioxidant properties on the stable 2,2-diphenyl-1-pyryl-hydrazyl (DPPH) free radical and on the arachidonic acid
metabolism. In addition, erycristagallin was tested on different experimental models of inflammation, such as the acute and chronic inflammation induced by the application of 12-O-tetradecanoylphorbol 13-acetate (TPA) on mice and the phospholipase A(2)-induced mouse paw oedema test. In the carrageenan-induced mouse paw oedema test, the ethyl acetate extract obtained from *E. mildbraedii* showed anti-inflammatory activity, and erycristagallin was isolated as the active principle. *In vivo*, erycristagallin significantly inhibited the phospholipase A(2)-induced mouse paw oedema as well as the mouse ear oedema induced by TPA (ID(50)<10 microg/ear). Moreover, it significantly reduced the chronic inflammation and leukocyte infiltration induced by repeated application of TPA. *In vitro*, erycristagallin inhibited the arachidonic acid metabolism via the 5-lipoxygenase pathway in rat polymorphonuclear leukocytes (IC_{50}=23.4 microM), but had no effect on cyclooxygenase-1 metabolism in human platelets, while showing antioxidant activity in the DPPH test. As with other phenolics, the anti-inflammatory activity of erycristagallin may be based on its capacity to inhibit the arachidonic acid metabolism via the 5-lipoxygenase pathway.

Njamen *et al.*, (2004) isolated two prenylated flavanones isolated from *Erythrina sigmoidea* Hua (sigmoidin A and sigmoidin B) studied for their ability to inhibit the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and arachidonic acid metabolism. In addition, the compounds were studied in two experimental models of inflammation induced in mouse ears by 12- O-tetradecanoylphorbol 13-acetate (TPA) and the phospholipase A (2)-induced mouse paw oedema. Both sigmoidins A and B proved to be potent scavengers of the DPPH radical, while the study of the inhibition of arachidonic acid metabolism demonstrated that these same compounds were selective inhibitors of 5-lipoxygenase, with no effect on cyclooxygenase-1 activity. Dose-response inhibitor potency was established for sigmoidin A (IC_{50} = 31 microM). In the assay of phospholipase A (2)-induced mouse paw oedema, only the sigmoidin B derivative inhibited oedema formation at 60 min, showing a percentage of inhibition below that obtained with cyproheptadine (59 % vs. 74 %). In the TPA test, sigmoidins A and B decreased the induced oedema by 89 % and 83 %, respectively. This is the first time that the anti-inflammatory activity and antioxidant properties of these prenylflavanones have...
been reported. The results indicate that the compounds have different mechanisms of action depending on whether one or two prenyl groups are present in ring B.

Neto *et al.*, (2005) conducted anti-inflammatory and antinociceptive effects of the crude hydroalcoholic extract (PE) of *Pfaffia glomerata* roots in the carrageenan-induced rat paw edema at the doses of 100, 200 and 300 mg/kg, using different animal models. The results indicate the potential of this plant extract to treat chronic inflammation.

Marchioro *et al.*, (2005) tested anti-nociceptive and anti-oedematogenic effects of the aqueous extract from the leaves of *Erythrina velutina* through experimental models of nociception in mice and paw oedema induced by carrageenin in rats. The extract (300 and 600 mg/kg) did not change the carrageenin-induced paw oedema. In the hot plate test the extract also did not alter the latency time for mice liking the rear paws. They demonstrated that the crude extract from the leaves of *E. velutina* has anti-nociceptive but not anti-oedematogenic properties.

Balamurugan *et al.*, (2010) screened ethanolic extract of the leaves of *Erythrina variegata* Linn leaves (Fabaceae) for its anti-inflammatory activity. The dried powder of leaves was extracted with ethanol (95%) for the present study. The extract showed a significant activity comparable to the standard drug Diclofenac sodium.

Kumar *et al.*, (2010) evaluated two different extracts of *Erythrina indica* (Family: Fabaceae) bark for antioxidant and anti-inflammatory activity. Standard methanolic and ethyl acetate extracts of *Erythrina indica* was tested *in vitro* for its anti-inflammatory activity and antioxidant activity using anti denaturation and reducing power methods respectively. Methanolic extract possess significant anti-inflammatory and antioxidant effect where as ethyl acetate extract possess moderate anti-inflammatory and antioxidant activity.

The leaf extract of *Erythrina stricta* (*ES*) was screened for anti-inflammatory activity in albino rats using acute carrageenan paw oedema, formalin induced paw oedema and sub acute granuloma pouch model by Subhashini *et al.*, (2011). The extract exhibit its anti-inflammatory action by means of inhibiting the synthesis, release or action of
inflammatory mediators viz, histamine, serotonin and prostaglandin might be involved in inflammation. From these results, it is suggested that anti oedematogenic effect of the ES on carrageenan and formalin induced oedema might be related to inhibition of inflammation mediator formation. So, our results strongly suggested that the extract of ES leaves showed anti-inflammatory activity in acute and sub acute administration in albino rats.

2.C. Wound Healing Activity:

The wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). These phases and their biophysiological functions must occur in the proper sequence, at a specific time, and continue for a specific duration at an optimal intensity (Table-1; Mathieu et al., 2006). There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair.

Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Non-healing wounds affect about 3 to 6 million people in the United States, with persons 65 years and older accounting for 85% of these events. Non-healing wounds result in enormous health care expenditures, with the total cost estimated at more than $3 billion per year (Mathieu et al., 2006; Menke et al., 2007).
Table 1. Normal Wound-healing Process

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cellular and Bio-physiologic Events</th>
</tr>
</thead>
</table>
| Hemostasis | 1. Vascular constriction  
               2. Platelet aggregation, degranulation, and fibrin formation (thrombus) |
| Inflammation | 1. Neutrophil infiltration  
                    2. Monocyte infiltration and differentiation to macrophage  
                    3. Lymphocyte infiltration |
| Proliferation | 1. Re-epithelialization  
                        2. Angiogenesis  
                        3. Collagen synthesis  
                        4. ECM formation |
| Remodeling  | 1. Collagen remodeling  
                        2. Vascular maturation and regression |

2.C.i. Wound healing process

Wound healing is a dynamic process consisting of four continuous, overlapping, and precisely programmed phases. The events of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or a non-healing chronic wound.

In adult humans, optimal wound healing involves the following events: (1) rapid hemostasis; (2) appropriate inflammation; (3) mesenchymal cell differentiation, proliferation, and migration to the wound site; (4) suitable angiogenesis; (5) prompt re-epithelialization (re-growth of epithelial tissue over the wound surface); and (6) proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue (Gosain and DiPietro, 2004; Mathieu et al., 2006). The first phase of hemostasis begins immediately after wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Once bleeding is controlled,
inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes (Gosain and DiPietro, 2004; Broughton et al., 2006; Campos et al., 2008). A critical function of neutrophils is the clearance of invading microbes and cellular debris in the wound area, although these cells also produce substances such as proteases and reactive oxygen species (ROS), which cause some additional bystander damage.

Macrophages play multiple roles in wound healing. In the early wound, macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thus paving the way for the resolution of inflammation. As macrophages clear these apoptotic cells, they undergo a phenotypic transition to a reparative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote tissue regeneration (Meszaros et al., 2000; Mosser and Edwards, 2008). In this way, macrophages promote the transition to the proliferative phase of healing.

T-lymphocytes migrate into wounds following the inflammatory cells and macrophages, and peak during the late-proliferative/early-remodeling phase. The role of T-lymphocytes is not completely understood and is a current area of intensive investigation. Several studies suggest that delayed T-cell infiltration along with decreased T-cell concentration in the wound site is associated with impaired wound healing, while others have reported that CD 4+ cells (T-helper cells) have a positive role in wound healing and CD8+ cells (T-suppressor-cytotoxic cells) play an inhibitory role in wound healing (Swift et al., 2001; Park and Barbul, 2004). Interestingly, recent studies in mice deficient in both T- and B-cells have shown that scar formation is diminished in the absence of lymphocytes (Gawronska-Kozak et al., 2006). In addition, skin gamma-delta T-cells regulate many aspects of wound healing, including maintaining tissue integrity, defending against pathogens, and regulating inflammation. These cells are also called dendritic epidermal T-cells (DETC), due to their unique dendritic morphology. DETC are activated by stressed, damaged, or transformed keratinocytes and produce fibroblast growth factor 7 (FGF-7), keratinocyte growth factors, and insulin-like growth factor-1, to support keratinocyte proliferation and cell survival. DETC also generate chemokines and cytokines that
contribute to the initiation and regulation of the inflammatory response during wound healing. While cross-talk between skin gamma-delta T-cells and keratinocytes contributes to the maintenance of normal skin and wound healing, mice lacking or defective in skin gamma-delta T-cells show a delay in wound closure and a decrease in the proliferation of keratinocytes at the wound site (Jameson and Havran, 2007; Mills et al., 2008).

The proliferative phase generally follows and overlaps with the inflammatory phase, and is characterized by epithelial proliferation and migration over the provisional matrix within the wound (re-epithelialization). In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury. Within the wound bed, fibroblasts produce collagen as well as glycosaminoglycans and proteoglycans, which are major components of the extracellular matrix (ECM). Following robust proliferation and ECM synthesis, wound healing enters the final remodeling phase, which can last for years. In this phase, regression of many of the newly formed capillaries occurs, so that vascular density of the wound returns to normal. One critical feature of the remodeling phase is ECM remodeling to an architecture that approaches that of the normal tissue. The wound also undergoes physical contraction throughout the entire wound-healing process, which is believed to be mediated by contractile fibroblasts (myofibroblasts) that appear in the wound (Gosain and DiPietro, 2004; Campos et al., 2008).

The role of stem cells (SC) in cutaneous wound healing and tissue regeneration is a topic of increasing research attention, with a focus on the role of adult stem cells such as epidermal stem cells and bone-marrow (BM)-derived cells (BMDCs). Epidermal stem cells reside in the bulge area of hair follicles and in the basal layer of the epidermis and give rise to the keratinocytes that migrate and epithelialize wounds. Normal skin is also a target organ for BMDCs. Two main stem cell populations are present in the bone marrow: hematopoietic SC (HSC) and mesenchymal SC (MSC). BM-MSCs are able to differentiate into a variety of cell types, including adipocytes, osteoblasts, chondrocytes, fibroblasts, and keratinocytes (Cha and Falanga, 2007; Rea et al., 2009). Endothelial progenitor cells (EPCs) derived from the HSC lineage are key cells that contribute to neovascularization.
Both BM-MSCs and EPCs are involved in the cutaneous wound-healing process. Wound-induced hypoxia triggers the mobilization of bone marrow EPCs to the circulation, playing a significant role in the process of neovascularization (Wu et al., 2007; Liu and Velazquez, 2008; Rea et al., 2009).

Several different cell types are involved in the wound-healing process, and, as described above, the cellular activities of any particular cell type may also vary during different stages of repair. The complexity and coordination of the healing process are major hurdles to therapeutic approaches, since any therapeutic must effectively be sequenced to the appropriate stage.

2.C.ii. Factors effecting wound healing process

Multiple factors can lead to impaired wound healing. In general terms, the factors that influence repair can be categorized into local and systemic. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal (Table-2). Many of these factors are related, and the systemic factors act through the local effects affecting wound healing.

a. Local Factors

Oxygenation

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction (Bishop, 2008; Rodriguez et al., 2008). In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels.

Due to vascular disruption and high oxygen consumption by metabolically active cells, the microenvironment of the early wound is depleted of oxygen and is quite hypoxic. Several systemic conditions, including advancing age and diabetes, can create impaired
vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg (Tandara and Mustoe, 2004).

Table-2. Factors Affecting Wound Healing

<table>
<thead>
<tr>
<th>Local Factors</th>
<th>Systemic Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenation</td>
<td>• Age and gender</td>
</tr>
<tr>
<td>Infection</td>
<td>• Sex hormones</td>
</tr>
<tr>
<td>Foreign body</td>
<td>• Stress</td>
</tr>
<tr>
<td>Venous sufficiency</td>
<td>• Ischemia</td>
</tr>
<tr>
<td></td>
<td>• Diseases: diabetes, keloids, fibrosis, hereditary healing disorders, jaundice, uremia</td>
</tr>
<tr>
<td></td>
<td>• Obesity</td>
</tr>
<tr>
<td></td>
<td>• Medications: glucocorticoid steroids, non-steroidal anti-inflammatory drugs, chemotherapy</td>
</tr>
<tr>
<td></td>
<td>• Alcoholism and smoking</td>
</tr>
<tr>
<td></td>
<td>• Immunocompromised conditions: cancer, radiation therapy, AIDS</td>
</tr>
<tr>
<td></td>
<td>• Nutrition</td>
</tr>
</tbody>
</table>

In wounds where oxygenation is not restored, healing is impaired. Temporary hypoxia after injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing (Bishop, 2008; Rodriguez et al., 2008). In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, tumor necrosis factor-α (TNF-α), and endothelin-1, and are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing (Rodriguez et al., 2008).
In normally healing wounds, ROS such as hydrogen peroxide ($\text{H}_2\text{O}_2$) and superoxide ($\text{O}_2^-$) are thought to act as cellular messengers to stimulate key processes associated with wound healing, including cell motility, cytokine action (including PDGF signal transduction), and angiogenesis. Both hypoxia and hyperoxia increase ROS production, but an increased level of ROS transcends the beneficial effect and causes additional tissue damage (Rodriguez et al., 2008).

In summary, the proper oxygen level is crucial for optimum wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, while oxygen is needed to sustain the healing process (Bishop, 2008). One therapeutic option that can sometimes overcome the influence of tissue hypoxia is hyperbaric oxygen therapy (HBOT) Rodriguez et al., 2008). While HBOT can be an effective treatment for hypoxic wounds, its availability is limited.

Infections

Once skin is injured, micro-organisms that are normally sequestered at the skin surface obtain access to the underlying tissues. The state of infection and replication status of the micro-organisms determines whether the wound is classified as having contamination, colonization, local infection/critical colonization, and/or spreading invasive infection. Contamination is the presence of non-replicating organisms on a wound, while colonization is defined as the presence of replicating micro-organisms on the wound without tissue damage. Local infection/critical colonization is an intermediate stage, with micro-organism replication and the beginning of local tissue responses. Invasive infection is defined as the presence of replicating organisms within a wound with subsequent host injury (Edwards and Harding, 2004).

Inflammation is a normal part of the wound-healing process, and is important to the removal of contaminating micro-organisms. In the absence of effective decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged
inflammation also leads to an increased level of matrix metalloproteases (MMPs), a family of proteases that can degrade the ECM. In tandem with the increased protease content, a decreased level of the naturally occurring protease inhibitors occurs. This shift in protease balance can cause growth factors that appear in chronic wounds to be rapidly degraded (Edwards and Harding, 2004; Menke et al., 2007). Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix (EPS; Edwards and Harding, 2004). Mature biofilms develop protected microenvironments and are more resistant to conventional antibiotic treatment. *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and β-hemolytic streptococci are common bacteria in infected and clinically non-infected wounds (Edwards and Harding, 2004; Davis et al., 2008).

*P. aeruginosa* and *Staphylococcus* appear to play an important role in bacterial infection in wounds. Many chronic ulcers probably do not heal because of the presence of biofilms containing *P. aeruginosa*, thus shielding the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds (Bjarnsholt et al., 2008).

b. Systemic Factors

1. Age
2. Sex Hormones in Aged Individuals
3. Stress
4. Diabetes

Multiple factors can cause impaired wound healing by affecting one or more phases of the process and are categorized into local and systemic factors. The influences of these factors are not mutually exclusive. Single or multiple factors may play a role in any one or more individual phases, contributing to the overall outcome of the healing process.

2.C.iii. Tensile strength of wound

This is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it. In the beginning a wound will be having little tensile
strength because the clot alone will be holding the edges together. Thereafter tensile strength increases rapidly as collagen deposition increases and cross linkages are formed between collagen fibers.

Wounds gain only about 20 percent of their final strength in the first three weeks, during which time fibrillar collagen has accumulated relatively rapidly and has been remodeled by contraction of the wound. Thereafter the rate at which wounds gain tensile strength is slow, reflecting a much slower rate of accumulation of collagen and, more important, collagen remodeling with the formation of larger collagen bundles and an increase in the number of intermolecular cross-links (Bailey et al., 1975). Nevertheless, wounds never attain the same breaking strength (the tension at which skin breaks) as in uninjured skin. At maximal strength, a scar is only 70 percent as strong as normal skin (Levenson et al., 1965). Extra cellular matrix involves five main components which include collagen, adhesive glycoproteins, basement membrane, elastic fibers and proteoglycans which are responsible for wound strength.

Several such plants have been used to promote wound healing. Many investigators reported the wound healing effect of the various plant extracts such as Aloe vera (Udupa, et al., 1994, Schmidt and Greenspoon, 1991); Trigonella foenum graecum (Taranalli and Kuppast, 1996); Hypericum mysoresne (Mukherjee and Suresh, 2000); Ginkgo biloba (Bairy and Rao, 2001); Gmelina arborea Roxb. (Shirwaikar, et al., 2002); Terminalia arjuna (Madhura and Sushma, 2003); Eucalyptus globulus (Kusum, et al., 2004); Saussurea lappa (Ganachari, et al., 2005); Plagiochasma appendiculatum (Meenakshi et al., 2006); Terminalia arjuna (Minakshi and Sushma, 2006); Embelia ribes (Kumaraswamy et al., 2007) and Lycopodium serratum (Manjunatha et al., 2007); Quercus infectoria (Umachigi et al., 2008); Lantana camara (Abdulla et al., 2009); and Cocos nucifera (Srivastava et al., 2008).

Even though the plant understudy Erythrina mysorensis related species have been reported in the traditional practices for wound healing purposes, but there are no scientific reports on their wound healing activity. Thus, this pharmacological activity is evaluated in this study.
Wound healing activity of plants

Ghosh et al., (2004) screened the ethanolic (50% v/v) extract of the leaves of Tagetes erecta Linn. (Family – Asteraceae) for wound-healing activity on adult albino rats by excision wound model and incision wound model respectively. The studies on excision wound model reveals significant wound healing activity of the extract, which is comparable with the reference control nitrofurazone. In the incision model, the tensile strength of the extract treated group is found to be highly significant \((p< 0.001)\) on 12\(^{th}\) post wounding day when compared with controls.

Mone et al., (2005) evaluated the wound healing effect of herbal ointments formulated with Napoleona imperialis (NI) embedded in different ointment bases (anionic, cationic and non-ionic) \(in vivo\) using the excision wound healing model, on guinea pigs. Napoleona imperialis was extracted using methanol and the extract formulated as herbal ointments. The wound healing effects of the formulations were compared to that of a standard antibiotic, Cicatrin\(^\circ\). The wound areas in the animals treated with the standard antibiotic, Cicatrin\(^\circ\) showed a 100% healing by the 19\(^{th}\) day, indicating that the plant extract, at that given concentration, had a better wound healing property than the standard antibiotic.

Shetty et al., (2006) investigated the wound healing properties of crude extract of Jatropha curcas linn. in Wister albino rats. The results of the study suggests that the crude bark extract of Jatropha curcas was very effective in accelerating wound healing process.

Tara et al., (2006) studied the wound healing effect of alcoholic extract of Kaempferia galanga (K. galanga) and its effect in dexamethasone suppressed wound healing was in Wistar rats. Three wound models viz. incision, excision and dead space wounds were used in their study. The parameters studied were breaking strength in case of incision wounds, epithelialization and wound contraction in case of excision wound and granulation tissue dry weight, breaking strength and hydroxyproline content in case of deadspace wound. The dexamethasone treated group showed a significant \((P<0.001)\) reduction in the wound breaking strength when compared to control group in incision type.
of wound model. Co-administration of *K. galanga* with dexamethasone had significantly ($P<0.001$) increased the breaking strength of dexamethasone treated group. In excision wound model, the percentage of the wound contraction was significantly ($P<0.05$) increased by *K. galanga* only on 16th day and also it reversed the dexamethasone suppressed wound contraction on the 16 day. *K. galanga* significantly ($P<0.001$) reduced the time required for epithelialization and reversed the epithelialization delaying effect of dexamethasone significantly ($P<0.001$).

Nayak *et al.*, (2007) reported the evaluation of the Wound-healing activity of ethanol extract of *Morinda citrifolia* L. (noni). Leaves of this plant are one of the most important traditional Polynesian medicinal plants. The primary indigenous use of this plant appears to be of the leaves, as a topical treatment for wound healing. The ethanol extract of noni leaves (150 mg kg$^{-1}$ day$^{-1}$) was used to evaluate the wound-healing activity on rats, using excision and dead space wound models. Enhanced wound contraction, decreased epithelialization time, increased hydroxyproline content and histological characteristics suggest that noni leaf extract may have therapeutic benefits.

Umachigi *et al.*, (2008) reported the studies on wound healing properties of *Quercus infectoria*. Ethanol extract of the shade-dried leaves *Quercus infectoria* was studied for its effect on wound healing in rats, using incision, excision and dead-space wound models, at two different dose levels of 400 and 800 mg/kg. The plant showed a definite, positive effect on wound healing, with a significant increase in the level of the antioxidant enzymes, superoxide dismutase and catalase, in the granuloma tissue.

Nalwaya *et al.*, (2009) evaluated the latex of *Calotropis gigantean* (200 mg/kg/day) for its wound healing activity in albino rats using excision and incision wound models. Latex treated animals' exhibit 83.42 % reduction in wound area when compared to controls which was 76.22 %. The extract treated wounds are found to epithelize faster as compared to controls. Significant ($p<0.001$) increase in granuloma breaking strength (485±34.64) was observed. The Framycetin sulphate cream (FSC) 1 % w/w was used as standard.
Palanimuthu et al., (2011) investigated wound healing property of bark extracts *Eugenia jambolana* on full thickness deep burn wound model in Albino rats, were used to study the healing efficiency. Formulations (10% ointment) of crude ethanolic extract of the *Eugenia jambolana* bark was applied tropically over thermal wound. It was found that ointment treated rats showed accelerated healing than the control. It was observed that 10% extract of the *Eugenia jambolana* bark has progressive effects on wound healing in the experimental groups. This study suggests that *Eugenia jambolana* bark powder could be developed as a therapeutic agent for wound healing.

Singh et al., (2011) explored the wound healing activity of ethanolic extract of *Plantago ovata* seeds in albino wistar rats. The extract was tested for wound healing activity by excision and incison wound model. *Aloe vera* ointment (10%w/w) was used as standard drug and the activity of the extract was in close proximity to standard. On the basis of the results it can be said that the extract of *Plantago ovata* seeds possess wound healing activity.

**2.D. Antimicrobial Activity:**

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found *in vitro* to have antimicrobial properties. (Dahanukar et al., 2000, Cowan, 1999). Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources especially plant sources are also being investigated. Second the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and national food stores and the self medication with these substances is a common practice to certain extent (Cowan, 1999).
Today, infectious diseases are the second major cause of death worldwide and third leading cause of death in economically advanced countries (Nathan, 2004). Bacterial pathogens are responsible for several serious diseases (Table-3). Strains are getting resistant to antibiotics in clinical use and hence posing threat to mankind. The ability of bacteria to deceive any kind of conventional therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide (Clark, 2003). Unnecessary use of antibiotics has further fuelled this problem.

Table- 3. Key bacterial pathogens and related diseases

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Infectious Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>Skin and wound infections, endocarditis</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>Upper respiratory tract infection, pneumonia, sinusitis, meningitis Pharyngitis</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>Peritonitis, urinary tract infection, bacteremia</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>Urinary tract infection, bacteremia, gastrointestinal infection</td>
</tr>
<tr>
<td>E. faecium</td>
<td>Bacteremia, pneumoni</td>
</tr>
<tr>
<td>E. coli</td>
<td>Respiratory tract infection, sinusitis, meningitis</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>Bacteremia, burn infection</td>
</tr>
<tr>
<td>H. influenza</td>
<td>Tuberculosis</td>
</tr>
</tbody>
</table>

The discovery of vancomycin resistant S. aureus (VRSA) and multiresistant S. aureus has generated worldwide concern. It has thus become evident that there is urgent need for novel antibacterial drugs with broader spectrum, lesser side effects, and without cross-resistance to antibiotics in use. More initiative is required to foster the responsible and appropriate use of antibiotic that is another issue. The traditional medicine system based on natural products continues to play an important role in treatment of many diseases especially the infectious diseases. According to the WHO estimation, approximately 80% of the world’s population relies mainly on traditional medicine for their primary health. Indian traditional medicine system relied on plants and their parts to
treat various infectious diseases (Table- 4). Hundreds of herbs are known to be used for various diseases including many infectious diseases. Acacia, garlic, turmeric, neem, ginger, clove, plum and pomegranate are only a few to name.

Table 4. Some Traditionally used Herbs and their Bioactivity

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant Parts</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triphala i.e. three fruits viz. Haritaki (<em>Terminalia chebula</em>), Bibhitaka (<em>Terminalia belerica</em>) &amp; Amalaki (<em>Emblica officinalis</em>)</td>
<td>90 % ethanolic &amp; aqueous extracts of Triphala</td>
<td>Significant activity on <em>S. aureus</em>, <em>E. coli</em> and <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Turmeric (<em>Curcuma longa</em>)</td>
<td>Leaf extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>Fenugreek (Methika) (Trigonella faenum-graecum)</td>
<td>Seed extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>Wildrue (<em>Peganum harmal</em>)</td>
<td>Aqueous seed extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>Gokama (<em>Clitoria Ternatea</em>)</td>
<td>Hexane &amp; methanolic root extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>Sharapunkha (<em>Tephrosia puropurea</em>)</td>
<td>Root extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>Brahmi (<em>Bacopa monnieri</em>)</td>
<td>Ethanolic extract of aerial parts</td>
<td>Antihelminth activity</td>
</tr>
<tr>
<td>Tulsi (<em>Ocimum sanctum</em>)</td>
<td>Leaf extract</td>
<td>Antibacterial and antifungal activity</td>
</tr>
</tbody>
</table>

Later on extracts from many of such plants and herbs have been screened by investigators inquest for potential and safer antibacterial agents (Nair, 2005, Dabur, 2007, Sukanya, 2009, Valsaraj, 1999, Thatoi, 2008). Many comprehensive review articles have been published on the role of natural products in the discovery of antibacterial agents (Nussbaum, 2006, Leeds, 2006, Singh, 2000, Shahid, 2009). The plethora of literature in the area indicates an urgent need for a coordinated effort for meaningful research and discovery of novel antimicrobial agents. Most of the antibacterial agents in use today are
either natural products or their semi-synthetic variations or improved subclasses. The success of natural products as guideposts to new drugs is most obvious in antibacterials. Over 75% of new chemical entities submitted between 1984 and 2004 were based on natural product lead structures (Newman, 2003).

Rosado-Vallado et al., (2000) screened the methanol and water extracts of six Fabaceae species, traditionally used in Mayan medicine for the treatment of diarrhoea and eye infections, for in vitro antimicrobial activity. Four species showed activity against Gram positive bacteria, five exhibited some activity against Candida albicans, two exhibited activity against Aspergillus niger and only one, Mimosa pigra, inhibited growth of Pseudomonas aeruginosa. None of the extracts was active against Escherichia coli.

Akinpelu Obuotor (2000) studied Piliostigma thonningii stem bark 60% methanolic extract antibacterial activity against six out of eight bacterial isolates at a concentration of 20 mg/ml. Ajali (2000) conducted preliminary antimicrobial screening of the petroleum ether, acetone, ethanol and methanol successive extracts of Alchornea cordifolia are reported.

Pillay (2001) screened aqueous, ethanolic and ethyl acetate extracts of the bark and leaves of five South African Erythrina species Erythrina caffra, Erythrina huneana, Erythrina latissima, Erythrina lysistem on and Erythrina zeyheri were screened for prostaglandin synthesis-inhibitory and anti-bacterial activity. The bark generally displayed higher activity than the leaves in both bioassays. The highest cyclooxygenase inhibiting activity and anti-bacterial activity was recorded for the ethanol and ethyl acetate bark extracts of E. caffra, E. latissima and E. lysistem on. An anti-bacterial compound, 4', 5, 7-trihydroxy-6-prenylisoflavone, was isolated by bioassay-guided fractionation from bark of E. lysistem on.

Schlemper et al., (2001) studied the antibacterial effects of extracts obtained from Persea cordata stem bark, employed in Brazil to treat infectious diseases, were studied. The ethyl acetate fraction of the hydroalcoholic extract showed activity against pathogenic bacteria which may justify the popular use of the plant. Audi et al., (2004) evaluated the antibacterial and hypotensive activities of an acetone: water and semipurified extracts
from the stem bark of *Stryphnodendron adstringens*. Both the crude and semipurified extracts showed activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It wasn’t possible to confirm the hypotensive activity. Quality control was determined, using the vegetable drug for two years, by means of harmacopoeial and chromatographic methods.

Yenesew *et al.*, (2005) investigated the chloroform extract of the stem bark of *Erythrina burttii* showed antifungal and antibacterial activities using the disk diffusion method. Flavonoids were identified as the active principles. Activities were observed against fungi and Gram(+) bacteria, but the Gram(-) bacteria *Escherichia coli* was resistant.

Rukachaisirikul *et al.*, (2007) isolated Seven pterocarpans, erybraedin B (1), erybraedin A (2), phaseollin (3), erythrabyssin II (4), erystaggallin A (5), erythrabissin-1 (6) and erycristagallin (7), two flavanones, 5-droxsophoranone (8) and glabrol (9), and one isoflavone, erysubin F (10), from the stems of *Erythrina subumbrans* (Leguminosae). Their structures were identified by means of spectroscopy. This is the first report of the isolation of the non-alkaloidal compounds from *Erythrina subumbrans* and the observed dehydration of 6a-hydroxypterocarpans 5 and 6 in CDC13 to the corresponding pterocarpenes 11 and 12, respectively. Compounds 8 and 9 were isolated for the first time from the genus *Erythrina*. Compounds 2 and 4 exhibited the highest degree of activity against *Streptococcus* strains with an MIC range of 0.78–1.56 μg/ml, whereas compound 7 exhibited the highest degree of activity against *Staphylococcus* strains, including drug-resistant strains (MRSA and VRSA), with an MIC range of 0.39–1.56 μg/ml. Interestingly, compounds 2, 4, 5 and 7 were more active against several strains of *Streptococcus* and *Staphylococcus* than the standard antibiotics vancomycin and oxacillin. Compound 7 showed the highest level of activity against all VRSA strains tested, with an MIC range of 0.39–1.56 μg/ml, which were resistant to both antibiotics. These compounds may prove to be potent phytochemical agents for antibacterial activity, especially against the MRSA and VRSA strains.
Togola et al. (2008) described ethnopharmacological knowledge on the uses of *Erythrina senegalensis* DC (Fabaceae) in traditional medicine in three different areas (Dioila, Kolokani and Koutiala) in Mali. Data were collected using interviews of traditional healers selected randomly. The main reported diseases for which *E. senegalensis* was used by the traditional healers were amenorrhea, malaria, jaundice, infections, abortion, wound, and body pain (chest pain, back pain, abdominal pain etc.). The fidelity level (which estimates the agreement of traditional healers on the same area about a reported use of the plant) was calculated to compare the results from the three areas. Certain differences were noticed, the most striking was the fact that amenorrhea was the most reported disease in Dioila and Kolokani with 21% of agreement for both areas, while this use was not reported in Koutiala at all. Similarities existed between the three areas on the use of the plant against malaria and infections, although with different degree of agreement among the healers. We also report the results of a literature survey on compounds isolated from the plant and their biological activities. A comparison of these results with the ethnopharmacological information from Mali and other countries showed that some of the traditional indications in Mali are scientifically supported by the literature. For instance, the use of *E. senegalensis* against infectious diseases (bilharzias, schistosomiasis, pneumonia etc.) is sustained by several antibacterial and antifungal compounds isolated from different parts of the plant. The comparison also showed that pharmacologists have not fully investigated all the possible bioactivities that healers ascribe to this plant.

Igbinosa et al. (2009) investigated the *in vitro* antimicrobial activity of crude ethanolic, methanolic and water extracts of the stem bark of *Jatropha curcas*. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 5 to 12, 8 to 20 and 0 to 8 mm for ethanol, methanol and water extracts respectively. All the extracts exhibited appreciable activity against all the fungal species investigated. Phytochemical screening revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts. The ability of the crude stem extracts of *J. curcas* to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.
Awal et al., (2010) studied in vitro antibacterial activity of ethanol extracts of leaf and root of *cassia fistula*. Five Gram-positive and 9 Gram-negative bacteria namely *Sarcina lutea, Bacillus megaterium, Bacillus subtilis, Streptococcus f3 - haemolyticus, staphylococcus aureus, Salmonella typhi, Shigella dysenteriae shigella boydii, shigella sonnei, shigella flexneri, shigella shiga, Escherichia coli, Pseudomonas aeruginosa and Klebsiella Pneumoniae* were tested. From the findings it is indicative that *Cassia fistula* may have antibacterial principles that could be useful in microbial diseases.

Chukwujekwu et al., (2010) investigated the antibacterial activity of the stem bark of *Erythrina caffra* Thunb. against different bacterial strains. The antibacterial activity was determined by a micro broth dilution assay. Four known flavonoids, abyssione-V 4'-O-methyl ether, 6, 8-diprenylgenistein, alpinumisoflavone and burttinone, were isolated. All the compounds were active against both Gram-negative and Gram-positive bacteria. This is the first report of antibacterial activity of burttinone and the isolation of these compounds from *E. caffra*.

Doughari (2010), experimented antimicrobial activity of organic (methanol and chloroform) and aqueous stem back extracts of *Erytrina senegalensis* against some pathogenic microorganisms was investigated using the filter paper disc diffusion method. Phytochemical studies revealed the presence of saponins, tannins, glycosides, phenols and alkaloids. The extracts demonstrated antimicrobial activity against both bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi, Pseudomonasa aeruginosa*) and fungi (*Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium notatum*). The methanol extracts demonstrated the highest activity while, the aqueous extracts demonstrated the lowest activity against all the test organisms. The results shows that *E. senegalensis* stem bark, if further purified can be used to source novel antibiotic substances for drug development against infections such as typhoid fever, urinary tract and wound infections, dysentery and mycotic infections.

Chandrashekar et al., (2010), studied the antimicrobial activity of the various extracts of the root bark of *Bauhinia purpuria* has been studied by agar cup plate diffusion method. Significant antibacterial and antifungal activity was shown by petroleum ether, chloroform and acetone extracts.
Innok et al. (2010) carried out chemical investigation of the stems of *Erythrina fusca* Lour. led to the isolation of three new pterocarpans, named fuscacarpans A–C (1–3), together with fourteen known compounds, sandwicensin (4), erythribyssin A (5), erythrabissin I (6), demethylmedicarpin (7), eryvarin D (8), erypoegin I (9), hydroxycristacarpone (10), orientanol A (11), scandenone (12), genistein (13), liquiritigenin (14), isoliquiritigenin (15), vestitone (16) and 3,7,4′-tri hydroxyflavone (17). Structures 1–3 were elucidated by spectroscopic and chemical methods. The isolates were evaluated for antibacterial, antiplasmodial and cytotoxic activities.

Sati et al. (2011) isolated 7-Methoxy kaempferol and 3-O- {{α-L rhamnopyranosyl- (1″→4″)}{α-L rhamnopyranosyl- (1″→6″)}}-β-D-glucopyranosyl quercetin were isolated from the ethanolic extract of stem bark of *Quercus leucotrichophora* along with β-sitosterol. The ethanolic extract exhibited a potent antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*.

Verma et al. (2011) screened the root bark extracts of the plant were screened for anti-bacterial activity of *Nyctanthes arbor-tristis* Linn. (Oleaceae). The test organisms were *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. The zone of inhibition were determined and compared with the standard drugs cefixatime.

**2.E. Cancer**

Cell growth and cell multiplication process is known as cell division. It must be extremely controlled that all the cells in the body should grow at the right place, and for all the organs and tissues to function properly. When the cells divide too quickly, consequences can be disastrous. When a cell divides, it first makes an exact copy of its DNA via a process called DNA replication, before splitting into half, to form two 'daughter' cells, that are genetically identical. Hundreds of proteins involve in Cell division. Some proteins inform the cell when or when not to divide. Others were responsible for making sure that the DNA is copied accurately. Yet more were involved physically by pulling the duplicated chromosomes apart as the cell to split into two.
Uncontrolled cell division may have many causes, to form any type of cell. But usually results from defects or damage from one or more of the genes involved in cell division. When those genes were damaged (mutated) on some way, for instance on exposure to cigarette smoke or ultraviolet radiation, the cell may start dividing uncontrollably. Those defective cells might multiply to form a lump of abnormal tissue called a tumour.

2.E.i. Cancer - Indian scenario

Every year about 8,50,000 new cancer cases being diagnosed, in India resulting about 5,80,000 cancer related deaths every year. India had the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India. In males, Oral, Lungs and Stomach cancers are the three most common causes of cancer incidence and death, whereas in females, Cervical, Breast and Oral cancers are the three main causes of cancer related illnesses and death. Overall, cervical cancer is the number one cause of cancer death in India. It is really unfortunate as cervical cancer can be easily prevented and also relatively easy to diagnose and treat at an early stage.

2.E.ii. Cancer - global scenario

Among all the cancers, Lung cancer is the most common worldwide and accounts for the major deaths annually. The Table-5 shows the global scenario for various types of cancer. The three leading cancer killers are different than the three most common forms, (i) Lung cancer responsible for 17.8 per cent of all cancer deaths, (ii) Stomach cancer 10.4 per cent, and (iii) Liver cancer 8.8 percent. Industrial nations with the highest overall cancer rates include USA, Italy, Australia, Germany, The Netherlands, Canada and France. Developing countries with the lowest cancer are in Northern Africa. Cancer rates could further increase by 50% to 15 million new cases in the year 2020. The report also provides clear evidence that healthy lifestyles, and public health action by governments and health practitioners, could stem this trend, thus prevent as many as one third of cancers worldwide.
2.E.iii. Role of Free radicals in cancer

A free radical is nothing more than a molecular structure which contains an unpaired electron. Electrons tend to stay in pairs. Electron pairs make up the chemical bonds which keep molecules from flying apart.

An unpaired electron, driven by a potent chemical force which compels it to find a mate. Thus molecular instinct that merges with another electron is so powerful that the searching molecule behaves erratically, moving about much like a weapon within cellular structures. Its random and wild molecular movements within cellular material can create cellular damage, which can eventually result in degeneration or mutation.

Table 5 Global Scenario on Types of Cancer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Type of Cancer</th>
<th>No. of Patients Affected /Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lung</td>
<td>1.2 MILLION</td>
</tr>
<tr>
<td>2</td>
<td>Breast Over</td>
<td>1 MILLION</td>
</tr>
<tr>
<td>3</td>
<td>Colorectal</td>
<td>9,40,000</td>
</tr>
<tr>
<td>4</td>
<td>Stomach</td>
<td>8,70,000</td>
</tr>
<tr>
<td>5</td>
<td>Liver</td>
<td>5,60,000</td>
</tr>
<tr>
<td>6</td>
<td>Cervical</td>
<td>4,70,000</td>
</tr>
<tr>
<td>7</td>
<td>Esophageal</td>
<td>4,10,000</td>
</tr>
<tr>
<td>8</td>
<td>Head and Neck</td>
<td>3,90,000</td>
</tr>
<tr>
<td>9</td>
<td>Urinary Bladder</td>
<td>3,30,000</td>
</tr>
<tr>
<td>10</td>
<td>Malignant Non-Hodgkin lymphomas</td>
<td>2,90,000</td>
</tr>
<tr>
<td>11</td>
<td>Leukemia</td>
<td>2,50,000</td>
</tr>
<tr>
<td>12</td>
<td>Prostate and Testicular</td>
<td>2,50,000</td>
</tr>
<tr>
<td>13</td>
<td>Pancreatic</td>
<td>2,16,000</td>
</tr>
<tr>
<td>14</td>
<td>Ovarian</td>
<td>1,90,000</td>
</tr>
<tr>
<td>15</td>
<td>Kidney</td>
<td>1,90,000</td>
</tr>
<tr>
<td>16</td>
<td>Endomaterial</td>
<td>1,88,000</td>
</tr>
</tbody>
</table>

A free radical can destroy a protein, an enzyme or even a complete cell. To make matters worse, free radicals can multiply through a chain reaction mechanism resulting in the release of thousands of the cellular oxidants. When it happens, cells can become so
badly damaged that DNA codes can be altered and immunity can be compromised. Contact with a free radical or oxidant on the scale can create cellular deterioration, resulting in diseases like cancer. Tissue breakdown from the oxidative stress can also occur, which contributes to aging, arthritis and a whole host of other degenerative conditions. Constant bombardment with free radicals had been likened to being irradiated at low levels all the time. Unfortunately, because of the damage free radicals cause within our cellular structures, the sad fact is that many of us will die prematurely from one of a wide variety of degenerative diseases. Free radical damage has been associated with over 60 known diseases and disorders. An important fact to remember is that the act of breathing oxygen activates those reactive chemical structures known as free radicals. To make matters worse, as because in our generation more than any other had been exposed to a number of potentially harmful environmental substances, free radical formation can reach what has been referred to as epidemic proportions. Some of the more dangerous free radical producing substances include: cigarette smoke, herbicides, high fats, pesticides, smog car exhaust, certain prescription drugs, diagnostic and therapeutic x-rays, ultra-violet light, gamma radiation, rancid foods, certain fats, alcohol some of our food and water supplies, stress, poor diets etc. Even exercising, as beneficial as it is, can initiate the release of free radicals within our cellular systems. Aerobic exercising produces damaging oxidation by-products. Many of these are not completely neutralized by internal safety mechanisms and an overload can occur. Supplementing the diet with effective antioxidant compounds. Numerous research studies support the fact that many cancers, in particular breast cancer-diet related. Moreover, the risks of certain kinds of cancer could be significantly reduced with dietary changes. While most of us are aware of the wonders of a low-fat diet, a tremendous amount of data conceding other cancer preventive nutrients never reaches the average consumer. For instance, recent studies suggested that just reducing dietary fat may not be enough to prevent certain cancers. Perhaps more and more research suggested that, lack of certain protective nutrients appeared to originate from dietary sources that increase risk of cancer and other degenerative diseases. The role of certain bioflavonoid compounds is the exceptional free radical scavengers that just begin to emerge, and the protective potential of those flavonoids is impressive, to say the least.
2.E.iv. Causes of cancer

1) Viruses such as *Epstein-Barr-Virus* (EBV), *Hepatitis-B-Virus* (HBV), *Human Papilloma Virus* (HPV).

2) Environmental and occupational exposure such as ionizing, UV radiation, exposure to chemicals including vinyl chloride, benzene and asbestos.

3) Lifestyle factors such as high-fat, low fiber diets, tobacco, ethanol etc.

4) Medication such as alkylating agents and immunosuppressant’s.

5) Genetic factors such as inherited mutations, cancer causing genes, defective tumor suppressor genes.

2.E.v. Plant Phytochemicals and Cancer Therapy

Plant materials have been used for the treatment of malignant diseases for centuries. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer often resulted in the isolation of principles with antitumour activity. An intensive survey of plants, microorganisms and marine animals for antitumour activity began in the later 1950s mainly because the United States National Cancer Institute (NCI) initiated and funded a major screening programme. Random selection screening programme was adopted, since novel compounds may be found anywhere from plant or animal kingdom.

There are ample reports to suggest that the phytochemicals do possess the ability to inhibit tumours or reduce the cancer risk.

Soybean phytochemicals such as genistein (4', 5, 7-trihydroxy isoflavone) inhibit the growth of transplantable human prostate carcinoma. (Ravindranath, *et al.*, 2004) Epidemiological studies have consistently shown that regular consumption of fruits and vegetables are strongly associated with reduced risk of developing chronic diseases such as cancer as the phytochemical extracts from it exhibit strong antioxidant activity. (Liu, *et al.*, 2004) *Andrographolide* is a potential cancer therapeutic agent isolated from *Andrographis paniculata*. (Kumar, *et al.*, 2004).
In the screening of Yemeni plants used in folk medicine for the anticancer potential, the methanolic extracts of *Dendrosicyos Socotrana*, *Withania aduensis*, *Withania riebeckii*, *Dracena Cinnabari* and *Buxus hildebrandlii* exhibited the highest toxicity on all tumor cell lines. (Mothana, *et al*., 2007) The four varieties of muscadine grape extract had the ability to inhibit the activity of matrix metalloproteinases implying that those could be good inhibitors of carcinogenesis (God, *et al*., 2007). The limonoids isolated from the methanol extract of *Khaya Senegalensis* proved good anticancer activity (Zhang, *et al*., 2007). The leaf extract of Ashwagandha selectively killed tumor cells and thus it was a natural source for safe anticancer medicine. (Widodo, *et al*., 2007) The fruit of deerberry (*Vaccinium stamineum*) exhibited the anticancer capability of human lung and leukemia cancer cells (Wang, *et al*., 2007). Polyphenolic extracts from *Vaccinium macrocarpon* inhibited the growth and proliferation of breast, colon, prostate, lung, and other tumors as do flavonols, proanthocyanidin, oligomers, and triterpenoids isolated from the fruits of the same (Neto, *et al*., 2007).

*Morinda citrifolia* is effective on both clinical practice and laboratory animal models (Wang, *et al*., 2001). An alcoholic extract of *Biorhythms sensitivum* for antitumor activity could inhibit the solid tumor development on mice induced with Dalton’s lymphoma ascites (DLA) cells and increase the life span of mice bearing Ehrlich ascites carcinoma (EAC) tumors. (Guruvayoorappan, *et al*., 2007) Edible fruits and berries served the source for novel anticancer agents, given that extracts of those foods have demonstrated cytotoxic activity against tumor cell lines (Ferguson, *et al*., 2006). Nimboide, a triterpenoid extract from the flowers of the neem tree was found to have antiproliferative activity against some cancer cell lines (Roy, *et al*., 2007). *Semecarpus anacardium* Linn nut milk extract exerts its anticancer effect through quenching - reactive oxygen species (Arulkumaran, *et al*., 2006). The cytotoxic activities of two medicinal herbs *Linum persicum* and *Euphorbia cheradania* that are native to Iran showed cytotoxic activity on tumor cell lines (Amirghofran, *et al*., 2005). The Pomegranate extracts inhibits the growth of breast cancer cells (Jeune., *et al*., 2005). Brassinosteroids, steroid plant hormones are promising leads for potential anticancer drugs (Malikovia, *et al*., 2007). The *Careya arborea* bark significantly reduced the solid tumor volume induced by DLA cells (Natesan, *et al*., 2007). The methanol extract of *Bauhinia racemosa* stem bark exhibited antitumor
effect in EAC bearing mice (Gupta, et al., 2004). The antitumor activity of the ethanol extract of *Indigofera aspalathoides* was established (Rajkapoor, et al., 2004). The extract of 12 Chinese medicinal herbs such as *Anemarrhena asphodeloides* (Root), *Artemisia argyi* (leaf), *Commiphora Myrrha* (Resin), *Duchesnea indica* (Aerial Plants), *Gleditsia sinensis* (Fruit), *Ligustrum lucidum* (fruit), *Rheumpalmatum* (Root and Rhizome), *Rubia cordifolia* (Root), *Salvia Chinesis* (Aerial parts), *Scutellaria barbata* (Aerial Parts), *Uncaria rhyphopylla* (Stem), *Vaccaria segetalis* (seed) showed anticancer effects in vitro and those effects were markedly greater on cancer cells compared with normal cells (Mark Shoe Maker, et al., 2005).

Phytoconstituents extracted from a large number of plants belonging to the genus *Hypericum* are known to possess potent anticancer nature (Dongre, et al., 2005). Cytotoxic activity of *Sarris cernuss* extract on human colon and breast carcinoma cultures is proved (Badisa, et al., 2007). The natural antioxidant gallic acid (GA) isolated from the fruits of an Indonasiian medicinal plant, *Phaleria macrocarpa* is proved to be a potent anticancer compound (Faried, et al., 2007). The rhizome *Zingiber officinalis*, one of the most widely used species of the ginger family is a common condiment for various foods and beverages. The pungent vallinoids i.e., 6-gingerol and 6-paradol, shogaols and zingerone attributed to the anticancer properties of ginger.

The antineoplastic activity of methanolic extracts of five medicinal plants that is native to Iran including *Galium mite*, *Ferula Angulata*, *Stachys obtuscrena*, *Grsium bracteosum*, and *Echinophora Cinerea* was investigated and proved to have anti tumor activity (Amirghofran, et al., 2006). *Panax ginseng* and its extracts have long been used for medical purposes and the increasing interest in developing ginseng products as cancer preventive agents (Wang, et al., 2007). Purified bioactive compounds derived from medicinal mushrooms were potentially important for new source of anticancer agents (Sullivan, et al., 2006).

The Saponins from the plant of China, *clematis manshrica* has obvious antitumor effects against various transplanted tumor on mice (Zhao, et al., 2005). The Embelin derivatives such as 1, 4 – benzoquinone derivative 5-0 ethyl embelin(1) and 5-0 methyl
embelin are promising antimitotic and anti cancer molecules (Xu, et al. 2005) Sesquiterpenes the class of naturally occurring molecules that are 15-carbon isoprenoid compounds have therapeutic potential in decreasing the progression of cancer (Modzelewska, et al., 2005)

The anticancer activity from Platycodon grandiflorum was proved and established (Lee, et al. 2004) The methanol extract of stem bark of Dillenia pentagons appears to be more active against Dalton’s lymphoma (Rosangkenia, et al., 2004) Limonium Vulgare, Artemisia maritima and Salicornia europaea showed antineoplastic activities The extracts of Ononis spinosa, Trifolium fragiferum and Trifolium repen showed tumor growth inhibiting activities (Lellau, et al., 2003) Methanol extract Ledum groelandicum Retzus (Labrador tea) leaf twig extract showed anticancer activity (Dufour, et al., 2007) The antineoplastic activity of guduchi (Tinospora cordifolii) on Ehrlich ascites carcinoma was proved (Jagetia, et al., 2006)

2.E.vi. Mechanism of Some Potential Active Ingredients

Phytochemicals isolated from folk medicines are found to act as potent antioxidants and free radical scavengers These natural products are supposed to minimize DNA damage by reacting with free radicals and in this way they could prevent cancer Some of the phytochemical antioxidants of folk medicines are inhibitors of lipoxygenase and urokinase Inhibition of these enzymes by folk medicines could prevent or reduce cancer growth and in this way their mechanism of action can be established Folk medicine phytochemicals such as flavonols and flavones were investigated to determine chemoprevention activity against cancer Urokinase plasminogen activator (uPA) or other proteolytic enzymes, such as metalloproteases, are commonly recognized as important factors in metastasis The urokinase activates plasminogen (nonactive form) turning it into its active form called plasmin Plasmin is a strong proteolytic enzyme and hydrolyzes proteins of connective tissue and basement membranes It activates other latent proteolytic enzymes, broadening the spectrum of proteins attacked Procollagenase is activated to collagenase in this way Plasmin is a key enzyme in the mechanism responsible for tissue remodeling, tumor invasion, angiogenesis and development of distant metastasis An
increased amount or activity of uPA, or urokinase plasminogen activator receptor (uPAR) per cell, has been found in human cancer cell lines with metastatic behavior (Conese and Blasi, 1995). Natural products of folk-medicine, for example, genistein and curcumin, are found to decrease Upa (Santibanez, 2000), thus helping to fight against cancer. Natural products of folk-medicine also affect lipoxygenase activity which is also a cause for cancer. Lipoxygenase enzymes are found in a wide variety of plant and animal tissues. These enzymes have a non-heme iron serving as a catalytic center for the stereo and regiospecific dioxygenation of select carbon atoms in polyunsaturated fatty acids for their metabolism. Eighteen carbon chain fatty acids (e.g. linoleate) are the primary substrates of the plant lipoxygenases while the mammalian isoymes mainly catalyze the metabolism of fatty acids of twenty chain carbon length (e.g. arachidonate). The mammalian fatty acid, arachidonic acid is metabolized by one of two enzyme pathways, cyclooxygenase (COX) or lipoxygenase (LOX) generating biologically active metabolites that are involved in carcinogenesis. It has been shown that the LOXs in particular are key regulators of cell survival and apoptosis in cells. It has been shown also that LOX is a regulator of human cancer development and it is over expressed in a variety of tumors including breast, colorectal, and prostate cancer, and cancer cell lines (Pidge et al., 2002). It has been reported that inhibition of oxidative enzymes such as 5-lipoxygenase and 12-lipoxygenase trigger tumor cell apoptosis, reduce tumor cell motility and invasiveness, or decrease tumor angiogenesis and growth (Nie et al., 2001). Phenols and polyphenols, the flavonoids and their derivatives, are ubiquitous in plants and more than 8,000 different compounds are included in this group and many of them are antioxidants. They have been associated with the inhibition of atherosclerosis and cancer (Martinez-Valverde et al., 2000) and other biological activities.

From the present review, it can be concluded that cancer is the leading cause of death in developing countries like India. As there is an enormous increase in the population day by day, the alternative therapy in the market is getting its glimpse. The cheap herbal drug treatment may highly be recommended to the rural and poor people to treat effectively the cancers of various types is an ideal choice. Based on that the siddha medicines are coming up in combination with metals and other essential supplements to improve the immune status of the cancer patients in India.
Table-6. Indian Medicinal Plants having Anticancer Activity

<table>
<thead>
<tr>
<th>SL.No.</th>
<th>Name of the Plant</th>
<th>Family</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calotrophis gigantea</td>
<td>Asclepiadaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>2</td>
<td>Cajanus cajan</td>
<td>Fabaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td>Butea monosperma</td>
<td>Fabaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>4</td>
<td>Bauhinia variegata</td>
<td>Caesalpinaceae</td>
<td>Root</td>
</tr>
<tr>
<td>5</td>
<td>Bacopa monnieri</td>
<td>Scrophulariaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>6</td>
<td>Azadirachta indica</td>
<td>Meliaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>7</td>
<td>Asparagus racemosus</td>
<td>Liliaceae</td>
<td>Root</td>
</tr>
<tr>
<td>8</td>
<td>Aphanantrix polystachya</td>
<td>Meliaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>9</td>
<td>Aloe barbadenss</td>
<td>Liliaceae</td>
<td>Leaf juice</td>
</tr>
<tr>
<td>10</td>
<td>Alium cepa</td>
<td>Liliaceae</td>
<td>Bulb</td>
</tr>
<tr>
<td>11</td>
<td>Acorus calamus</td>
<td>Araceae</td>
<td>Rhizome</td>
</tr>
<tr>
<td>12</td>
<td>Cassia absus</td>
<td>Caesalpinaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>13</td>
<td>Cassia aurulata</td>
<td>Caesalpinaceae</td>
<td>Root</td>
</tr>
<tr>
<td>14</td>
<td>Cassia senna</td>
<td>Caesalpinaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>15</td>
<td>Catunaregum spinosa</td>
<td>Rutaceae</td>
<td>Bark/Fruit</td>
</tr>
<tr>
<td>16</td>
<td>Citrullus colocynthis</td>
<td>Cucurbitaceae</td>
<td>Root</td>
</tr>
<tr>
<td>17</td>
<td>Citrus medica</td>
<td>Rutaceae</td>
<td>Root</td>
</tr>
<tr>
<td>18</td>
<td>Cissus quadrangularis</td>
<td>Vitaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>19</td>
<td>Clerodendrum serratum</td>
<td>Verbanaceae</td>
<td>Root</td>
</tr>
<tr>
<td>20</td>
<td>Clerodendrum viscosum</td>
<td>Verbanaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>21</td>
<td>Crinum asiaticum</td>
<td>Amaryllidaceae</td>
<td>Bulb</td>
</tr>
<tr>
<td>22</td>
<td>Daucus carota</td>
<td>Apiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>23</td>
<td>Flacourtia jangomos</td>
<td>Flacourtiaceae</td>
<td>Bark/Leaf</td>
</tr>
<tr>
<td>24</td>
<td>Jatropha curcas</td>
<td>Euphorbiaceae</td>
<td>Leaves, seed, oils</td>
</tr>
<tr>
<td>25</td>
<td>Lens culinaris medikus</td>
<td>Fabaceae</td>
<td>Seed</td>
</tr>
<tr>
<td>26</td>
<td>Limonia acidissima</td>
<td>Rutaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>27</td>
<td>Macrotyloma uniflorum</td>
<td>Fabaceae</td>
<td>Seed</td>
</tr>
</tbody>
</table>

The above survey reveals the role of Indian medicinal plants and the various phytochemicals may be used to treat the cancer effectively. The available literature finds to be very impressive which may give an indication for the therapeutic usefulness. There are hundreds of plants unexplored needs much detailed survey to check the anti-cancer efficacy. There are few plants with known anti-cancer properties have been shown in
Table-6. The isolation, identification of active principles and pharmacological studies of the active phytoconstituents may be considered and studied elaborately to treat effectively for various types of cancers.

Nkengfack et al., (2001) carried out Bioassay-directed fractionation of the CH2Cl2-MeOH (1:1) extract of the stem bark of *Erythrina indica*, that has resulted in the isolation of two new isoflavone derivatives named indicanines D and E together with 11 known compounds including: six isoflavones (genistein, wighteone, alpinumisoflavone, dimethylalpinumisoflavone, 8-prenyl erythrinin C, and erysenegalensein E), one cinnamate (erythrinassinate B), two pentacyclic triterpenes (oleanolic acid and erythrodiol), and two phytosterols (stigmasterol and its 3-O-β-D-glucopyranoside). The structures of the new compounds were elucidated by means of spectroscopic analysis. The *in vitro* cytotoxic activity against KB cells of some of the isolated compounds are also reported.

Suresh et al., (2010) evaluated the effect of methanolic extract of stem bark of *Nyctanthes arbortristis linn* (EENA) against Dalton’s Ascitic Lymphoma (DAL) in Swiss Albino mice. DAL cells were injected intraperitonially (10^6 cells) to the mice. 5-fluorouracil (20 mg/kg) was used as reference drug. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with EENA. The haematological parameters were also normalized by EENA in tumour-induced mice. These observations are suggestive of the protective effect of EENA against Dalton’s Ascitic Lymphoma (DAL).

Enos et al., (2009) evaluated the anti cancer properties of the diethylether extract of Sunkun (*Arotocarpus altitis* Wood) the extract was tested in human T47D breast cancer cells and examined for its effect on cell viability, nuclear morphology and subGi formation and showed decreasing cell viability in a concentration dependent manner. The data demonstrated that Sunkun wood extract induced apoptosis and G1 phase formation in breast cancer (T47D) cells, and therefore has potential as an anticancer agent.

Osman et al., (2011) evaluated the *in vivo* antitumour activity of the ethyl acetate extract of stem bark of *M. zapota L.* (EASM) at 50, 100 and 200 mg/kg b.w against EAC
using mean survival time. Significant efficacy was observed for EASM at 100 mg/kg dose (P<0.05). It can be concluded that the ethyl acetate extract of stem bark of *M. zapota L.* possesses significant antitumour activity.

Kumar *et al.* (2011) have evaluated its traditionally claimed cytotoxic effect of 50% methanolic extract of stem bark of *Bauhinia racemosa* (MERB) on four human cancer cell lines scientifically. The cytotoxicity of MERB was assessed by Sulforhodamine B (SRB) assay method. It is suggested that 50% methanolic extract of stem bark of *Bauhinia racemosa* demonstrate cytotoxicity on the four cancer cell lines tested but the antiproliferative effect appears to vary depending upon tumor cell type and MERB concentration; this should be further investigated for the possible mechanism behind the cytotoxic effect of MERB.

Kumar and Vikram (2011) designed to evaluate the *in vitro* cytotoxicity activity of methanolic extract of *Glochidion zeylanicum* (Gaertn.) root. In this study the extract was tested using human cancer cell lines HepG2, HT-29 and PC-3 for its effects on cell viability, growth inhibition and cell morphology. The data demonstrated that methanolic extract of roots of *Glochidion zeylanicum* (Gaertn.) has a potential cytotoxicity activity on HepG2, HT29 and PC3 cell lines but the effect was more significant on PC3 cell lines.

Zanq (2009) has studied the anti-tumor activities and mechanism of *Erythrina variegata* L. extract. The MTT method was used to evaluate the inhibitory activity of the *Erythrina variegata* L. extract on proliferation of cancer cell lines. Moreover, in order to determine its anti-tumor effect *in vivo*, the Lewis lung cancer mice model was established. By comparing the relative tumor proliferation rates, growth curves, inhibition rates of different groups, the anti-tumor effect was evaluated. Furthermore, the anti-tumor mechanism of *Erythrina variegata* L. extract was studied by using G-quadruplex stability experiment. In the *in vitro* anti-liver cancer experiment, the *Erythrina variegata* L. extract has shown obvious anti-tumor effect on various tumor cells. And in the *in vivo* experiment, it exhibited significant anti-tumor effect. Besides, from the result of G-quadruplex stability experiment, we can see that the quadruplex structure showed increasing T(m) values with increasing amounts of *Erythrina variegata* L. extract.
Sismindar (2003), demonstrated the methanol extract isolated from *E. fusca* leaves had a selective cytotoxicity effect, as indicated by the level of the IC$_{50}$ which was higher to myeloma compared to HeLa S-3 cell-line, and had much less cytotoxic on normal mononuclear cells.

Baskar (2010), evaluated the effect of methanolic extract of root bark of *Erythrina variegata* (MEEV) against Dalton’s Ascitic Lymphoma (DAL) in swiss Albino mice. DAL cells were injected intraperitoneally (10$^6$ cells) to the mice. Two days after cells injection the animals were treated with 250 and 500 mg/kg of MEEV for 8 days. 5-fluorouracil (20 mg/kg) used as a reference drug. On day 11, cancer cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span and haematological parameters were evaluated and compared with the same parameters in control. A significant increase in the life span and decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with MEEV. The haematological parameters were also normalized by MEEV in tumour-induced mice. These observations are suggestive of the protective effect of MEEV against Dalton’s Ascitic Lymphoma (DAL).

In the present investigation stem bark extracts have been used to check the anti-tumor effects of *Erythrina mysorensis*. As large population use root and bark in ayurvedic medicine worldwide, there is an urgent need for additional, carefully conducted, high-quality intensive research to evaluate its efficacy and to develop this discipline to meet ever-new challenges of modern medicine in the field of oncology. Standardization and quality production of herbal products may allow us to develop low cost therapies with reduced risk over pharmaceuticals. In any case, studies on anticancer ayurvedic drugs will be popular from the economy point of view because cancer is becoming the major cause of death (Premalatha and Rajagopal, 2005).

2.F. Epilepsy:

2.F.i. Definition

A seizure (from the Latin sacire, “to take possession of”) is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of CNS neurons. Depending on the distribution of discharges, this abnormal CNS activity can have
various manifestations, ranging from dramatic convulsive activity to experimental phenomena not readily discernible by an observer (Daniel, 2005). Seizure often is described as convulsive or non-convulsive, depending on the prominence of motor features. This distinction omits the diversity of non-convulsive seizures and more important distinction between seizures of focal and non-focal cortical origin. Furthermore, seizures with an immediate and proximate cause, such as an acute metabolic disturbance, infection, or head trauma, can be considered symptomatic or provoked. In other instances, seizures may be the result of past brain injury. Such episodes can be described as remote symptomatic. In large number of seizures, a cause cannot be identified. These seizures can be called idiopathic or cryptogenic. Idiopathic seizures are presumed to have a genetic basis. They occur in partial or generalized epileptic syndromes that have particular clinical and EEG characteristics. The term cryptogenic, however, implies a symptomatic cause that cannot be diagnosed with currently available medical technology (Carl et al., 2005).

Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process. This definition implies that a person with a single seizure, or recurrent seizures due to correctable or avoidable circumstances, does not necessarily have epilepsy. Epilepsy refers to a clinical phenomenon rather than a single disease entity, since there are many forms and causes of epilepsy. However, among the many causes of epilepsy there are various epilepsy syndromes in which clinical and pathologic characteristics are distinctive and suggest a specific underlying etiology (Daniel, 2005).

2.F.ii. Epidemiology

Although a variety of factors influence the incidence and prevalence of seizures, 5 to 10% of the population will have at least one seizure, with the highest incidence occurring in early childhood and late adulthood (Daniel, 2005). About 4% of persons living to an age of 74 have at least one unprovoked seizure. When provoked seizures (i.e., febrile seizures or those related to an acute illness) are included, the likelihood of experiencing a seizure by the age of 74 increases to at least 9%. The risk of developing epilepsy is about 3% by the age of 74 (Sander, 2003). In most developed countries, incidence rates range from 40-70 per 100,000, but in developing countries, the rates may
be as high as 100-190 per 100,000. Similarly, the prevalence of active epilepsy, defined as persons who take anticonvulsant drugs or who have had a seizure in the past 5 years, ranges from 4-10 per 10,000 in developed countries and up to 57 per 10,000 in developing countries. Studies have estimated that 1.5-5.0% of any population will have a seizure at some time. Partial seizures, with or without secondary generalization, are the most common seizure type, followed by generalized tonic-clonic seizures. Other seizure types, such as absence, pure tonic, atonic, or myoclonic, are relatively uncommon (Bittemcourt et al., 1996).

2. F.iii. Classification of Epileptic Seizure

I. Partial (focal) seizures
   A. Simple partial seizures (consciousness not impaired)
   B. Complex partial seizures (consciousness is impaired)
   C. Partial seizures evolving to secondarily generalized seizures

II. Generalized seizures of nonfocal origin (convulsive or nonconvulsive)
   A. Absence seizures
   B. Myoclonic seizures
   C. Tonic-Clonic seizures (may include clonic-tonic-Clonic seizures)
   D. Tonic seizures
   E. Atonic seizures

III. Unclassified epileptic seizures (Neonatal seizures, rhythmic eye movement, chewing, swimming movements).

Despite the shortcomings of the current classifications, they remain widely accepted guides for clinical management and research. (Carl et al., 2003). Determining the type of seizure that has occurred is essential for focusing the diagnostic approach on particular etiologies, selecting the appropriate therapy and providing potentially vital information regarding prognosis. Partial seizures are those in which the seizure activity is restricted to discrete areas of the cerebral cortex. Generalized seizures involve diffuse regions of the brain simultaneously. Partial seizures are usually associated with structural
abnormalities of the brain. In contrast, generalized seizures may result from cellular, biochemical, or structural abnormalities that have a more widespread distribution (Daniel, 2005).

2.F.iv. Role of Neurotransmitter in Epilepsy

Various animal models of epilepsy helped for the study and understanding of the human epilepsies and showed evidence that the neurotransmitters glutamate and GABA (Gamma-aminobutyric acid) are centrally involved in the development of epilepsy. Bradfor (1995) GABA is the principal inhibitory transmitter in the mammalian brain. GABA maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is perturbed, seizures may ensue. GABA is formed within GABAergic axon terminals and released into the synapse, where it acts upon its receptors: GABAA, to increase membrane chloride conductance and thereby stabilize or hyperpolarize the resting membrane potential and GABAB, which increases potassium conductance, decreases calcium entry, and inhibits the presynaptic release of other transmitters. GABAA-receptor binding influences the early portion of the GABA-mediated inhibitory postsynaptic potential, whereas GABAB binding influences the late portion. GABA is rapidly removed by uptake into both glial and presynaptic nerve terminals and then catabolized by GABA transaminase. It has been found that:

Abnormalities of GABAergic function have been observed in genetic and acquired animal models of epilepsy; Reductions of GABA-mediated inhibition, activity of glutamate decarboxylase, binding to GABAA and benzodiazepine sites, GABA in cerebrospinal fluid and brain tissue, and GABA detected during microdialysis studies have been reported in studies of human epileptic brain tissue; GABA agonists suppress seizures, and GABA antagonists produce seizures; Drugs that inhibit GABA synthesis cause seizures and Benzodiazepines and barbiturates work by enhancing GABA-mediated inhibition. Finally, drugs that increase synaptic GABA are potent anticonvulsants. (Treiman, 2001)

Excitatory glutamatergic neurotransmission is responsible for the initiation and spread of seizure activity, even if it is not necessarily the primary underlying pathogenic
mechanism. Similarly, GABA-mediated synaptic inhibition is known to be critical in regulating epileptic activity, as even a minor disinhibition can trigger hyperexcitability. Thus a dysfunction in either GABA or glutamate availability will have important consequences regarding seizure genesis. The duration of excitation during glutamatergic neurotransmission relies on specific transporters, which terminate the action of glutamate and control its extracellular level by clearing the synaptic cleft, thus preventing excitotoxicity and hyperexcitability (Bacci et al. 2002).

2. F.V. Targets for antiepileptic drug action

1. Ion channels

Na+ channels: The neuronal Na+ channel represents one of the most important targets for AED action. In the nervous system, voltage-dependent Na+ channels are responsible for the upstroke of the neuronal action potential, and ultimately control the intrinsic excitability of the nervous system. At normal membrane potentials, most Na+ channels exist in a closed, resting state. Upon depolarization, the channel activates, facilitating ion flux. Thereafter, the Na+ channel enters an inactivated state, from which it is not readily re-activated. Repolarisation of the neuronal membrane rapidly converts the channel back to a resting state, from which it can respond to subsequent depolarization.

Ca2+ channels: Voltage-sensitive Ca2+ channels can be broadly classified into low or high threshold. The low-threshold T-type Ca2+ channel is expressed predominantly in thalamocortical relay neurones, where it is believed to be instrumental in the generation of the rhythmic 3-Hz spike-and-wave discharge that is characteristic of generalized absence seizures. High threshold Ca2+ channels are sub classified by their pharmacological properties into L-, N-, P-, Q-, and R-types. The N-, P-, and Q-type channels, in particular, have been implicated in the control of neurotransmitter release at the synapse. Several AEDs have been reported to block voltage-sensitive Ca2+ channels in a subtype-specific manner, an effect that may contribute to their antiepileptic actions.

K+ channel: Neuronal K+ channels are responsible for the action potential down stroke or, more specifically, repolarisation of the plasma membrane in the aftermath of Na+
channel activation. Direct activation of voltage dependent K+ channels hyperpolarizes the neuronal membrane and limits action potential firing. Accordingly, K+ channel activators have anticonvulsant effects in some experimental seizure models, whereas K+ channel blockers precipitate seizures.

2. **GABA-mediated inhibition** GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, where it is released at up to 40% of all synapses. Impairment of GABA function is widely recognized to provoke seizures, whereas facilitation has an anticonvulsant effect.

3. **Glutamate-mediated excitation**

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Focal injection of glutamate induces seizures in animals, and over-activation of glutamatergic transmission or abnormal glutamate receptor properties are observed in certain experimental seizure models and human epilepsy syndromes. Inhibition of the neuronal release of glutamate and blockage of its receptors has received considerable attention in the search for novel AEDs. Glutamate is synthesised from glutamine by the action of the enzyme glutaminase in glutamatergic neurones. Following synaptic release, glutamate exerts its pharmacological effects on several receptors. Although none of the commonly used AEDs exert their pharmacological effects solely by an action on the glutamate system, blockade of glutamate receptors is believed to contribute to the antiepileptic activity of several compounds. (Kwan et al., 2001).

Ambawade et al., (2002) have assessed the anticonvulsant activity of ethanolic extract of roots and rhizomes of *Glycyrrhiza glabra* (10, 30, 100 and 500 mg/kg, i.p.) in mice using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using albino mice. The lithium-pilocarpine model of status epilepticus was also used to assess the anticonvulsant activity in rats. The ethanolic extract of *G. glabra* inhibits PTZ and lithium-pilocarpine-induced convulsions but not MES-induced convulsions.
Dantas et al. (2004) studied the aqueous extract (AE) of *Erythrina velutina* prolonged the sleep duration induced by sodium pentobarbital. The crude extract of *Erythrina velutina* at lower doses interferes with mnemonic process for different tasks, while at higher doses, the sedative and neuromuscular blocking actions are the main effects.

Tandon and Gupta (2005) have seen Maximal electroshock seizures (MES) in albino rats and pentylenetetrazole (PTZ) induced seizures in albino mice to study anticonvulsant activity of *Vitex-negundo* leaf extract. The findings suggest that *Vitex-negundo* possesses anticonvulsant activity particularly against PTZ induced convulsions.

Buznego and Pe`rez-Saad (2006), investigated the potential behavioral and antiepileptic effect of decoctions (1, 5, and 30%, intraperitoneally) of the dried roots of *Echinodorus berteroi*. The results shows that, the root decoctions of *E. berteroi* paradoxically exhibited neuroleptic and antiepileptic actions.

Vasconcelos et al., (2007) have examined the anticonvulsant effects of hydroalcoholic extracts (HAEs) from the stem bark of *Erythrina velutina* and *Erythrina mulungu* on pentylenetetrazole (PTZ) and strychnine-induced seizure tests and the potentiation of pentobarbital-induced sleeping time in mice with the extracts. The extracts of these plants (intraperitoneally) when administered in mice at single doses did not exhibit any protector effect in PTZ-induced seizures, at any dose, an increase in the latency of convulsion and in the death time was observed with both doses and routes of *Erythrina velutina* and at higher dose of *Erythrina mulungu*, in strychnine-induced seizure. No alteration was observed with *Erythrina velutina* and *Erythrina mulungu* on sleeping latency at both doses as compared to control (362.8+/−59.5). However, the sleeping time was increased in both plants as compared to control (943.8+/−129.6). The results suggests that the hydroalcoholic extracts of *Erythrina velutina* and *Erythrina mulungu* have anticonvulsant effects only in the strychnine-induced seizure model, suggesting their possible action in glycine system and a potentiation of pentobarbital sleeping time, suggesting depressant action in the central nervous system.
Similarly, Pe’rez-Saad, Buznego (2008) investigated the potential neuropharmacological action profile of decoctions obtained from dry leaves of the plant *Cestrum nocturnum* is a garden shrub from the family Solanaceae. Decoctions were tested in different neuropharmacological models—Irwin test, exploratory behavior, tests for analgesia, isoniazid- and picrotoxin-induced convulsions, and maximal electroshock seizures—in mice, as well as in amphetamine-induced stereotypies and penicillin epileptic foci in rats. The decoctions were not effective against pharmacologically induced convulsions. However, repeated administration of five doses of D5, at 1-hour intervals, reduced the amplitude of penicillin-induced epileptic spikes in both primary and secondary foci, in curarized rats.

Nanda *et al.,* (2010) investigated anticonvulsant effect of the petroleum ether, benzene, chloroform, ethanol and triple distilled water extract of whole parts of the plant of the *Sphaeranthus indicus* Linn. (Compositae) on electrically and chemically induced seizures. The data suggest that the ethanolic root extract of *S.indicus* may produce its anticonvulsant effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazol and picrotoxin.

Debnath *et al.,* (2010) evaluated the anti-epileptic activity of hydroalcoholic extract of the bark of *Erythrina fusca* Lour. (HAEEF) using different *in vivo* experimental models. Animals are used for the demonstration of an injury by exogenous agents of epileptic seizure on the brain with its physiological significance. Epileptic seizure challenged animals treated with HAEEF at doses of 250 mg/kg and 500 mg/kg showed reduction of maximal electro shock (MES) -, picrotoxin (PTX) - and pentylenetetrazole (PTZ) - induced epileptic seizure. Seizure duration was found to be lower and onset of seizure found to increase in the extract treated animals compared to control group. Thus, it can be inferred that the hydro-alcoholic extract of *Erythrina fusca* Lour. bark possesses antiepileptic effect against the animal models of epilepsy. Thus, the result suggested that the HAEEF possess anticonvulsive activity.
Garg and Paliwal (2011) studied anticonvulsant activity against maximal electroshock (MES) and Pentylenetetrazol (PTZ) induced convulsions in mice. The extract suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protector effect in PTZ-induced seizures. In conclusion, we showed that the ethanolic extract of *Cynodon dactylon* has anticonvulsant effect in the both models, suggesting their possible depressant action in the central nervous system.

Sadiq *et al.*, (2011) evaluated the antiepileptic activity of eugenol excluded clove extract. Wister albino rats are used in this study for evaluation. In pentylenetetrazol [PTZ] induced convulsion model, aqueous clove extract had significantly delayed the onset of clonic and tonic convulsions, indicating the anticonvulsant activity. In maximal electric shock [MES] induced convulsions also, aqueous clove extract had significantly decreased the duration of hind limb tonic extensor phase which is an observational parameter confirming anticonvulsant activity.

Hegde *et al.*, (2011) studied the ethanol extract of the roots of *C. spinarum* (100, 200 and 400 mg/kg, *p.o.*) for its anticonvulsant effect on maximal electroshock, pentylenetetrazole and picrotoxin-induced seizures in mice. The data suggest that the ethanol extract of the roots of *C. spinarum* may possess significant anticonvulsant activity via non-specific mechanisms, since it has been shown to delay the latency of seizures produced by the convulsive models affecting electrical discharge in the brain, gabaergic system and glutaminergic systems.

Saini *et al.*, (2011) screened different extracts (Petroleum ether, methanolic, aqueous) of *Nerium oderum* at a dose level of 400 mg/kg were screened for the anticonvulsant activity using Maximum Electroshock Induced Seizures & Pentylene Tetrazole Induced Seizures test models. In this study the petroleum ether extract of *N. oderum* showed better anticonvulsant activity as compared to other extracts.

### 2.G. Anxiety:

The term anxiety is commonly used for physical, mental and behavioural changes which automatically occur in the face of threat. It is a naturally healthy coping reaction to a
threatening or dangerous situation. People feel apprehensive, ‘on edge’ and may worry. They may also find it difficult to concentrate on anything other than the threat. In their brain signals, release of hormones which trigger activation of the sympathetic nervous system, commonly referred to as the fight-or-flight response, generates following several physical resources in the body to attack or run away from the threat:

1. Blood clotting ability increases, preparing for possible injury
2. Heart-rate speeds up and blood pressure rises
3. Sweating increases to help cool the body
4. Blood is diverted to the muscles, which tense, ready for action
5. Digestion slows
6. Saliva production decreases, causing a dry mouth
7. Breathing rate speeds up—nostrils and air passages in lungs open wider to take in air more quickly
8. Liver releases sugar to provide quick energy
9. Sphincter muscles contract to close bowel and bladder openings

Immune responses decrease (useful in the short-term to allow massive response to immediate threat, but potentially harmful over a long period). This fight-or-flight response is useful in the short-term especially if the threat requires a physical response, since it allows us to respond quickly and mobilize our physical resources. But in today’s world most threats cannot be dealt with by fighting or running away. Threats in human society (e.g. of redundancy) are often long-lasting and beyond the power of the individual to resolve, and may result in states of chronic anxiety (Bourne, 2005).

2. G.i. Classification of Anxiety Disorders

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) anxiety disorders are classified as: (Daniel, 2007)

1) Panic disorder with or without agoraphobia
2) Phobias: a) specific phobia b) Social phobia
3) Obsessive-compulsive disorder (OCD)
4) Generalized anxiety disorder (GAD)
5) Post traumatic stress disorder (PTSD)
6) Acute stress disorder
7) Adjustment disorder
1) Panic disorder with or without agoraphobia

**Panic disorder:** it is characterized by sudden episodes of acute apprehension or fear occurring without any apparent cause (free-floating). Panic feelings usually last only five to ten minutes, but can return in ‘waves’ for periods of up to two hours. These are associated with persistent worry about additional attacks, worry about the implications of attacks or their consequences (e.g. losing control, having a heart attack, ‘going crazy’), and a significant change in behaviour related to attacks (Roy-Byrne *et al.*, 1993).

**Agoraphobia:** it is defined as the experience of person feels anxious about situations from which escape might be difficult (or embarrassing), or in which help might be unavailable. Difficult situations often include being at home alone, being in a crowd, travelling alone in a car (American Psychiatric Association, 2000).

2) Phobias

It is defined as an intense and irrational fear of a specific object or situation that evokes profound negative responses and compels the person to avoid it. Some phobias are related to activities or objects that involve some risk (e.g. flying or driving) but many are focused on harmless animals or other objects. (Terry, 2009).

a) **Specific phobia**

It is also called as simple phobia, associated with a marked fear of a specific object or situation, that the person themselves recognizes is excessive and irrational. Common fears are of animals, insects, lightning, blood, injections, injury, bridges, and closed spaces.

b) **Social phobia (social anxiety disorder)**

Social phobia is a chronic disorder which may fluctuate over time. Typically, onset is in the mid-teens, and with a history of social inhibition or shyness. Onset may follow a stressful or humiliating experience, or be more insidious. It is a marked and persistent fear
Review of Literature

of social or performance settings in which the person feels exposed to the scrutiny of others. They fear they will act in an embarrassing way or show anxiety symptoms, and others will judge them. Exposure to feared social settings triggers anxiety including situationally bound panic attacks. Although the sufferer recognizes these fears as irrational they are compelling and intrusive. Situations commonly feared by individuals with social phobia include:

a. Eating in public
b. Speaking in public
c. Using public toilets

People with social phobia are more likely to present with co-morbid agoraphobia, alcohol abuse and depression (Schneier et al., 1992).

3) Obsessive-compulsive disorder (OCD)

OCD is characterized by obsessions and/or compulsions severe enough to be time consuming i.e. their performance takes more than an hour a day and causing marked distress or significant impairment. At some point the sufferer recognizes their obsessions/ compulsions are excessive or irrational but feel powerless to resist.

Obsessions are defined in the DSM-IV as:

a. Recurrent and persistent thoughts, impulses or images which are intrusive and inappropriate, and cause marked anxiety or distress
b. Thoughts, impulses or images which are not simply excessive worries about real-life problems
c. An attempt by the sufferer to ignore or suppress such thoughts, impulses or images, or neutralize them with some other thought or activity
d. Recognition that the obsessional thoughts, impulses or images are a product of the sufferer’s mind (not imposed from outside, as in thought insertion)

Compulsions

Compulsions are defined as repetitive behaviors, (e.g. hand-washing, ordering, checking), or mental activities (e.g. counting, silently repeating words), which the person
feels driven to perform in response to either an obsession or to rigidly applied rules (Jenike, 1996).

Behaviors or mental acts designed to prevent or reduce distress, or prevent a dreaded event or situation, but which are not realistically connected with what they are designed to neutralize or prevent, or are clearly excessive. Although the repetitive behaviours are intended to alleviate anxiety, relief is usually short-lived, and they must be repeated many times. Rituals often begin to lose their power to relieve anxiety so people develop increasingly complex rituals, or take hours to perform them perfectly.

4) **Generalized anxiety disorder (GAD)**

Generalized anxiety is characterized by excessive worry and apprehension, occurring more days than not for at least six months. (Wells et al., 1997) This is accompanied by at least three additional symptoms in the categories of (Schniering et al., 2000)

- **Motor tension** e.g. muscle tension, trembling, restlessness and fatigue
- **Autonomic hyperactivity** e.g. shortness of breath, rapid heartbeat, dry mouth, cold hands and dizziness (but not to the degree of qualifying as panic symptoms)
- **Vigilance and scanning** e.g. feeling ‘keyed up’ all the time, difficulty concentrating, startling easily and insomnia or irritability

5) **Post traumatic stress disorder (PTSD)**

The essential feature of PTSD is development of psychological symptoms after direct or indirect exposure to an extreme stress or outside the normal range of human experience. Symptoms can occur soon after the event (e.g. sexual abuse, car accident, robbery).

People with PTSD will often be permanently affected by the personal meaning they attribute to the trauma (e.g. someone who suffered childhood abuse may feel guilt and blame, believing they are not a valuable person or it would not have happened to them, or becoming distrustful of others). They may have, among other symptoms, impaired self-
esteem, poorer social functioning, difficulties in establishing or maintaining personal relationships, and be at significantly higher risk of suicide (Barbara et al., 2009).

6) Acute stress disorder

Acute stress reaction is a transient disorder developed in response to a traumatic event. Its essential features mimic those of PTSD except that symptoms appear, at most, four weeks after the trauma, and last a maximum of another four weeks. Most commonly reported reactions include:

i. Anxiety or fear of being alone or in other frightening situations, of danger to self or loved ones, or of a similar event happening again

ii. Avoidance of situations or thoughts reminiscent of the traumatic event

iii. Being easily startled by loud noises or sudden movements

iv. Flashbacks, when images of the traumatic event suddenly come to mind

v. Sleep problems

Not everyone will experience these reactions, and they should wear off in a matter of weeks. If the person continues to experience them a month after the event, a diagnosis of PTSD should be considered.

7) Adjustment disorder

Adjustment disorders (manifested as either depression and/or anxiety) are characterized by a short period of emotional and behavioural disturbance in response to a significant life change or stress. An adjustment disorder is only diagnosed, if distress exceeds expected levels, given the cause, or if it causes significant impairment in social or occupational functioning. Typical stressors or life events include business difficulties, redundancy, re-entry culture shock (e.g. getting out of jail, leaving hospital, returning to the home country after a long period of absence), or family life-cycle changes (e.g. marriage, birth of a baby, children going to school, leaving home, retirement). Common symptoms associated with this disorder can include: (Hunt et al., 1995)

i. Depressed mood

ii. Tearfulness and feelings of hopelessness

iii. Insomnia
iv. Reverting to bed-wetting, thumb-sucking or other regressive behaviors is common in children.

2.G.ii. Pathophysiology

Fowling neurochemicals models involve in pathophysiology of anxiety disorder. (Barbara et al., 2009).

1) Noradrenergic model

This model suggests that the autonomic nervous system of anxious patients is hypersensitive and overreacts to various stimuli. The locus ceruleus may have a role in regulating anxiety, as it activates norepinephrine release and stimulates the sympathetic and parasympathetic nervous systems. Chronic noradrenergic over activity down regulates α2-adrenoreceptors in patients with generalized anxiety disorder (GAD) and posttraumatic stress disorder (PTSD). Patients with social anxiety disorder (SAD) appear to have a hyper responsive adrenocortical response to psychological stress.

2) γ-Aminobutyric acid (GABA) receptor model.

GABA is the major inhibitory neurotransmitter in the CNS. Many antianxiety drugs target the GABAA receptor. Benzodiazepines (BDZs) enhance the inhibitory effects of GABA, which has a strong regulatory or inhibitory effect on serotonin (5-HT), norepinephrine, and dopamine systems. Anxiety symptoms may be linked to under activity of GABA systems or down regulated central BDZs receptors. In patients with GAD, BDZs binding in the left temporal lobe is reduced. Abnormal sensitivity to antagonism of the BDZs binding site and decreased binding was demonstrated in panic disorder. Growth hormone response to baclofen in patients with GAD and SAD suggests an abnormality of central GABAB receptor function. Abnormalities of GABA inhibition may lead to increased response to stress in PTSD patients.

3) Serotonin (5-HT) model

GAD symptoms may reflect excessive 5-HT transmission or over activity of the stimulatory 5-HT pathways. Patients with SAD have greater prolactin response to buspirone challenge, indicating an enhanced central serotonergic response. The role of 5-
HT in panic disorder is unclear; but it may have a role in development of anticipatory anxiety. Preliminary data suggest that the 5-HT and 5-HT2 antagonist metachlorophenylpiperazine causes increased anxiety in PTSD patients.

4) Patients with PTSD have a hypersecretion of corticotropin-releasing factor but demonstrate subnormal levels of cortisol at the time of trauma and chronically. Dysregulation of the hypothalamic-pituitary-adrenal axis may be a risk factor for eventual development of PTSD.

5) Functional neuroimaging studies suggest that frontal and occipital brain areas are integral to the anxiety response. Patients with panic disorder may have abnormal activation of the parahippocampal region and prefrontal cortex at rest. Panic anxiety is associated with activation of brain stem and basal ganglia regions. GAD patients have an abnormal increase in cortical activity and a decrease in basal ganglia activity. In patients with SAD, there may be abnormalities in the amygdala, hippocampus, and various cortical regions. Lower hippocampal volumes in patients with PTSD may be a precursor for subsequent development of PTSD.

2.G.iii. Neuroanatomy of Anxiety Disorder

Amygdala, an area of the brain responsible for the acquisition and expression of fear conditioned, located within the medial temporal lobe, it is comprised of thirteen nuclei, three of which are basal amygdala (BA), lateral amygdale (LA), and central nuclei, involved in the pathways of fear response. Stimuli received by the sensory thalamus are transmitted to the LA, and then they are transferred to the central nucleus (CA) (short loop pathway). The BA also serves as a connection between the LA and central nucleus. The long loop pathway sends signals to the LA from the sensory cortex (Insula, and prefrontal cortex) (figure.4). From there, the information projects to the effectors’ sites in the brain stem and hypothalamus, produces the autonomic and behavioural manifestations of the acute fear response. It has been shown that the LA is the area responsible for memory consolidation and plasticity in fear conditioning. Disruption or lesions of the LA and CA can disrupt the acquisition of conditioned fear and long-term contextual fear memory; there is evidence that lesions of the BA can affect fear responses. The molecular mechanism by
which fear acquisition occurs in the LA is long-term potentiation (LTP). It is proposed that consolidation of memory occurs during a process in which calcium enters the cell via N-methyl-D aspartate (NMDA) receptors and through voltage-gated calcium channels (VGCCs). Blockage of the VGCCs will disrupt short-term memory but not long-term memory, indicating that this pathway requires only NMDA receptors to be active. Some animal studies have shown that blockage of NMDA receptor by the antagonist D, L-2-amino-5-phosphonovaleric acid (APV, AP5); will block fear acquisition, but not expression, although more recently studies have shown that both processes are inhibited. Gene studies have shown high expression of receptors in the hippocampus as well, indicating the importance of this brain structure in Pavlovian conditioning. As in the amygdala, blockage of these receptors will inhibit conditioned fear responses. The application of these preclinical findings to humans is currently limited, but potential avenues include the use of NMDA receptor antagonists and calcium channel blockers to impair memory consolidation and thereby treat anxiety symptoms (Amir et al., 2006).

![Figure.4: Fear Conditioning Circuitry](image)

In auditory fear conditioning, animals learn to fear an innocuous tone. By pairing tone and shock, the tone acquires the capacity to elicit defensive reactions, such as freezing
(arrow pointing up). Tone and shock stimuli converge in the lateral amygdala (LA), resulting in associative plasticity in the tone-LA pathway. Subsequent presentations of the tone can now activate LA neurons. The LA then communicates with the central nucleus (CE), which controls the expression of fear by way of connections to specific circuits that mediate freezing behaviour. The LA connects with CE directly and by way of connections to other amygdala areas, including the intercalated cell masses (ICM), which gate the output, and the basal nucleus (B), which processes contextual information from the hippocampus.

Krishna et al., (2001) evaluated the anxiolytic-like behavioural effects of four calcium channel antagonists (CCAs) belonging to different classes in rats. Study was conducted using male Wistar strain albino rats in two non-conflicting models, elevated plus maze and light-dark arena. Among the CCAs tested only verapamil, diltiazem and flunarizine have anxiolytic-like activity comparable to diazepam.

Shafaghi et al., (2002) investigated putative activity of hydroalcoholic and aqueous infusion extracts of *Echium amoenum* L. in mice using the rotarod model of motor coordination and the elevated plus maze model of anxiety. Preliminary phytochemical study of the plant, with standard procedures, showed that it contains saponins, flavonoids, unsaturated terpenoids and sterols. There was no evidence of tannins, alkaloids and cyanogenic glycosides. It can be concluded that single administration of aqueous extract of *Echium amoenum* L. produces a significant but mild to moderate anxiolytic effect.

Sousa et al., (2005) presented the behavioral effects of riparin I (methyl ether of N-benzoyl tyramine) from unripe fruit of *Aniba riparia* (Lauraceae) on the elevated plus maze, open field, rota rod and hole board tests in mice. Riparin I was administered acutely by intraperitoneal (i.p.) and oral routes to male mice at doses of 25 and 50 mg/kg. The results showed that riparin I by both administration routes has effects on the central nervous system with antianxiety effects on the plus maze and hole board tests. The substance is devoid of myorelaxant effects.

Ribeiro et al., (2006) studied *Erythrina velutina* (EV) and *Erythrina mulungu* (EM), popularly used in Brazil as tranquilizing agents. The effects of acute and chronic
oral treatment with a water: alcohol extract of EV (7:3, plant grounded stem bark; acute = 100, 200, 400 mg/kg; chronic = 50, 100, 200 mg/kg) were evaluated in rats (N = 11-12) submitted to the elevated T-maze (for avoidance and escape measurements) model of anxiety. Animals were treated with the same doses of EV and EM (water: alcohol 7:3, inflorescence extract) and submitted to the forced swim test for the evaluation of antidepressant activity (N = 7-10). These results were not due to motor alterations since no significant effects were detected in an open field. The observations suggest that EV exerts anxiolytic-like effects on a specific subset of defensive behaviors which have been associated with generalized anxiety disorder.

Kumar and Sharma (2006) carried out investigation of Turnera aphrodisiaca Ward (Turneraceae) by isolate the bioactive constituent(s) from T. aphrodisiaca using bioactivity-guided fractionation. Antianxiety activity guided fractionation of methanol extract of the plant led to isolation of 5,7,40 -trihydroxy flavone apigenin. Its structure was elucidated by UV and NMR data. Apigenin exhibited significant anxiolytic activity at a dose of 2mg=kg, p.o., in mice using elevated plus maze model of anxiety. It is concluded that apigenin is responsible for anxiolytic effects of this traditionally used plant.

Abid et al., (2006) carried out the screening of ethanol and chloroform extracts of Pachyrhizus erosus seeds for central nervous system (CNS) depressant activity. The different activities studied were potentiation of pentobarbitone-induced sleep, test for locomotor activity, and effect on muscle co-ordination, antiaggressive and antianxiety activities. The result of the study reflected that ethanol extract of the seeds (150 mg/kg, p.o) decreased locomotor activity, produced muscle relaxation and showed antianxiety and antiaggressive activity.

Krishna et al., (2006) studied the anxiolytic-like activity of NR-ANX-C, a polyherbal product, in rats. Inbred, male, Wistar albino rats weighing between 150 and 180 g were used. The standard anxiolytic, diazepam (0.5 mg and 1 mg/kg), and the test drug, NR-ANX-C powder (5, 10 and 20 mg/kg), were dissolved/suspended in 1% gum acacia solution and administered orally. In present study NR-ANX-C exhibited anxiolytic-like activity comparable to that of diazepam.
Raupp *et al.*, (2008) evaluated the effect of acute and chronic (23–26 days) administrations of the hydroalcoholic extract of the stem bark of *Erythrina velutina* (orally) in mice submitted to the following tests: elevated plus-maze, forced swim, spontaneous locomotor activity, and habituation to active chamber. Chlordiazepoxide and imipramine were used as standard drugs. The results suggest that chronic administration of the hydroalcoholic extract of the stem bark of *Erythrina velutina* exerts an anxiolytic-like effect on mice, and it could serve as a new approach for the treatment of anxiety, although it may have an amnesic effect at low doses.

Lollato *et al.*, (2010) evaluated behavioral effects of aqueous (AE) and dichloromethane (DCM) extracts of *Erythrina speciosa* Andrews, leaves in male mice, as well as their lethal dose 50% (LD50). LD50 for both extracts were higher than 2000 mg/kg. The results showed that AE and DCM extracts of *E. speciosa* leaves do not produce anxiolytic effect in the elevated plus maze nor seems to depress the Central Nervous System. However, since serotonergic mechanisms may be involved in the pharmacological action of *Erythrina* plants and the elevated plus maze test is not adequate to evaluate serotonergic drugs, our results do not invalidate the use of this plant in folk medicine but suggest that the mechanism involved in a possible central action of *Erythrina* needs to be clarified.

Similarly, Serrano *et al.*, (2011) isolated two alkaloids, erysodine (1) and erysothrine (2) from the flowers of a Pakistani medicinal plant, *Erythrina suberosa*. These compounds were investigated for anxiolytic properties, and the results showed significant effect, in an acute oral treatment with 1-2, which were suspended in saline (NaCl 0.9%) plus DMSO 1%, and evaluated in 122 Swiss male mice exposed to two tests of anxiety - the elevated plus-maze (EPM) and the light/dark transition model (LDTM).

Singh *et al.*, (2011) evaluated the petroleum ether (PE), chloroform (CE), ethanol (EE) and water extractives (WE) of stem, leaf and stem calli of *O. sanctum* for antianxiety activity against standard drug, diazepam using elevated plus maze apparatus and albino mice. The ethanol extractive of callus tissue (6-month-old) exhibited maximum significant antianxiety activity out of all extractives studied followed by ethanol extractive of stem (8-
(month-old) of *O sanctum*. Chemically the extractives of stem and leaf showed the presence of saponins, sterols, triterpenoids, carbohydrates, tannins and proteins in stem, leaf and stem cultures. While flavonoids were detected in stem and Leaf only. The results indicate that both the original plant and callus developed from stem can be considered as potential candidate for bioactivity guided isolation of natural antianxiety agents.

2.H. Anthelmintic Activity:

Helminth infections are among the most common infections in man, affecting a large proportion of the world’s population. In developing countries they pose a large threat to public health, and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas, and some of them can be developed in template climates. Parasitic diseases causing severe morbidity include lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences. (Bundy, 1994). The World health Organisation (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs for their primary health care needs. The use of medicinal plants is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs (Rajesh et al, 2010). Among the most common infections of digestive system in human beings are helminth infections. In developing countries they pose a large threat to the society. Such parasitic diseases cause severe morbidity, including lymphatic filariasis, onchoserciasis and schistosomiasis. These infections can affect most of the population in endemic areas with major economic and social consequences. Ayurveda provides many herbal preparations to overcome alimentary canal infections with negligible side effects. Anthelmintics are drug that either kill (vermin – cides) or expel (vermifuge) infesting helminthes. Helminthic infections are now being recognized as a cause of many acute as well as chronic ill healths among the various human beings as well as cattle’s. The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials and insecticides. (Akhtar, 2000).
Nirmal et al., (2007) studied the leaves, wood, seed; bark and pericarp of the fruit of *Pongamia glabra*. Anthelmintic activity was evaluated on Indian adult earthworms, *Pherentima posthuma*. They concluded that anthelmintic activity of the seed of *P. glabra* is due to the active principles present mostly in the ethyl acetate and petroleum ether extracts.

Rastogi et al., (2009) studied the ethanolic extracts of *Moringa oleifera* and *Vitex negundo* for anthelmintic activity against Indian earthworm *Pherentima posthuma*. Various concentrations of both extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as a control group. Dose dependent activity was observed in both plant extracts but *Moringa oleifera* shows more activity as compared to *Vitex negundo*.

Jesupillai, (2009) carried out anthelmintic activity of ethanol, chloroform and ethyl acetate extracts leaves of *Erythrina indica* against *Pherentima posthuma*. The coarsely powdered leave materials were extracted exhaustively with ethanol (95%), chloroform and ethyl acetate by using Soxhlet apparatus. These extracts were concentrated under reduced pressure and preserved in desiccators until further use.

Dwivedi et al., (2009) evaluated the crude extracts of *Balanites aegyptiaca* (L.) Delile (Balanitaceae) for anthelmintic activity using adult earthworms; the bark extract exhibited a dose-dependent inhibition of spontaneous motility (paralysis). The result shows that the aqueous extract possesses wormicidal activity and thus, may be useful as an anthelmintic.

Deore et al., (2009) investigated alcohol and aqueous extracts from the seeds of *Cassia tora* for their anthelmintic activity against *Pherentima posthuma* and *Ascaridia galli*. Three concentrations (25, 50 and 100 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml.
Kushwaha and Aind (2010) investigated the anthelmintic activity of different herbs in polyherbal combination. The aqueous and ethanolic extract of the different concentrations were tested which involved determination of paralysis time and time to kill the worms. Piperazine citrate was used as standard and it was found that the PHF ethanolic extract activity is higher than PHF aqueous extract.

Sutar et al., (2010) investigated ethanol extract from the leaves of *Platycladus orientalis* were for their anthelmintic activity against *Pheretima posthuma*. Three concentrations (1%, 2.5% and 5%) of extract were studied in activity, which involved the determination of time of paralysis and death of the worm. The extract exhibited significant dose dependent anthelmintic activity. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The anthelmintic activity of ethanol extract of *Platycladus orientalis* has therefore been demonstrated for the first time.

Barnabas et al., (2011) screened extracts of *Celosia laxa*, *Neocarya macrophylla* and *Zanthoxylum zanthoxyloides* leaves for anthelminthic activities on *Ascaris lumbricoides*. *Celosia laxa* appeared to be more potent against *A. lumbricoides* with rapid recovery of weight while *Z. zanthoxyloides* has the lowest potency. The effects of these plant extracts on *Ascaris lumbricoides* suggest that they could serve as an alternative source of anthelminthic agent.

Basuri and Vishal (2011) investigated the petroleum ether, chloroform & ethanol extracts of the aerial parts of stem bark of *Sterblus asper* (Moraceae) for activity against Indian earthworms *Pheretima posthuma*. Various concentrations (5-25mg mL-1) of each extract were tested, which involved determination of time of paralysis and time of death of the worms. All three extracts exhibited considerable anthelmintic activity. All the extracts (i.e., petroleum ether, chloroform and ethanol) at the tested dose (5-25 mg mL-1) level produced significant activity (p < 0.001) when compared with piperazine citrate (15 mg mL-1) and albendazole (10 mg mL-1) which were included as standard reference and normal saline as control. The present study indicates the potential usefulness of *Sterblus asper* stem bark as anthelmintic activity.
Kirubha et al., (2011) evaluated the anthelmintic activity of the alcoholic and aqueous extracts of roots and rhizomes of *Corallocarpus epigaeus* was evaluated on adult earthworms – *Lampito maruti*, *Eudrillus eugine*, and *Eisenia foetida* using piperazine citrate as the reference standard. The extracts caused paralysis followed by death of the worms at all tested dose levels.

Lasisi and Kareem (2011) investigated anthelmintic properties of the stem bark extract and compounds isolated from *Bridelia ferruginae*. In relation to the traditional use of *B. ferruginae* against gastro-intestinal infections, bioactivity-guided fractionations of the CHCl₃ - and CH₂Cl₂ - soluble fractions of the 80% MeOH extract from the stem barks of *B. ferruginae* yielded two known triterpenoids: betuline (1), glucoside of betulinic acid (2) and other two known flavonoids: quercetin (3) and kaempferol (4). Structures of compounds 1 to 4 were elucidated by spectroscopic studies and comparison with related compounds in literature. The time of paralysis and death of the parasitic worms: *Fasciola gigantical* (liver fluke), *Taenia solium* (tape worm) and *Pheritima posthuma* (earthworm, Annelid) were determined at 25, 50, 80 and 100 mg/ml. The stem barks extract of *B. ferruginae* and isolated compounds demonstrated concentration-dependent anthelmintic potencies against parasitic worms assayed. Structural-activity relationship is explained.

Rabiu et al., (2011) investigated the aqueous extract of *Cinnamomum camphor* leaves for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Railletina spiralis*) and roundworms (*Ascaridia galli*). Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at the concentration of 50 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as an anthelmintic.