REVIEW OF LITERATURE
II. REVIEW OF LITERATURE

Indian traditional medicine is based on various systems including Ayurveda, Siddha, and Unani. These traditional systems of medicine are unique but there is a common thread in their fundamental principles and practices. The materia medica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. With the emerging worldwide interest in adopting and studying traditional systems for exploiting their potential based on different health care systems. The government and the private sectors in India are now exploring all of the possibilities for the perfect evaluation of these systems in order to effectively adopt the therapeutic approaches available in Indian systems of medicine as well as to help in generating data to restore these products on the national health program.

Natural products are historically core of the medicines and they are still a major source of drug leads, which in term used to describe compounds that may be developed into medicines. For example, paclitaxel, marked as Taxol is the best selling drug obtained from natural products. This drug was developed by ‘Bristol-Myers Squibb’ and marketed for the treatment of ovarian and mammary cancers and became available for use in the USA in 1993. The compound was initially isolated from the bark of the Pacific yellow tree Taxus brevifolia and demonstrated that the best possible qualities of natural products being highly functional and potent remedies for various ailments. The large proportion of natural products in drug discovery has stemmed from the diverse
structures and intricate carbon skeletons. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more “drug-likeness and biological friendliness than totally synthetic molecules” (Koehn and Carter, 2005), making them good candidates for further drug development (Balunas and Kinghorn, 2005; Drahl et al., 2005).

In India, Department of Biotechnology has launched a Bio-prospecting and Molecular Taxonomy programme which opens new avenues for sustainable utilisation and bio-prospecting of medicinal plant, genetic resources and commercialization of the drugs originated from the medicinal plants. This has resulted in the characterization of intraspecific genetic diversity of many medicinal plants (Symlocos laurina, S. racemosa, Gaultheria fragrantissims, Eurya nitida, Vitex negundo, Podophyllum hexandrum, Rhododendron nilgiricum etc) (Anonymous, 2000). As a result of many bio-prospecting projects carried out worldwide, many therapeutic compounds have been discovered and introduced to the national as well as the international markets. But so far, only 90,000 natural compounds have been well studied which represent about 40% of total possible new drugs. However, the increasing need for phytochemicals as a safe alternative or an adjunct to modern medicine is seriously felt particularly to the widely perceived biohazardous side effects of the synthetic drugs.

A survey of pharmacopeia showed the natural products have a key role in biologically activity. Infact it has been estimated that 50-55% of all
medicines are derived from such sources. Some of the drugs derived from the natural product are listed in the Table

**Commercialization of drugs derived from natural products.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
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<tbody>
<tr>
<td>Silymarin</td>
<td>Hepatoprotective</td>
</tr>
<tr>
<td>Acetyl salicylic</td>
<td>Analgesic,</td>
</tr>
<tr>
<td>acid (Aspirin)</td>
<td>Antiphlogistic etc</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>Acarbose</td>
<td>Antidiabetic</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>Immunosuppressant</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Anticancer</td>
</tr>
<tr>
<td>FK 506</td>
<td>Immunosuppressant</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Anticancer</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Antihyperlipidemic</td>
</tr>
<tr>
<td>Morphine</td>
<td>Analgesic</td>
</tr>
<tr>
<td>Miglitol</td>
<td>Antidiabetic</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Antibacterial</td>
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<tr>
<td>Streptomycin</td>
<td>Antibacterial</td>
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<tr>
<td>Chloroquine</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Anticancer</td>
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<tr>
<td>Topotecan</td>
<td>Anticancer</td>
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</table>

India is a known mega-diversity centre harbouring a multitude of medicinal plant species. Each presumably studied with as yet unknown genetic
and chemical variations of economic importance. Out of an estimated 17,000 higher plant species occurring in India, more than 1000 species are used over several centuries in the traditional systems of medicine viz. Ayurveda, Siddha, Unani and Amchi. The villagers and tribal folks spread across the length and breadth of the country make use of more than 7000 plant species through oral traditions (Pushpangadan et al., 1997). Nearly 3/4\textsuperscript{th} of the herbal drugs and perfumery products used in the world are available in natural state in India. Therefore, the rich and varied plant diversity, especially the genetic diversity of medicinal and aromatic plants, is one of India’s important strengths and is the bedrock for all future bioindustrial developments.

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 Ghat 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 deciduous to dry deciduous and scrub jungles. It is one of the richest biodiversity centers and is considered as one among the eighteenth hot spot of the world. This region comprises about 4000 species of angiosperms of which 2,280 species are endemic to this region.

In the present investigation, an endemic medicinal tree *Semecarpus anacardium* have been selected for phytochemical and pharmacological investigations.
Semecarpus anacardium is a medium size tree with grey bark and simple alternate, obviate blong leaves. The flowers are greenish white and the fruit ia a drupe born on the false fruit of the thalamus and popularly called as nut. Many investigators explored the nut and nut milk of this plant for phytochemical and pharmacological investigations.

2.1 Phytococonstituents reported from S. anacardium:

The review of phytochemical analysis of S. anacardium has showed the presence of biflavonoids, phenolic compounds (Prakasa Rao et al., 1973), bhilawanols, sterols and glycosides (Indap et al., 1986; Nagabhushana et al., 2002) and anacardic acid (Chattopadhyaya and Khare 1969) in S. anacardium nuts. Drug distillation studies of the S. anacardium gave rise to catechol and a hydrocarbon (Premalatha, 2000), and monophenolic compounds known as semecarpol (C_{17}H_{28}O) have also been isolated. Vitamins and linoleic, myristic, oleic, palmitic and stearic acids are present in nut milk (Rai et al., 2000). Vijayalakshmi et al. have reported the presence of carbohydrates, phenols and flavonoids in the milk extract, and it is non-toxic up to 2,000 mg/kg body weight. The presence of the above-said compounds was confirmed by the TLC, HPLC and HPTLC analysis of the nut and milk extract (Shin et al., 1999; Aravind et al., 2008; Mythilypriya et al., 2008; Sahoo et al., 2008; Raveedran Nair et al., 2009). On the basis of chemical and spectral data, several biflavonoids have been isolated from the alcoholic fraction of nut shells and characterized, namely semecarpuflavonone, jeединflavanone, galluflavanone,
nallaflavanone, semecarpetin and anacarduflavanone (Murthy 1992; Murthy, 1983 a; Murthy, 1985 a; Murthy, 1983 b; Murthy, 1983 c; Murthy, 1985 b).

Amentoflavone, is one of the active components belongs to the biflavonoid class of flavonoids. It is well known for displaying a remarkable wide spectrum of biological activities, and has a potential use in pharmaceutical, food and cosmetic areas. Amentoflavone is the chief flavonoid constituent isolated from the nut shell and from the leaves of *Semecarpus anacardium*.

2.2 Pharmacological properties of *S. anacardium*:

antiarthritic, antioxidant (Bose et al., 1967; Premlatha et al., 2000; Verma et al., 2009; Sahoo et al., 2008), hypolipidemic, hypoglycemic (Tripathi, et al., 2004; Arul, et al., 2004; Kangralkar, et al., 2009; Haseena Banu, et al., 2012), antifungal, antifertility and neuroprotective activities (Sharma et al., 2003; Sharma et al., 2002; Shukla, et al., 2000) protection against FeSO₄-induced lipid peroxidation (Vijayalaxmi et al., 1997), anticonvulsant activity from nut extract (Basavaraj et al., 2011). Cytotoxicity activity (Krishnaraju et al., 2005), antimutagenic effect (Prabhu et al., 2005), Nephrotoxicity (Choudhari et al., (2007), antibacterial activity (Nair et al., 1996; Mohanta et al., 2007) CNS activity (Farooq et al., 2007), cyclooxygenase (COX) inhibitory biflavonoid from the seed (Selvam, et al., 2004), Apoptotic effect on T47D breast cancer cell line (Mathivadhani et al., 2007).

2.2.1 Wound Healing Activity:

The main function of the skin is to serve as a protective barrier against the external environment. Loss of the integrity of large portions of the skin as a result of injury or illness may lead to major disability or even death.

a) Anatomy and physiology of normal skin

The total surface area of the skin ranges from 0.2 to 0.3m² in an average newborn and 1.5 to 2.0 m² in an adult. The skin is consisted of two layers: the epidermis, ranging from 0.05 mm thick (in areas such as the eyelids) to over 1 mm thick on the soles; and the dermis, usually at least 10 times thicker than the associated epidermis. As a result, the average total skin depth is 1-2 mm (Tian
Jun, 2004). The epidermis, originating from ectoderm, is composed primarily of epithelial cells, specifically keratinocytes. These include melanocytes, whose function is to produce pigment (melanin) for the purpose of protection from ultraviolet radiation; Langerhans cells, which capture foreign proteins and present them to the immune system. The Langerhan cells play a role in protecting the skin against cancers, infection and also in skin allergies. Merkel cells which serve as mechanoreceptors. The basal layer of keratinocytes is called the stratum germinativum and contains immature cells, which are mitotically active, providing generation of outwardly migrating epidermal cell layers. From this layer, cells migrate outward and mature along the way to become the stratum spinosum, a layer where mitosis no longer occurs but protein synthesis is prominent.

The next outward layer of maturation is the stratum granulosum where specialization into keratin production predominates. The next stage of migration is the stratum lucidum, where cells lose their nuclei and flatten, evolving into a dead layer called the stratum corneum. This layer is a compact and relatively impervious layer, which eventually desquamates. The entire process of epidermal maturation and turnover from the basal layer to desquamation takes approximately 2-4 weeks. Epidermal appendages (i.e. hair follicles, sebaceous glands, and sweat glands) are comprised of special cell types but are also lined with epidermal cells. These structures extend from the epidermis downward, residing mostly in the dermis.
The dermis, derived from mesoderm, is a relatively thick layer comprised of fibrous connective tissue. The primary cell type is the fibroblast, a spindle-shaped cell which does not frequently replicate but is very active in producing extracellular protein, primarily collagen and elastin. Collagen is secreted into the intercellular matrix, where it undergoes maturation (crosslinking and coiling) into strong fibers oriented so as to allow stretchability while providing tensile strength. Elastin is processed to form elastic fibers, imparting a degree of resting tension to the skin. Constant turnover occurs at a low rate in unstressed skin and at higher rates when chronic mechanical stress is applied or when healing is occurring. The ground substance of the skin is a nonfibrous bulk of glycosaminoglycan. The ground substance provides a semifluid matrix that lubricates the cellular and fibrillar components. It is through this ground substance that inflammatory and other cells may migrate and nutrients diffuse.

The dermis is subdivided into a thin, superficial layer known as the papillary dermis and a thicker, deep portion called the reticular dermis. The subdermal plexus, sends vessels upward to form a plexus between the reticular and papillary dermis. Other structures found in the dermis include lymphatics and nerve fibers. At the dermoepidermal junction exists a basement membrane, a layer composed of mucopolysaccharides but also rich in fibronectin. Cells of the basal epidermal layer are joined to the basement membrane by hemidesmosomes. The basement membrane, in turn, is anchored to the dermis with the aid of specialized fibrils.
b) Biology of wound healing process:

The ultimate goal for wound healing is to minimize scarring and maximize the functional and cosmetic outcome. The management of the wounds depends on the depth and extent of the injury. Those wounds that are superficial need only to reepithelialize. Small but deep wounds heal by scar formation and contraction, while larger wounds require grafting. These processes are beneficial at times, but detrimental at other times. The primary goals of the treatment of wounds are rapid wound closure and a functional and aesthetically satisfactory scar. Recent advances in cellular and molecular biology have greatly expanded our understanding of the biologic processes involved in wound repair and tissue regeneration (Clark, 1996) and have led to improvements in wound care. The review of healing, traumatic and nontraumatic wounds, discuss the use of skin substitutes and growth factors to promote wound healing. Wound healing has three phases; inflammation, tissue formation and tissue remodeling that overlap in time.

c) Inflammatory phase:

Tissue injury causes the disruption of blood vessels and extravasations of blood constituents. The blood clot reestablishes hemostasis and provides a provisional extracellular matrix for cell migration. Platelets not only facilitate the formation of a hemo-static plug but also secrete several mediators of wound healing, such as platelet-derived growth factor, that activate macrophages and fibroblasts (Heldin and Westermark, 1996). However, in the absence of hemorrhage, platelets are not essential in wound healing. Numerous vasoactive
mediators and chemotactic factors are generated by the coagulation that activates the complement pathways by injured or activated parenchymal cells. These substances recruit inflammatory leukocytes to the site of injury (Clark, 1996). Infiltrating neutrophils cleanse the wounded area of foreign particles including microbes and are then extruded with the eschar or phagocytosed by macrophages. In response to specific chemoattractants, such as fragments of extracellular-matrix protein, transforming growth factor β, and monocyte chemoattractant protein 1, monocytes infiltrate the wound site and activate the macrophages that release growth factors such as platelet-derived growth factor and vascular endothelial growth factor, which initiate the formation of granulation tissue. Macrophages bind to specific proteins of the extracellular matrix by their integrin receptors, which stimulate the phagocytosis of microorganisms and fragments of extracellular matrix by the macrophages (Brown, 1995). Adherence to the extracellular matrix also stimulates monocytes to undergo metamorphosis into inflammatory or reparative macrophages. Adherence induces monocytes and macrophages to express colony-stimulating factor 1, a cytokine necessary for the survival of monocytes and macrophages; tumor necrosis factor α, a potent inflammatory cytokine and platelet-derived growth factor, a potent chemoattractant and mitogen for fibroblasts.
d) Epithelialization:

Reepithelialization of wounds begins within hours after injury. Epidermal cells from skin appendages such as hair follicles quickly remove clotted blood and damaged stroma from the wound space. At the same time, the cells undergo marked phenotypic alteration that includes retraction of intracellular tonofilaments (Paladini et al., 1996); dissolution of most intercellular desmosomes, which provide physical connections between the cells and formation of peripheral cytoplasmic actin filaments, which allow cell movement (Goliger and Paul, 1995; Gabbiani et al., 1978). Furthermore, epidermal and dermal cells no longer adhere to one another, because of the dissolution of hemidesmosomomal links between the epidermis and the basement membrane, which allows the lateral movement of epidermal cells. The expression of integrin receptors on epidermal cells allows them to interact with a variety of extracellular-matrix proteins (e.g., fibronectin and vitronectin) that are interspersed with stromal type I collagen at the margin of the wound and interwoven with the fibrin clot in the wound space (Clark, 1990). The migrating epidermal cells dissect the wound, separating desiccated eschar from viable tissue. The path of dissection appears to be determined by the array of integrins that the migrating epidermal cells, express on their cell membranes.

One to two days after injury, epidermal cells at the wound margin begin to proliferate behind the actively migrating cells. The absence of neighbor cells at the margin of the wound (the “free edge” effect) may signal both migration and proliferation of epidermal cells. Local release of growth factors and
increased expression of growth-factor receptors may also stimulate these processes. Leading contenders include epidermal growth factor, transforming growth factor α, and keratinocyte growth factor (Werner et al., 1994). As re-epithelialization ensue, basement-membrane proteins reappear in a very ordered sequence from the margin of the wound inward in a zipper like fashion (Clark et al., 1982). Epidermal cells revert to their normal phenotype, once again firmly attaching to the reestablished basement membrane and underlying dermis.

e) Wound contraction and extracellular-matrix:

- Reorganization:

Wound contraction involves a complex and superbly orchestrated interaction of cells, extracellular matrix and cytokines. During the second week of healing, fibroblasts assume a myofibroblast phenotype characterized by large bundles of actin-containing microfilaments disposed along the cytoplasmic face of the plasma membrane of the cells and by cell-cell and cell-matrix linkages (Welch et al., 1990; Desmoulière and Gabbiani, 1996). The appearance of the myofibroblasts corresponds to the commencement of connective-tissue compaction and the contraction of the wound. Collagen remodeling during the transition from granulation tissue to scar is depends on continued synthesis and catabolism of collagen at a low rate. The degradation of collagen in the wound is controlled by several proteolytic enzymes termed matrix metalloproteinases, which are secreted by macrophages, epidermal cells and endothelial cells, as well as fibroblasts (Mignatti et al., 1996).
• **Tensile strength of wound:**

The strength of a healing wound 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 of 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 uninjured skin. At maximal strength, a scar is only 70 percent as strong as normal skin (Levenson *et al.*, 1965). Extracellular matrix involves five main components which include collagen, adhesive glycoproteins, basement membrane, elastic fibers and proteoglycans which are responsible for wound strength.

**f) Wound healing plants of the Western Ghats:**

In the traditional systems of medicine, various 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); *Trigonella*
foenum graecum (Taranalli and Kuppast, 1996); Hypericum mysorensense (Mukherjee and Suresh, 2000); Nelumbo nucifera (Mukherjee et al., 2000); Ginkgo biloba (Bairy and Rao, 2001); Aegle marmelos (Jaswanth et al., 2001); Opuntia ficus-indica (Park and Chun, 2001); Gmelina arborea Roxb. (Shirwaikar et al., 2002); Bryophyllum pinnatum (Mahamood and Patil, 2002); Eucalyptus globulus (Hukkeri et al., 2002); Terminalia arjuna (Madhura and Sushma, 2003); Cinnamomum zeylanicium (Kamath et al., 2003); Eucalyptus globulus (Kusum et al., 2004); Trigonella foenum-graecum (Taranalli et al., 2004a); Oxalis corniculata (Taranalli et al., 2004b); Merremia tridentata (Hatapakki et al., 2004); Diospyros cordifolia (Mankani et al., 2004); Saussurea lappa (Ganachari et al., 2005); Plagiochasma appendiculatum (Meenakshi et al., 2006); Terminalia arjuna (Minakshi and Sushma, 2006); Embelia ribes (Kumara Swamy et al., 2007). Allium cepalin (Chitra et al., 2009), Mussaenda frondosa (Suhas et al., 2011), Heliotropium indicum, Plumbago zeylanium, Acalypha indica (Suresh et al., 2002), aristolochia bracteata, cassia tora (Jayasutha et al., 2011), Tecomaria capensis (Saini et al., 2012), Anthocephalus Cadamba (Kishore et al., 2007), Ageratum conyzoides (Jain et al., 2009), Moringa oleifera (Rathi et al., 2006), Buteamono sperma (Sumitra et al., 2005), Morinda citrifolia (Nayak et al., 2009), Boesenbergia rotunda (Mahmood et al., 2010), Quercus infectoria (Umachigi et al., 2008), Catharanthus roseus (Nayak et al., 2006), Lycopodium serratum (Manjunatha et al., 2007), Radix paeoniae (Malviya et al., 2009), Morindaci trifolia (Nayak et al., 2009), Termina liabellirica (Choudhary et al., 2008).
2.2.2 Antioxidant Activity:

a) In vitro antioxidant activity:

Oxidation involves the transfer of electrons from one atom to another. The oxidized molecule loses an electron while the receiving molecule is reduced. Oxidation reactions are an essential part of aerobic metabolism, since oxygen is an electron acceptor in the electron flow system that produces energy (Lee et al., 2003). Oxidation becomes a problem when reactions become uncoupled and free radicals are formed. Free radicals are molecules that are highly reactive and unstable because they contain an unpaired electron. Electrons are most stable in pairs, hence the free radicals tend to attach to or receive hydrogen ions from molecules with lower bond dissociation energy like unsaturated fatty acids or phenolic antioxidants. Reactive oxygen species (ROS) are oxygen-centered free radicals. Examples of the ROS species are superoxides \((O_2^-)\), peroxyls \((ROO^+)\), alkoxyls \((RO^+)\), hydroxyls \((HO^+)\) and nitric oxides \((NO^+)\) (Pietta, 2000). Some of the ROS, such as singlet oxygen \((O_2)\), hydrogen peroxide \((H_2O_2)\), and hypochlorous acid \((HOCl)\) do not contain free radicals and are classified as non-radical ROS species. The ROS may react in various environments.

• Methods for measuring oxidation:

There are many ways to measure the extent of oxidation in an oxidized system. Oxidation can be evaluated by estimating the 1, 1-Diphenyl 2-Picryl Hydrazyl (DPPH) radical scavenging activity. This assay provides information on the reactivity of compounds with a stable free radical. Because of the odd
electron, DPPH shows a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes and the resulting decolourisation is stochiometric with respect to the number of electrons taken up (Shirwaikar et al., 2006).

Superoxide anion (O₂) is a reactive free radical produced naturally in the body. It is the key molecule initiating the propagation of other ROS production. Several systems can generate superoxide anion including mitochondria respiration, cytochrome P₄₅₀ 2E1, redox cycling drugs, iron overload, oxidases, and inflammatory factors. The produced superoxide anion is rapidly converted into other kinds of free radicals e.g. hydrogen peroxide (H₂O₂) and hydroxyl radical (OH). These radicals can trigger activation of transcription factors leading to gene regulation.

- **Role of Antioxidants:**

In order to maintain freshness in foods and lengthen their shelf-life, antioxidants are often employed to prevent or delay lipid oxidation. There are two primary mechanisms by which antioxidants can reduce autoxidation. One mechanism is to hinder the development of free radicals at the initiation step, while the other is to stop propagation by quenching free radicals. In most of the food products, the latter method is used, the antioxidant acting either as a hydrogen donor or free radical acceptor (Nawar, 1996). There are many synthetic and natural antioxidants used in foods, most of which are phenolic
compounds that can easily donate an H⁺ to quench the lipid radical. Some of the natural antioxidants currently used which includes ascorbic acid, citric acid and α-tocopherol. Some synthetic antioxidants that are commonly used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) (Nawar, 1996).

- **Plants as potent antioxidants:**

  Several investigators have worked on medicinal plants which possess potent antioxidant activity *viz.*, *Rubus idaeus, Rubus occidentalis*, and *Fragaria ananassa* (Shiow and Hsin-Shan, 2000); *Cordyceps sinensis* (Li et al., 2001); *Emblica officinalis* (Kaur and Kapoor, 2002); *Morinda officinalis* (Soon and Tan, 2002); *Satureja hortensis* (Güllüce et al., 2003); *Allium cepa, Illicium religiosum, Fagopyrum esculentum, Origanum officinalis, Rosmarinus officinalis, Pyrus pyrifolia, Acanthopanax senticosus, Eugenia caryophyllata* and *Erigeron annuus* (Young and Kyong, 2003); *Ardisia compressa* (Sonia and de Mejia, 2004); *Theobroma cacao* (Osman et al., 2004); *Aframomum danielli, Allium cepa, Allium sativa, Capsicum frutescens, Citrus sinensis, Curcuma longa, Justicia flava, Ocimum gratissimum, Piper guineense* (Odukoya et al., 2005); *Fagopyrum esculentum* (Ting and Chi-Tang, 2005); *Rhodiola sacra, Polygonum multiflorum* and *P. multiflorum* (Chi-Chun et al., 2006); *Garcinia mangostana* (Weechargasen et al., 2006); *Gentiana lutea* (Kussar and Zupaneie et al., 2006); *Lycium barbarum* (Li et al., 2007); *Ocimum basilicum* (Ihami et al., 2007) and *Zanthoxylum piperitum* (Yamazaki et al., 2007), *Vigna*
unguiculata (Siddhurst et al., 2007), Lamiaceae herbal extracts (Lopez et al., 2007), Stevia rebaudiana (Tadhani et al., 2007), Mangifera indica (Ajila et al., 2008), Ligustrum vulgare (Agati et al., 2009), Populus tremuloides Michx (Diouf et al., 2009), Casuarina equisetifolia (Zhang et al., 2010); Carissa spinarum (Hegde and Joshi, 2010), Pithecellobium dulce (Megala et al., 2010), Etlingera genus (Chan et al., 2011).

b) In vivo antioxidant activity:

Carbon tetrachloride (CCL₄) is widely used in animal models to induce acute liver injury (Mizuoka et al., 1999; Rao et al., 1997; Czaja et al., 1995). It is generally believed that the toxicity of CCL₄ results from its reductive dehalogenation by the cytochrome P₄₅₀ enzyme system into the highly reactive free radical trichloromethyl radical (Recknagel et al., 1989). Liver fibrosis induced by the CCL₄ leads to the impairment in hepatocellular functions this in turn causes obstruction in detoxification mechanism leads to the clinical conditions such as hyperbilirubinemia, hypoproteinaemia etc.

Oxidative stress contributed to the transition of non-fatal hepatic steatosis to steatohepatitis with progressive fibrosis (Starkel et al., 2003). The review of oxidative stress plays an important role in different types of liver injuries (Choi and Ou, 2006; Chowdhury et al., 2006; Wheeler et al., 2001b) and contributed to the transition of non-fatal hepatic steatosis to steatohepatitis with progressive fibrosis (Starkel et al., 2003).

Although, serum AST, ALT and ALP activities are used as indicators for liver injury, the elevations of these enzymes in the serum are not specific to
liver damage. For example, elevated serum AST activity has also been observed during myocardial damage (Nekrassova, 1963). Hence, an additional parameter is required to confirm the presence of liver injury. Several studies have demonstrated or suggested that the pathological effects of CCl₄ are mediated by induction of oxidative stress (Cotran et al., 1994; Kaplowitz et al., 1986). Among the various mechanisms involved in hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generation (DeLeve and Kaplowitz, 1995; Farrel, 1998) and antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous substances. They can react with reactive lipids including cholesterol, unsaturated fatty acids, and glycolipids, leading to lipid peroxidation (Gioratti, 1998). Lipid peroxides are commonly found in oxidative stress-mediated liver injury (Jayatilleke and Shaw, 1998; Sadrzadeh et al., 1994). In order to probe the possible mechanism by which active constituents prevent hepatic damage caused by CCl₄ and to examine the presence of oxidative stress in CCl₄ treated rat livers, investigation on lipid peroxidation and glutathione were carried in the liver homogenate. CCl₄ is capable of generating highly reactive free radicals, inhibiting glutathione (GSH) synthesis, producing glutathione loss from the tissue, increasing malondialdehyde (MDA) levels and impairing antioxidant defense systems in humans and experimental animals.

In our laboratory, many investigators evaluated the in vivo antioxidant property of the medicinal plants of the western ghats such as, Clerodendrum serratum (Vidya et al., 2007) etc.,
2.2.3 Antidiabetic activity:

Numerous animal models have been developed for the past few decades for studying diabetes mellitus and testing anti-diabetic agents that include chemical, surgical and genetic manipulations (Srinivasan et al., 2007). One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan (Etuk, 2010). It is a well-known diabetogenic agent that is used to induce Type I diabetes in experimental animals (Viana et al., 2004). Alloxan is a urea derivative which causes selective necrosis of the β- cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used (Etuk, 2010). As it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals.

a) Mechanism:

The mechanism of alloxan action has been thoroughly studied which currently can be characterized quite well. Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment (Szkudelski et al., 1998; Iachin and Reza, 2012). This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used (Kliber et al., 1996). Further, the alloxan action in the
pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups (Lenzen et al., 1991; Zhang et al., 1992). Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result of alloxan reduction, dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals (Munday, 1998; Das et al., 2012). The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions (Sakurai and Ogiso, 1995). In addition, superoxide radicals undergo dismutation to yield hydrogen peroxide (H2O2) in the presence of superoxide dismutase. As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of ferrous and H2O2. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. The fragmentation of DNA takes place in the beta cells exposed to alloxan that causes DNA damage, which stimulates poly ADP-ribosylation, a process participating in DNA repair. Antioxidants like superoxide dismutase, catalase and the non enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity (Ebelt et al., 2000). In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an
important step in the diabetogenic action of alloxan. It has been noted that alloxan elevates cytosolic free Ca$^{2+}$ concentration in the beta cells of pancreatic islets (Park et al., 1995). The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca$^{2+}$ ion further contributes to supraphysiologica l insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets (Etuk, 2010; Szkudelski, 2001; Lenzen, 2008).

b) Biological effects:

Alloxan is a hydrophilic and unstable chemical compound which has similar shape as that of glucose, which is responsible for its selective uptake and accumulation by the pancreatic beta cell.41 Similarity in the shape allows it to transports into the cytosol by the glucose transporter (GLUT2) in the plasma membrane of beta cell Gorus et al., 1982; Elsner et al., 2002). Another biological effect of alloxan has been attributed to the thiol group reactivity that allows selective inhibition of glucose-induced insulin secretion through inhibition of glucokinase. This inhibition of glucose-induced insulin secretion has been regarded as the major pathophysiological effect of alloxan, which results from the thiol group reactivity of alloxan. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Dunn et al., 1943; Szkudelski 2001;
Dhanesha et al., 2012). The underlying mechanism involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells (Lenzen, 2008; Viswanathaswamy et al., 2001).

There are number of different types of diabetes drugs are available in market with having similar ways of acting. Drugs which act similarly to each other are put into the same class of drugs they are Insulin, Biguanides/Metformin, Sulphonylureas, Meglitinides/Prandial glucose regulator/Glinides, Alpha-glucosidase inhibitors, Thiazolidinedione/ Glitazones, DPP-4 inhibitors/Gliptins, Incretin mimetics/GLP-1 analogues, Amylin analogues (Ogbonnia et al., 2008).

c) Plants having antidiabetic property:

Arul et al. (2004) investigated the effect of ethanolic extract from dried nuts of Semecarpus anacardium on both normal and streptozotocin-induced diabetic (antihyperglycemic) rats. The ethanolic extract of S. anacardium (100 mg/kg) reduced the blood glucose level significantly after the streptozotocin treatment and antihyperglycemic activity of S. anacardium was compared with tolbutamide, a sulfonyl urea derivative used in diabetes mellitus. Ali et al. (2012) evaluated the antidiabetic effect of ethanol extract of stem bark of Semecarpus anacardium was by measuring fasting blood glucose level and oral glucose tolerance test (OGTT) in normal and alloxan induced diabetes animal
models. The experimental animal study indicates that *S. anacardium* stem-bark ethanolic extract possesses antidiabetic properties.

2.2.4 Antipyretic activity:

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body’s natural function to create an environment where infectious agents or damaged tissues cannot survive (Chattopadhyay *et al.*, 2005).

a) The pathogenesis of fever:

The critical “endogenous pyrogens” involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to effect a fever response (Luheshi, 1998). Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacterial outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1b (14).

These signals trigger the release of other mediators, most notably prostaglandin E2 (PGE2), in the region of the POAH (Saper *et al.*, 1980). PGE2 is believed to be the proximal mediator of the febrile response. Preoptic neurons bearing E-prostanoid receptors alter their intrinsic firing rate in response to PGE2, evoking an elevation in the thermoregulatory set point.
There are four known cellular receptors for PGE2: EP1 through EP4 (Ushikubi et al., 1998). The particular receptor subtype involved in pyrogenesis is unknown. Although mice lacking the neuronal PGE2 receptor subtype EP3 demonstrate an impaired febrile response to both exogenous (endotoxin) and endogenous yrogens (Ushikubi et al., 1998), studies in rats appear to implicate the EP4 receptor (Oka et al., 2000).

The intracellular events riggering pyrexia after PGE2-EP receptor coupling among species are unclear. Fever is tightly regulated by the immune response. Inflammatory stimuli triggering the generation of propyretic messages provoke the release of endogenous antipyretic substances (Kluger et al., 1998). Substances such as arginine vasopressin (AVP), α-melanocyte stimulating hormone, and glucocorticoids act both centrally and peripherally to limit pyrexia (Kluger et al., 1998). The cytokine interleukin-10 (IL-10) has numerous anti-inflammatory properties, including fever suppression (Pajkrt et al., 1997; Leon et al., 1999). In addition, a class of lipid compounds known as epoxyeicosanoids generated by certain cytochrome P-450 enzymes plays an important role in limiting the fever and inflammation (Kozak et al., 2000a, 2000b). Analogous to a biochemical feedback pathway, fever itself appears capable of countering the release of pyrogenic cytokines (Jiang et al., 1999; Jiang et al., 2000). For example, febrile temperatures augment early TNF release in endotoxin-challenged mice, yet limit its prolonged (and perhaps detrimental) expression after either lipopolysaccharide injection or bacterial infection (Jiang et al., 1999; Jiang et al., 2000).
Many medications which have antipyretic effects are useful for fever including Ibuprofen, Naproxen sodium, Ketoprofen, Aspirin, Choline salicylate, Magnesium salicylate, Sodium salicylate. Paracetamol, Metamizole, Nabumetone, Nimesulide, Phenazone and Quinine. But, these drugs have some side effects. Hence, natural antipyretic agent with reduced or no toxicity is essential.

b) Evaluation of antipyretic properties of plants:

Madhavan et al. (2010) studied the two botanical sources of Murva, viz. Wattakaka volubilis and Maerua oblongifolia, for antipyretic activity by yeast-induced pyrexia in Wistar albino rats. Alcohol and aqueous extracts of both species significantly reduced the elevated rectal temperature in febrile rats within 30 min of their administration. The results of these studies support the traditional use of these two botanical sources of the drug Murva in the treatment of fever. Rajesh Gupta et al. (2012) reported the in vivo evaluation of antipyretic activity of methanol extract on rats by using yeast-induced pyrexia method. The effect of methanol extract of Bark shows that different dose caused lowering of the body temperature up to 2h following its administration. Similarly, antipyretic properties have been investigated on many plant species such as, Cocculus hirsutus (Jain et al., 2007), Dalbergia sissooleaves (Hajare et al., 2000), Buddleia cordata (Martinez-Vazquez et al., 1996), Mezoneuron benthamianum (Mbagwu et al., 2007), Nigella sativa (Al-Ghamdi, 2001), Enicostema littorale (Jude et al., 2012), Cuscuta reflexa (Sanjib Bhattacharya, 2010).
2.2.5 Analgesic Activity:

Pain is part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. This concept involves 2 components, nociception and perception. Pain perception is an integrative function modulated by emotional, motivational, psychological conditions and individual’s past history. Nociception or the nociceptive sensation results from the activation of specific primary sensory neuron subpopulations that transmit the nociceptive information to the spinal cord from where it is relayed to supra spinal levels (Verri et al., 2006; Millan et al., 1999). In a general manner, there are four types of pain:

- Nociceptive pain, due to excessive stimulation of nociceptors localized in the skin, viscera, and other organs.
- Neurogenic pain, pain reflecting damage to neuronal tissue in the periphery or CNS.
- Neuropathic pain, due to a dysfunction of, or damage to, a nerve or group of nerves.
- Psychogenic pain, not due to an identifiable, somatic origin and which may reflect psychological factors.

a) Mechanism :

Pain is usually elicited by the activation of specific nociceptors (nociceptive pain). However, it may also result from injury to sensory fibres or from damage to the CNS itself (neuropathic pain) (Calixto et al., 2000). In
addition, some disorders commonly occur in patients who experience pain such as hyperalgesia (extreme sensitivity to pain stimuli), allodynia (pain in response to a nonnoxious mechanical stimuli), and hyperesthesia (abnormal sensitivity to sensory stimuli) (Besson, 1999). Nociception is a mechanism by which noxious stimuli are transmitted to the central nervous system (CNS) (Furst, 1999). The nociceptors are pain-sensitive neurones located in the skin, vessels, muscles, fascia, joints and viscera. They are predominantly myelinated (Ad) or non-myelinated (C) fibres activated by noxious stimuli (mechanical, heat, cold and chemical) that conduct these signals to the CNS (Grubb, 1998; Russo and Brose, 1998; Besson, 1999; Furst, 1999; Millan, 1999).

The molecular complexity of the primary afferent nociceptor is illustrated by its response to inflammatory mediators released at the site of tissue injury. Some of the main components of the ‘inflammatory soup’ are shown, including peptides (bradykinin), lipids (prostaglandins), neurotransmitters (serotonin (5-HT) and ATP) and neurotrophins (NGF). The acidic nature of the inflammatory soup is also indicated. Each of these factors sensitize (lower the threshold) or excite the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Examples of these factors and representative molecular targets are indicated in the box. Activation of the nociceptor not only transmits afferent messages to the spinal cord dorsal horn (and from there to the brain), but also initiates the process of neurogenic inflammation. This is an efferent function of the nociceptor whereby release of neurotransmitters, notably substance P and calcitonin gene
related peptide (CGRP), from the peripheral terminal induces vasodilation and plasma extravasation (leakage of proteins and fluid from postcapillary venules), as well as activation of many non-neuronal cells, including mast cells and neutrophils. These cells in turn contribute additional elements to the inflammatory soup.

The neurotransmitters involved in this descending noxious inhibition (such as endogenous opioids, serotonin, noradrenaline) all seem to inhibit the firing of the second-order neurones in the presence of noxious stimuli (Russo and Brose, 1998; Furst, 1999; Millan, 1999). However, nociception is not a uniform sensation, and both the quality of pain and the initiation of protective responses are determined by many factors within the spinal cord and in higher brain structures involved in the integration and modification of nociceptive signals.

Many drugs used to relieve the pain and a few drugs like morphine (Brune, 1990) and aspirin (Willete et al., 1987) have been significantly used for the last three decades. Most of the pain-relieving chemicals produced pronounced side-effects on the physiology of the body and also most reported analgesic drugs produce their effects by modulating the release of endogenous analgesic mediators or inhibiting algogenic neurotransmitters, through either pre- or post-synaptic mechanisms at central and peripheral levels (Dray, 1997; Grubb, 1998; Sawynok, 1998; Besson, 1999; Furst, 1999; Millan, 1999; Urban and Gebhart, 1999).
b) Analgesics activity of plants:

2.2.6 Anticonvulsant activity:

Epilepsy is a one of the serious neurological disorders characterized by paroxysmal dysrhythmia, seizure or excessive discharge of cerebral neurons (Sudha et al., 2005). Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000 (Fisher et al., 2005; Poole et al., 2000).

Around 75-80% of epileptic patients may be provided with adequate seizure control with the help of antiepileptic drugs such as Carbamazepine, ethosuximide, phenobarbital, phenytoin, and valproate. Deckers et al. (2000) proposed a classification of antiepileptic drugs based upon mechanisms.

- First group consists of antiepileptics (Carbamazepine, Gabapentin, Lamotrigine, Oxcarbazepine, Phenobarbital, Phenytoin, Topiramate, Valproate) which block sustained repetitive firing in individual neurons, this effect being mainly due to the blockage of voltage-dependent sodium or calcium channels. These drugs are effective against generalized tonic-clonic and partial seizures.

- The second group includes drugs enhancing inhibitory events mediated by gamma aminobutyric acid (GABA): benzodiazepines, gabapentin, phenobarbital, tiagabine, topiramate, vigabatrin, and valproate.

- The Third group consists of Ethosuximide and Zonisamide which attenuate the glutamate-mediated excitatory neurotransmission. (Sobieszek et al., 2003).
A separate category of drugs may be also suggested – these antiepileptic drugs reduce events mediated by excitatory amino acids (glutamate) and at present three antiepileptics meet these criteria: felbamate, phenobarbital, and topiramate (Deckers et al., 2000).

a) **Excitatory neurotransmission:**

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following release from glutamatergic nerve terminals, it exerts its effects on three specific subtypes of ionotropic receptor in the postsynaptic membrane, designated according to their agonist specificities; AMPA, kainate and NMDA. These receptors respond to glutamate binding by increasing cation conductance resulting in neuronal depolarization or excitation. The AMPA and kainate receptor subtypes are permeable to sodium ions and are involved in fast excitatory synaptic transmission. In contrast, the NMDA receptor is permeable to both sodium and calcium ions and, owing to a voltage-dependent blockade by magnesium ions at resting membrane potential, is only activated during periods of prolonged depolarisation, as might be expected during epileptiform discharges. Metabotropic glutamate receptors perform a similar function to GABAB receptors; they are G-protein coupled and act predominantly as autoreceptors on glutamatergic terminals, limiting glutamate release. Glutamate is removed from the synapse into nerve terminals and glial cells by a family of specific sodium-dependent transport proteins (EAAT1–EAAT5) and is inactivated by the enzymes glutamine synthetase (glial cells only) and glutamate dehydrogenase.
b) Anticonvulsant activity of plants:

infortunatum (Das et al., 2010), Clinopodium mexicanum (Estrada-Reyes et al., 2010),

Basavaraj et al. (2011) evaluated the anticonvulsant activity of Semecarpus anacardium (LNN.) nut extract (Petroleum ether, Chloroform and Alcohol) using maximal electroshock (MES)-induced convulsion (MESIC), pentalenetetrazol (PTZ)-induced convulsion (PTZIC), strychnine-induced convulsion (SIC), picrotoxin-induced convulsion (PIC), isoniazid (INH)-induced convulsion (IIC) and 4-amino pyridine (4-AP)-induced convulsion (4-APIC) in mice. The results suggest that significant reduction in locomotor scores were recorded with a diazepam (5 mg/kg) but not with CHSA (Chloroform extract of S. anacardium) (100, 200 & 400 mg/kg). In MESIC, a phenytoin (25 mg/kg) was possessed anticonvulsant activity by decreased duration of tonic extension phase of the animals but not with CHSA. In PTZIC, SIC PIC, IIC and 4-APMIC models, a diazepam exhibited anticonvulsant effect, the CHSA was ineffective among all those paradigms. Increased GABA level in the mice serum was observed with a standard drug, gabapentin (20 mg/kg) treated group, in CHSA such effect was not observed. Hence it concluded that Semecarpus anacardium (Linn.) not possesses sedative and anticonvulsant properties.

2.2.7 Evaluation of antimicrobial activity:

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections (Service, 1995), the need to discover new antimicrobial substances for use in
combating such microorganisms become pertinent. Thus there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). Plant based natural constituents can be derived from part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, etc (Gordon and David, 2001); i.e. any part of the plant may contain active components. Infections due to multidrug-resistant gram-negative microorganisms pose an important clinical problem, resulting in significant morbidity and mortality worldwide (Carmeli et al., 1999; Hsueh et al., 2002; Livermore, 2002).

The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time (Weisser et al., 1966). Extra-chromosomal genes were found responsible for these antimicrobial resistant phenotypes that may impart resistance to an entire antimicrobial class. These resistant genes have been associated with plasmids which are large, transferable, extra-chromosomal DNA elements. Other DNA mobile elements, such as transposons and integrons, are present on plasmids. These DNA mobile elements transmit genetic determinants for antimicrobial resistance mechanisms and may cause rapid dissemination of resistance genes among different bacteria (McDermott et al., 2002). The emergence of multiresistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefsen and Miller, 2000).
a) Microbial pathogens selected in this study:

*Pseudomonas aeruginosa* and *Klebsiella pneumonia* are the important gram-negative human pathogenic bacteria that cause nosocomial infections. In particular, *P. aeruginosa* is a predominant respiratory pathogen among cystic fibrosis patients producing chronic pulmonary infection and progressive deterioration in lung infection (Van Delden, 2004; Van Delden and Iglewski, 2005). The *Pseudomonas aeruginosa* has been implicated in infections of respiratory and urinary tract, supportive infections in sinuses and middle ear, septicemia, etc., whereas *Klebsiella pneumonia* has been associated with a range of clinical conditions which include, infections of wounds, infections of urinary tract and eyes, septicemia etc. and Gram-positive *Staphylococcus aureus* has been implicated in Abscess in immunodeficiency, Septicemia Food poisoning patients and five clinically isolated pathogenic fungi such as *Aspergillus niger, Aspergillus fumigates, Aspergillus flavus and Candida albicans* were implicated in infections of Cutaneous mycoses, Scarring of the scalp, Ringworm infections and Opportunistic mycoses candidosis.

b) Antibiotic property of the medicinal plants:

Antibiotic resistance of *P. aeruginosa* is caused by environmental factors such as exposure to subinhibitory concentrations of antibiotics or limiting concentrations of divalent cations (Gunn *et al.*, 1998; Livermore, 2002). Several investigators has worked on antimicrobial activity using medicinal plant extracts viz., *Eupatorium perfoliatum* (Habtemariam and Maepherson, 2000); *Mezoneuron benthamianum* (Binetu and Cordell, 2000);
Pterocarpus osun (Ebi and Ofoefule, 2000); Dichrostachys cinerea (Eisa, 2000); Enantia polycarpa (Ajali, 2000); Ricinus communis (Parameswari and Tulasi Latha, 2001); Drynaria quercifolia (Ramesh et al., 2001); Eupatorium cannabinum (Senatore et al., 2001); Bixa orellana (Castello et al., 2002); Datura alba (Priya et al., 2002); Feronia limonia (Mukhlesur and Alexander, 2002); Aerva lanata (Chowdhury et al., 2002); Embelia ribes (Chitra et al., 2003); Crotalaria pallida (Muthusamy et al., 2003); Cocos nucifera (Srinivas et al., 2003); Artemisia douglasiana (Setzer et al., 2004); Melissa officinalis (Mimica-Dukie, 2004); Eupatorium glandulosum (Sasikumar et al., 2005); Quercus infectoria (Basri and Fan, 2005); Vigna angularis (Hori et al., 2006); Bauhinia variegata and Plumbago zeylanica (Jigna et al., 2006); Mentha longifolia, Melissa officinalis and Rosa damascene (Bassam et al., 2006); Cleome rutidosperma (Bose et al., 2007); Combretum molle, Peltophorum africanum, Piper capense, Terminalia sericea and Zanthoxylum davyi (Steenkamp et al., 2007).

The plants possess innumerable number of secondary metabolites which are usually produced under stress conditions and often in response to infections. These secondary metabolites possess profound antimicrobial potency. Many workers have isolated different types of active constituents and studied for their antimicrobial potency. Alkaloids (Burdick, 1971); phenolic compounds (Mason and Wasserman, 1987); tannins (Scalbert, 1991); flavanones and flavonoids (Panilio et. al., 1992); sesquiterpenes (Topcu et. al., 1993); anthroquinone (Kazmi, 1994); flavonoid glycosides (Hasan and Ahmad,
1996); triterpene acid glycosides (Kirmizigul et. al., 1996); monoterpenes (Meng et. al., 2000); diterpenes (Ulubelen et. al., 2000); triterpenes (Akbar and Malik, 2002). These active constituents isolated from medicinal plants showed significant antibacterial effect.

Nair et al. (1996) reported that the alcoholic extract of dry nuts of Semecarpus anacardium showed bactericidal activity in vitro against three gram negative strains (Escherichia coli, Salmonella typhi and Proteus vulgaris) and two gram positive strains (Staphylococcus aureus and Corynebacterium diphtheriae). Subsequent studies have shown that the alcoholic extracts of different parts of the plant (leaves, twigs and green fruit) also possess antibacterial properties, especially the leaf extract.

Mohanta et al. (2007) reported that the antimicrobial activity (disc diffusion method) of Semecarpus anacardium with different extract. The petroleum ether and aqueous extract fractions of Semecarpus anacardium showed inhibitory activity against Staphylococcus aureus (10 mm) and Shigella flexneri (16 mm) at 100 mg/ml concentration. While chloroform extract showed inhibition against Bacillus licheniformis, Vibrio cholerae and Pseudomonas aeruginosa, the ethanol extract showed inhibition to Pseudomonas aeruginosa and S. aureus.

Sharma et al. (2010) investigate that nut oil of Semecarpus anacardium shows significant anti-microbial activity against several Gram positive (Bacillus subtilis, Staphylococcus aureus) and Gram negative (Proteus vulgaris, Escheria coli) Bacteria.
Zabin et al. (2012) The petroleum ether nut extract of *Semecarpus anacardium* exhibited antibacterial property against gram positive bacteria and gram negative bacteria by Agar well method. The nut extract showed inhibitory activity against test organisms like *Escherichia coli* (19 mm), *Micrococcus luteus* (23 mm), *Salmonella typhi* (26 mm), *Bacillus subtilis* (14 mm) and *Klebsiella pneumonia* (22 mm). The antibacterial activity of nut extract of *Semecarpus anacardium* is due to Petroleum Ether extractable compounds.

Sharma et al. (2002) also found that due to presence of flavonoid, alcoholic extract of dry nuts of *Semecarpus anacardium* show antifungal activity (*Aspergillus fumigatus* and *Candida albicans*) at 400 mg/ml concentration. Both the fungi were show inhibition in growth, reduction in size of cells and Sporulation also decreased.

2.2.8 Evaluation of anti cancer activity :

Cancer is a very widespread disease, which is responsible for millions of deaths each year worldwide. Chemotherapy is an essential strategy for the treatment of disseminated cancers. This observation stimulates the search for new anticancer agents, and in this regard, the investigation of naturally originating compounds could be very valuable. However, as a contribution towards the chemotherapy of cancer, plant secondary metabolites in particular play a very important role (Cragg, 1997; Shu, 1998; Cragg, 2001; Newman, 2000). After several plant-derived natural products demonstrated prominent anticancer activity in patients with advanced malignancies in the 1950s and 1960s, the microtubule was recognized as a subcellular target of major strategic
importance with regard to anticancer therapeutics. The first widely used class of antimicrotubule agents, the Vinca alkaloids (Cragg, 2001), has been the mainstay of both palliative and curative regimens for treating both hematopoietic and lymphoid malignancies for several decades. More recently, a large number of plant derived compounds such as the epothilones, camptothecin and discodermolide have been identified, with yet even more distinctive antimicrotubule and anticancer activities (Newman, 2000). Camptothecin isolated from the wood and bark of a Chinese tree, *Camptotheca acuminata* by Wall and Wani in 1966 (Tang, 1992). It is a pyrrolo [3,4-b]-quinoline alkaloid that was extracted using ethanol from the stem-wood of the plant (Ghisalberti, 1993). They stabilize topoisomerase-DNA cleavable complexes by hindering the DNA relegating step of the catalytic reaction (Wall, 1966; Giovanella, 1989), thus resulting in DNA cleavage stimulation, which lead to apoptosis (Binaschi, 1995). Topotecan and irinotecan are the two synthetic analogs of Camptothecin (Grabley, 1999) which shows activity towards ovarian cancer (topotecan) and colorectal cancer (irinotecan) (Creemers, 1996; Bertino, 1997). Flavopiridol is a flavone inhibitor of the cyclin-dependent kinase (CDK) family that was semi-synthesized from rohitukine, a plant derived natural product (da Rocha, 2001). It appears to be non-selective towards any particular CDK. The drug is in the early stages of clinical trials, but it is creating excitement because of its interesting mechanism of action (Newman, 2000). The progression of the cell cycle is blocked during stages of growth after the compound interferes with the kinase phosphorylation
step (Kelland, 2000) which ultimately causes cell death. Homoharringtonine obtained from the seeds of a Chinese evergreen (*Cephalotaxus harringtonia*) widely used in China for traditional medicine and known for efficiency as a cytotoxic anti-leukemia drug (Powell, 1970; Warber, 1999). This drug was a product of discovery through an extensive research program carried out by the National Cancer Institute in the 1960s, and in 1993, it was classified as one of the NCI's investigational new drugs (Warber, 1999; Cordell, 1993). Homoharringtonine is thought to function during the cell cycle when proteins are being elongated by peptidyl transferase (Warber, 1999). This interruption of protein synthesis leads to apoptosis and differentiation of cancer cells (Warber, 1999; Zhou, 1995). A non-alkaloid bioactive compound from different plant species like *Podophyllum* and *Juniperus* that deserves some attention is podophyllotoxin. It is isolated from the roots and identified as an antitumor dimeric lignan in 1880 (Kuo, 2001). The epimer of podophyllotoxin is epipodophyllotoxin, giving rise to two semi-synthetic compounds with high activities and clinical applications, etoposide and teniposide (Williams, 1987). Like many anticancer drugs, etoposide functions by inhibiting topoisomerase II (Liu, 1989).

a) *In vitro* cytotoxicic property of medicinal plants:

*In vitro* cytotoxicity was screened against three human cancer cell lines and one normal cell line (lung carcinoma cell line COR-L23, the human breast adenocarcinoma cell line MCF-7 and human colon adenocarcinoma cell line LS-174T and normal human keratinocytes SVK-14) of 11 Thai medicinal plant
species (Arunporn Itharat et al., 2004), 76 ethanolic extracts of medicinal herbs from the Jordanian flora, belonging to 67 species and 34 families, were evaluated for their antiproliferative activity on a breast cancer cell line (MCF7). (Rana Abu-Dahab and Fatma Afifi, 2007), In vitro cytotoxic activity of Lantana camara Linn was screened by Raghu et al., 2004. The in vitro antiproliferative activity of crude methanol extracts of three traditional Korean medicinal plants: Achyranthes fauriei, Epimedium koreanum Nakai and Scutellaria baicalensis were screened on four human cancer cell lines: lung cancer cells (Lu1), colon cancer cells (Col2), oral epidermoid carcinomas (KB) and hormone-dependent prostate cancer cells (LNCaP). (Hye Hyun Yoo et al., 2007). Anthrapyrazoles are potent cytotoxic agents that intercalate into DNA, causing DNA strand breaks, inhibition of DNA synthesis and topoisomerase II was investigated the in vitro cytotoxic activity of two anthrapyrazole analogues (AP-10 and AP-11) in human prostate (DU-145) and testicular (NTERA-2) carcinoma cells (Cuevas and Seilheimer, 2008).

b) In vitro cytotoxicicitic property reported on Semecarpus ancardium:

Mathivadhani et al., (2007) reported the Semecarpus ancardium nut extract for inhibitory effect on human breast cancer cells (T47D). Cytotoxicity analyses suggested that these cells had become apoptotic. Semecarpus anacardium was discovered to induce rapid Ca (2+) mobilization from intracellular stores of T47D cell line, and its cytotoxicity against T47D was well correlated with altered mitochondrial transmembrane potential. At the molecular level, these changes are accompanied by decrease in Bcl(2) and
increase in Bax, cytochrome c, caspases and PARP cleavage, and ultimately by internucleosomal DNA fragmentation. Taken together, the results provide unprecedented evidence that Semecarpus ancardium triggers apoptotic signals in T47D cells.

Sugapriya et al., (2008) showed restoration of energy metabolism in leukemic mice treated by Semecarpus ancardium nut milk extract. Leukemia-bearing mice showed a significant increase in LPOs, glycolytic enzymes, a decrease in gluconeogenic enzymes and significant decrease in the activities of TCA cycle and respiratory chain enzymes as compared to control animals. Semecarpus ancardium treatment was compared with standard drug imatinibmesylate. Semecarpus ancardium administration to leukemic animals resulted in clearance of the leukemic cells from the bone marrow and internal organs.

Prabhu et al., (2005) studied the antimutagenic effect of Semecarpus ancardium under in vivo condition. Mice were intraperitoneally treated with 500 and 250 mg/kg of Semecarpus ancardium, which showed a significant inhibition of induced aberrations at the 12 h pretreatment period. The results on the reduction of induced chromosome aberrations clearly show that Semecarpus ancardium serves as an antioxidant because of the presence of flavonoids which scavenge free radicals. The action of Semecarpus ancardium oil extract has definite beneficial role against mitomycin-C induced mutagenicity and its administration may be protective and therapeutic. Krishnarajua et al., (2005) found that aqueous extracts of medicinal plants were
screened for their cytotoxicity using brine shrimp lethality test. Out of the 120 plants tested, *Semecarpus ancardium* (Anacardiaceae) showed significant cytotoxicity with LC50 29.5 μg, respectively.

The present investigation was undertaken because, so far, no systematic study has been reported regarding the cytotoxic activity of *S. ancardium stem bark*. In the present study, an effort has been made to screen *in vitro* cytotoxic activity of extracts and isolated constituents of *S. ancardium* stem bark using sulforhodamine- B (SRB) assay.

c) **Pharmacological property of Amentoflavone**:

The Pharmacological properties of amentoflavone have been reported for anti-inflammatory, anti-ulcerogenic activities (Gambhir *et al.*, 1987). Kamil *et al.* (1987) reported the isolation of 7"-O-Methyl tetrahydro amentoflavone, together with 7"-O-methyl ochnaflavone, ochnaflavone and tetrahydroamentoflavone, from the leaves of Ochna pumila. The isolation of tetrahydroamentoflavone and 7"-O-methyl tetrahydro amentoflavone from *O. pumila* constitutes the first report of their occurrence as a new series of biflavanones.

Hee Kee Kim *et al.* (1998) reported that amentoflavone possess a potent anti-inflammatory activity by intraperitoneal injection on animal models of acute inflammation. Nahrstedt *et al.* (1997) reported principle biflavanoid constituents namely, quercetin, quercitrin, amentoflavone from *Hypericum perforatum*. 
Nielsen et al. (1988) and Baureithel et al. (1997) focused their investigations on the biflavone amentoflavone, which bound to the brain benzodiazepine receptors with an affinity comparable to diazepam. Lin et al. (1999) reported that the five groups of biflavonoids (amentoflavone, agathisflavone, robustaflavone, rhusflavanone and succedaneflavanone) were isolated from medicinal plants of Rhus succedanea and Garcinia multiflora, and exhibited various antiviral effects against a number of viruses including respiratory viruses (influenza A, influenza B, parainfluenza type 3, RSV, adenovirus type 5 and measles) and herpes viruses (HSV-1, HSV-2, HCMV and varicella zoster virus, VZV). Krazu-Baranowska et al. (1999) reported the antifungal activity of amentoflavone from three different plants, including Cupressocyparis leylandii, Taxus baccata, and Ginkgo biloba, and it exhibited antifungal activity against Altemana altemate, Cladosporium oxysporum, Fusarium culmorum, and Fusarium avenaceum. Ma et al. (2001) reported the amentoflavone and three other flavonoids were isolated from the ethanol extract of Selaginella sinensis. Amentoflavone showed potent antiviral activity against respiratory syncytial virus (RSV), with an IC50 of 5.5 µg/ml. The contents of amentoflavone in nine species of Selaginella were determined by reversed-phase HPLC. Goutam Brahmachari (2001) reported the flavonoid compounds namely, luteolin 7-O-glucoside, luteolin and amentoflavone and evaluated their effect in reducing the blood glucose levels by inhibiting the absorption of glucose in intestinal brush border or flavonoids may act on
tyrosine phosphorylation of the insulin receptor and insulin receptor substrate to mimic the insulin.

Amentoflavone had been reported for antiviral activity against influenza, herpes, and respiratory syncytial virus (RSV) (Lin et al., 1999; Ma et al., 2001). Matsuoka et al. (2002) reported the antifungal effect of amentoflavone against Candida albicans. The growth of C. albicans cells which causes candidiasis was also inhibited by amentoflavone. It also demonstrates that amentoflavone can serve as an antifungal agent against human infectious fungi namely, Aspergillus flavus which causes aspergillosis. Uddin et al. (2004) reported the amentoflavone, in the presence of copper ions, can damage DNA and give rise to reactive oxygen species. Cardoso et al. (2006) reported the aglycone quercetin and amentoflavone were mutagenic to strain TA98, with and without metabolic activation.

Xulin Pan et al. (2005) reported the structure–activity relationship (SAR) and binding mechanism of three biflavones, amentoflavone (AMF1), 4000-methylamentoflavone (AMF2) and 700, 4000-dimethylamentoflavone (AMF3), isolated from Taxodium mucronatum by us as novel natural inhibitors of human CatB with strong inhibitory activities at IC50 values of 1.75, 1.68 and 0.55 μM, respectively.

Jung et al. (2006) reported the cytotoxic effect against the human tumor cell line. Amentoflavone can also promote mutagenic activity (Cardoso et al., 2006), inhibit human cathepsin β (Pan et al., 2005), interact at GABA_A receptors (Reena et al., 2005), exhibit strong neuroprotection against cytotoxic
insults induced by oxidative stress and amyloid β (Kang et al., 2005). Cassia et al. (2006) evaluated the putative mutagenic effect of Byrsonima crassa extracts, fractions and isolated compounds (quercetin-3-O-β-d-galactopyranoside, quercetin-3-O-β-l-arabinopyranoside, amentoflavone, methyl gallate and (+)-catechin) from the methanolic extract of the leaves, which are commonly used to treat diarrhoea.

Guruvarapu et al. (2007) investigated of the antimetastatic activity of amentoflavone using B16F-10 melanoma-induced experimental lung metastasis in C57BL/6 mice. Aravind et al. (2008) reported that the quality evaluation and standardization of polyherbal formulations of S. anacardium, an important medicinal plant with wide medicinal properties, is frequently used in a large number of traditional herbal preparations.

Ying Jing et al. (2010) reported the isolation of amentoflavone from the plant of Selaginella tamariscina and determination of in vitro anticancerous activity. The extracts mainly contained biflavonoids. The structure of amentoflavone was elucidated by spectral examinations. Amentoflavone and the extracts were screened against five cancer cells, including HeLa (human cervical carcinoma cells), BEL-7402 (human hepatoma carcinoma cells), MCF-7 (human breast cancer cells), PANC-1 (human pancreatic cancer cells) and HL-60 (human leukemia cells). The anticancer activity were determined by means of MTT assay and Trypan Blue cytometry. Assays in vitro showed that they were effective to inhibit the proliferation of HL-60, MCF-7, HeLa, BEL-7402, PANC-1 and had reliable activity against HL-60.
Siveen et al. (2011) reported the effect of amentoflavone, a biflavanoid isolated from *Biophytum sensitivum*, on cell cycling distribution and apoptosis in B16F-10 melanoma cells. Treatment of B16F-10 melanoma cells with amentoflavone (10 μg/mL) increased cells in the sub-G0/G1 phase accompanied by a decrease in G0/G1 phase cells in a time-dependent manner.

Eunjung Lee et al. (2012) investigated the mechanism of action of amentoflavone in cancer cells and demonstrated that amentoflavone showed strong cytotoxicity against MCF-7 and HeLa cancer cell lines. Their study revealed that hPPARγ expression in MCF-7 and HeLa cells is specifically stimulated by amentoflavone, and suggested that amentoflavone-induced cytotoxic activities which are mediated by activation of hPPARγ in these two cancer cell lines. Moreover, amentoflavone increased PTEN levels in these two cancer cell lines, indicating that the cytotoxic activities of amentoflavone are mediated by increasing of PTEN expression levels due to hPPARγ activation.

Eunjung et al. (2012) reported the anti-inflammatory activity of amentoflavone in LPS-stimulated macrophages and its mode of action were examined. Using LPS-stimulated RAW264.7 macrophage cells.

Ji Hong et al., (2013) reported the antibacterial effects of amentoflavone, ampicillin, cefotaxime, and chloramphenicol, the antimicrobial susceptibility testing was conducted against bacterial strains including *E. faecium*, *S. aureus*, *S. mutans*, *E. coli* O-157, *E. coli*, and *P. aeruginosa*, using the CLSI method. The results showed that amentoflavone, with MIC values of 4-32 μg/ml, had remarkable antibacterial activity against Gram-positive and
Gram-negative bacteria. The bacterial strains showed MIC values ranging from 2-8 μg/ml for ampicillin and cefotaxime, or 2-16 μg/ml for chloramphenicol. Amentoflavone and the antibiotics showed varying antibacterial activities against different bacterial species. Amentoflavone and all of the antibiotics were less effective against *S. mutans* than other bacterial strains. The Gram-positive bacterium *E. faecium* and the Gram-negative bacteria *E. coli O-157* and *P. aeruginosa* were significantly sensitive to cefotaxime. Amentoflavone had equal MIC levels for *E. faecium*, *E. coli O-157*, and *P. aeruginosa*. These results suggested that amentoflavone had potent and similar antibacterial activity as the antibiotics (ampicillin, cefotaxime, and chloramphenicol).