Pharmacognosy

The word "Pharmacognosy" was derived from the Greek words pharmakon (drug) and gnosis (knowledge). The term pharmacognosy was used for the first time by the Austrian physician Schmidt in 1811. A "crude drug" means a dried unprepared natural material of plant, animal or mineral origin, which is used for medicine. The term "Pharmakognosie" and its discipline developed in German speaking areas of Europe - where it is a synonym of "Drogenkunde" ("science of the crude drugs").

Pharmacognosy is basically divided into conventional and modern pharmacognosy. Conventional pharmacognostical study is based on macroscopic, microscopic and quantitative microscopy. Macroscopic characters include shape, size, colour and texture of the drug in crude or powdered form. Microscopic characters include the anatomical details of drug producing plant as seen in transverse, longitudinal sections, maceration study and the size measurement of various types of cells. Quantitative microscopy includes the vein islet number, palisade ratio, stomatal number, stomatal indices and so restricted to leaf drug only.

The modern pharmacognosy utilizes characteristics of analytical, phytochemical and certain physical constant values over the traditional science of taxonomy in plant systematics. Most of the botanical, chemical, physical and microbial techniques employed in pharmacognosy are applicable to the analysis of drugs and therefore, used by public analysts, forensic scientists and quality control chemists associated with industries.
Pharmacognostical studies on some important medicinal plants

The plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. Large numbers of plants are constantly being screened for their possible pharmacognostical characters. The macroscopic and microscopic characters, physical constant values, extractive values, ash values and the behavior of powdered drug on treatment with different chemical reagents were conducted to characterize some pharmacognostical parameters of Curcuma amada rhizome (Chitra and Thoppil, 2002). Pharmacognostical and preliminary phytochemical studies on the fruit wall powder of Mallotus philippensis was reported by Thirumurugan et al. (2008).

The pharmacognostical investigations were carried out on the leaves of Viburnum punctatum by Prabhu et al. (2009). Badmanaban et al. (2009) carried out to provide requisite pharmacognostical details about Lagenaria siceraria leaves. Pharmacognostical evaluation including examinations of macroscopical and microscopical characterization of Euphorbia rothiana was carried out by Rani et al. (2009). Chenthurpandy et al. (2009) investigated the pharmacognostical studies of Hiptage benghalensis leaf. Kalidass et al. (2009a) reported the pharmacognostical studies of the root and stem of Ichnocarpus frutescens. Subramanian et al. (2009) investigated the pharmacognostical studies on the trunk bark of Mitragyna parvifolia. Kalidass et al. (2009b & c) determined the pharmacognostic studies on the whole plant of Asclepias curassavica and Capparis sepiaria.

Marimuthu et al. (2009) reported the pharmaco-chemical characterization of leaves of Saraca asoca, Vitex negundo and the whole plant of Tribulus terrestris.
Thirumurugan et al. (2009) investigated the pharmaco-chemical characterization of whole plant of *Andrographis paniculata*, *Alpinia calcarata* and *Hiptage benghalensis*.

Pharmacognostical parameter of the bark of *Artocarpus hirsutus* was studied by Dibinlal et al. (2010). It was observed that the bark of *A. hirsutus* showed the presence of laticifers, phloem cells and phloem fibres. Rajesh et al. (2010) evaluated the various pharmacognostical procedures in the leaves of *Capparis sepiaria*. Shruthi et al. (2010) reported the pharmacognostical profile of leaves of *Kirganelia reticulata*. Kalidass and Mohan (2010) studied the pharmacognostical and phytochemical investigation of aerial parts of *Gymnema sylvestre*. Tresina et al. (2010) studied the pharmacognostical characterization of leaves of *Cryptolepis buchanani*, *Cylista scariosa* and *Syzygium aromaticum*. Mohan et al. (2010) determined the pharmacognostic and phytochemical investigation of leaf, petiole, stem, peduncle and root of *Elephantopus scaber*.

Amish et al. (2010) reported the pharmacognostical investigation of leaf, stem and root of *Hedyotis puberula*. Rashmi et al. (2010) studied the pharmacognostical and phytochemical properties of aerial parts of *Peristrophe bicalyculata*. Mohan et al. (2010) investigated the pharmacognostical and phytochemical screening of whole plant of *Blepharis maderaspatensis*. Lalitharani et al. (2010) reported the pharmacognostical and phytochemical studies of *Pothos scandens* leaf. Kala et al. (2010) carried out the pharmaco-chemical characterization of *Eugenia singampattiana* leaf.

Mohan kumar et al. (2011) investigated the pharmacognostical studies of the plant *Wedelia chinensis*. Jaliwala et al. (2011) carried out the investigations on bark of *Ficus arnottiana* to establish methods for quality control of drugs, botanical
evaluation which comprises of macroscopic, physicochemical parameters like loss on drying, extractive values and ash values. Amrish and Tarasingh (2011) reported the pharmacognostical evaluation of the crude drug powder of *Albizia odoratissima* bark. Irudayaraj and Johnson (2011) studied the pharmacognostical characters viz., morphological and physicochemical characteristics of three rare medicinally important spleenworts viz. *Asplenium affine*, *Asplenium decrescens* and *Asplenium zenkeranum*. Sutha et al. (2011) reported the pharmaco-chemical characterization like physicochemical constant, fluorescence analysis, preliminary phytochemical analysis, macroscopic and microscopic features of leaf of *Alstonia venenata*.

Pillai et al. (2011) evaluated the toxicity of the methanol leaf extract of a traditionally used plant *Plectranthus amboinicus*. Plant material was analysed for various pharmacognostical parameters as per WHO guidelines i.e., foreign matter, microscopical sections, loss on drying, extractive value, total ash, acid soluble ash, phytochemical analysis and toxicity studies. The pharmacognostical parameters of aerial parts of *Jatropha maheswarii* were carried out by complete botanical evaluation which includes macroscopic, microscopic, physicochemical and phytochemical analysis (Uthayakumari and Sumathy, 2011). Kannan and Babu (2011) described the pharmacognostical characteristics of rhizomes, stem and inflorescence of *Balanophora fungosa*. Kalidass et al. (2011) investigated the pharmacognostical studies on a multipurpose medicinal plant *Leptadenia reticulata*.

Kadam et al. (2012) reported that the microscopic examination of the bark powder of *Mimusops elengi* showed fragments of cork cells, vessels and fibres of various thickness, tannin cells, stone cells, solitary crystals and other cell contents. The pharmacognostic investigations of *Dalbergia sissoo* leaves were carried out in
terms of organoleptic, microscopic and physical parameters. Powder characters such as epidermal cells with rubiaceous stomata, uniseriate trichomes, polygonal parenchyma cells, sclerenchyma fibres, vascular bundles, lignified xylem fibres were noticed in *Dalbergia sissoo* (Rashida *et al*., 2012). Nedelcheva (2012) reported the pharmacognostical profile of *Achillea clypeolata*, including macroscopic and microscopic characteristics.

The fresh leaf and flower were studied for pharmacognostic evaluations including examination of morphological and microscopic characters, determination of ash values and extractive values. The powder microscopy of *Quisquails indica* leaf revealed the presence of fragments of unicellular covering and glandular trichomes, phloem fibres, parenchyma cells, calcium oxalate crystals, numerous xylem vessels of spiral type and epidermal cells with anomocytic stomata (Bairagi *et al*., 2012). Shantha *et al*. (2012) studied the pharmacognostic evaluation on the leaves of *Ximenia americana* include morphological, microscopical characters, powder microscopy and physicochemical characters like ash values and loss on drying. Pharmacognostical evaluation of corm of *Amorphophallus paeonifolius* was investigated by Madhavan *et al*. (2012). Kewatkar (2012) determined the physicochemical constants and preliminary phytochemical screening of different extracts of *Cassia obtusifolia*.

Chothani and Patel (2012) documented the detailed pharmacognostic profile and physicochemical evaluation of leaves of *Gmelina arborea*. The pharmacognostical features of fresh and dried bark of *Toona ciliata* were evaluated by Singh *et al*. (2012). Organoleptic (colour, odour, taste), microscopic (size, shape, texture, fracture) and powder microscopic characteristics evaluations were performed
to establish the qualitative and diagnostic features. The various physicochemical parameters (loss on drying, foreign matter, extractive values and ash values) were also determined for the effective standardization for this plant material.

Agarwal et al. (2013) selected Ocimum tenuiflorum and Ocimum gratissimum for the pharmacognostical studies due to their medicinal importance. Sharma et al. (2013) reported the phytochemical and pharmacognostical study of Pongamia pinnata seed. Chanda (2014) discussed the need and the importance of pharmacognostic study of medicinal plants and evaluated the parameters such as organoleptic characters, macroscopic study, microscopic study, powder study, physicochemical analysis, phytochemical analysis and fluorescence analysis. Sutha et al. (2014) reported the pharmacognostical investigation of leaf and stem of Erythrophalum scandens. Sarada et al. (2014) studied the pharmacognostical characterization of Naringi crenulata bark.

**Phytochemical screening**

**Qualitative and Quantitative analysis**

Plants are good source of biologically active compounds known as phytochemicals. The phytochemicals act as antioxidants by scavenging free radicals and many have therapeutic potential for free radical associated disorders (Lee et al., 2000). Phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds in the plants serve a defense mechanism against prediction by many microorganisms, insects and other herbivores (Shihabudeen et al., 2010).
The phytochemicals are grouped into two main categories (Krishnaiah et al., 2009) namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc. (Krishnaiah et al., 2007; Edeoga et al., 2005). Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidal, antibacterial, antifungal (Lemos et al., 1990), anticonstipative (Ferdous et al., 1992), spasmolytic (Sontos et al., 1998), antiplasmodial (Benoitvical et al., 2001) and antioxidant (Vardar-Unlu et al., 2003) activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contain.

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated by several workers (Kokate et al., 1998). Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993). Different types of solvents play an important role in extractability of different phytochemical. Flavonoids were found to be extractable in all the solvent system such as petroleum ether, methanol and water (Andrei et al., 2000; Zafar and Mujeeb, 2002). Several workers reported the isolation of steroids by using petroleum ether as a solvent system (Komarov, 1968).

**Phytochemical screening on some medicinal plants**

Faraz et al. (2003) carried out phytochemical screening in fifty five Iranian plants belonging to 21 families. Theeshan et al. (2005) studied the phytochemical
constituents of *Cassia fistula*. Falodun *et al.* (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol esters in the aqueous and methanol extracts of *Euphorbia heterophylla*. Two new homoisoflavonoids were isolated from *Caesalpinia pulcherrima* by Maheswara *et al.* (2006). Raghavendra *et al.* (2006) examined different solvent extracts of the powdered leaf of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins.

Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al.* (2007) for their phytochemical properties. Onwukaeme *et al.* (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. Umadevi *et al.* (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated to detect the presence of various phytochemicals by Vaghasiya and Chanda (2007) and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins.

Dhanabalans *et al.* (2008) showed the presence of eight phytochemicals such as alkaloid, tannin, saponin, steroid, phlobatannin, terpenoid, flavonoid and cardiac glycoside from the methanol extract of leaves of *Tridax procumbens*. Jude *et al.* (2009) reported six phytochemicals from the leaves of *Tridax procumbens*. Das *et al.* (2009) determined eight secondary metabolites from the aqueous and methanol leaf extract of *Tridax procumbens*. Igbionosa *et al.* (2009) revealed the presence of saponin, steroid, tannin, glycoside, alkaloid and flavonoid in the stem bark extracts of *Jatropha curcas*. Krishna *et al.* (2009) conducted preliminary phytochemical studies and also estimated the total phenolics and flavonoid contents in the methanol extract.
of *Justicia gendarussa*. A comparative phytochemical study between six Malaysian medicinal plants, belonging to different families, was carried out by Krishnaiah *et al.* (2009).

Mungole *et al.* (2010) reported the preliminary phytochemical screening of *Ipomoea obscura* leaf, stem and seed extracts in different solvents like petroleum ether, absolute alcohol, chloroform, acetone and water. Sutharsingh *et al.* (2011) studied the phytochemical and antioxidant properties of aerial parts of *Naravelia zeylanica*. Hussain *et al.* (2011) carried out to assess the phytochemical and antimicrobial bioassay of five medicinal plants, *Lepidium sativum, Nerium oleander, Ranunculus repens, Tecoma stans* and *Urtica dioca*. Mungole and Chaturvedi (2011) studied the phytochemical screening of *Hibiscus sabdariffa*.

Pascaline *et al.* (2011) assessed the phytochemical constituents of ten medicinal plants belonging to different families used by the Nandis of South Nandi district. Qualitative and quantitative phytochemical analysis were carried out in seven plants, *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia* and *Xanthium strumarium* of Northeastern region of India (Yadav and Agarwala, 2011). Vaghasiya *et al.* (2011) investigated total phenols and flavonoids content of 53 traditionally used medicinal plants of western region of India. Khan *et al.* (2011) investigated the crude phytochemical and antimicrobial activities of selected medicinal plants of Peshawar region.

Quantitative phytochemical analysis was conducted on *Cissus populnea* with the aim of identifying and determining the actual phytochemicals and quantity of each constituent present in the leaves of the plant (Soladoye and Chukwuma, 2012).
Qualitative and quantitative analysis were carried out in four wetted plants such as *Marsilea quadrifolia, Centella asiatica, Trapa natans* and *Ipomea aquatica* using five different solvents viz. petroleum ether, chloroform, acetone, ethanol and distilled water (Pepsi *et al.*, 2012). Badugu (2012) analyzed the extracts of leaves of *Cyamopsis tetragonoloba* for the presence of various phytoconstituents.

The preliminary screening of pulp of fruits of *Terminalia belerica* was evaluated for its phytochemical constituents by using generally accepted laboratory technique for qualitative determination which showed the presence of phytosterols, carbohydrates, flavonoids, phenolic compounds and tannins (Manohar *et al.*, 2012). Joshi *et al.* (2013) carried out phytochemical investigation of the ethanol extract of stem bark of *Terminalia tomentosa* belonging to the family Combretaceae. The phytochemical constituent and some antioxidant indices of ethanol leaf extract of *Azadirachta indica* were evaluated by Oladipupo (2014).

**HPTLC analysis**

HPTLC is an inexpensive method for separation, qualitative identification or semi-quantitative analysis of samples and it can be used to solve many qualitative and quantitative analytical problems in a wide range of field including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis (Jain *et al.*, 2009).

Arokiyaraj *et al.* (2008) evaluated methanol extract of *Pterocarpus santalinus* leaf to detect the presence of various phytochemicals by using HPTLC finger print technique. Bhise and Salunkhe (2009), by using TLC and HPTLC techniques, screened the phytochemical components from Ashwagandha, Tulsi, Mulethi, Awala,
Shatavari, Gokharu, Arjun, Giloy, Safed musli, Kalimirchi, Haldi and Jaiphal. Methanol extract of *Ocimum basilicum* was analysed by TLC / HPTLC techniques to detect the phytochemicals by Maria *et al.* (2009).

The preliminary phytochemical screening was made in *Pergularia daemia*. Separation and identification of compounds were done from the crude extract of leaves using TLC, HPLC and HPTLC by Karthishwaran *et al.* (2010). HPTLC fingerprint was drawn for the phytochemicals derived from the methanol leaf extract of *Acacia nilotica* by Venkataswamy *et al.* (2010). Abirami and Murugan (2011) quantified flavonoids in *Cassia occidentalis* by HPTLC. Alam *et al.* (2011) isolated swertiamarin in 60% methanol extract of *Enicostemma littorale* by high performance thin layer chromatographic densitometric method.

Quantitative determination of phytochemicals by HPTLC was done by Verma *et al.* (2011) in *Eucalyptus hybrida* leaves. Optimization and development of a sensitive HPTLC method for estimation of wedelolactone in different extracts of *Eclipta alba* was established by Savita and Prakashchandra (2011). Patel *et al.* (2012) examined the phytochemical parameters of *Aloe vera* extract by HPTLC techniques. Prasanth *et al.* (2012) determined the flavonoid content in *Clerodendrum viscosum* roots by HPTLC techniques.

**GC-MS analysis**

Wang *et al.* (2003) isolated the active principles from selected Chinese herbs and used Gas Chromatography-Mass Spectrometric analysis for structure elucidation. Rahaman *et al.* (2006) reported 3, 5, 7, 4 - tetrahydroxy flavone from the leaves of *Cassia alata*. Sixty two compounds were identified by Chowdhury *et al.* (2007),
from the leaves of *Lantana camara* using GC-MS technique. Ivana *et al.* (2008) used GC-MS technique to analyze the chemical composition of the leaf extracts of *Stevia rebaudiana*. The extracts of two varieties of *Aloe greatheadii* were examined, quantified and compared for the phytochemical contents using GC-MS technique (Lisa *et al.* 2008). Ayo *et al.* (2009) studied the phytochemicals present in the methanol leaf extract of *Cassia nigricans* by GC-MS technique.

Hassanzadeh *et al.* (2010) obtained the essential oils from the leaves of *Cupressus lusitanica* by hydro distillation method and their chemical nature were analyzed by GC-MS. Thirty four compounds were identified in *Syzygium aromaticum* using GC-MS analysis by Hema *et al.* (2010).

The essential oil of the leaves of *Psidium guajava* was extracted and analyzed by gas chromatography coupled with mass spectrometry (Nisha *et al*., 2011). Charles *et al.* (2011) identified the bioactive phytocomponents of the bark extract of *Alseodaphne semecarpifolia* through GC-MS analysis. Velmurugan and Kamaraj (2011) investigated the phytochemical analysis of alcohol leaf extract of *Cadaba trifoliata*. Preliminary studies showed the presence of tannins, steroids, alkaloids, glycosides, flavonoids and phenolic compounds. In the GC-MS analysis, 19 bioactive phytochemical compounds were identified in the alcohol extract. Hexane extract of the leaves of *Ehretia laevis* was analyzed to identify the phytoconstituents by GC-MS analysis (Torane *et al*., 2011).

The bioactive compounds of *Vernonia cinerea* have been evaluated using GC-MS by Abirami and Rajendran (2012). Mohan *et al.* (2012) designed to investigate the preliminary phytochemical, antibacterial and GC-MS analysis of ethanol extract of *Acalypha indica*. Rajasekaran *et al.* (2012) reported the GC-MS
analysis and identification of phytochemicals present in the leaves of *Beloperone plumbaginifolia*. Kalpanadevi *et al.* (2012) determined the possible bioactive components of seed of *Entada pursaetha* using GC-MS. Mamza *et al.* (2012) documented the possible bioactive components of leaves of *Phyllanthus amarus* using GC-MS analysis. The chemical compositions of the ethanol extract of whole plant of *Sarcostemma secamone* was investigated using Gas Chromatography - Mass Spectrometry (Thanga Krishnakumari *et al.*, 2012).

Banu and Nagarajan (2013) analyzed major bioactive compounds present in the leaf extract from *Wedelia chinensis* by GC-MS. Twenty two phytoconstituents were identified in the ethanol extract of leaf of *Canthium parviflorum* by GC-MS analysis (Purushoth *et al.*, 2013). Sheela and Uthayakumari (2013) carried out to determine the bioactive phytoconstituents in the ethanol extracts of leaf and stem of *Sesuvium portulacastrum*.

The phytochemicals of *Acacia nilotica* were identified, such as 3-picoline-2-nitro, 1-acetyl-beta-carboline, hydroxycitronellal, trans-decalone, propionic acid-2-chloro, ethyl ester, lavandulyl acetate and D-glucoronic acid by GC-MS analysis (Hemamalini *et al.*, 2013). The GC-MS analysis of the ethanol extract of *Calotropis gigantea* revealed the presence of 14 major compounds. This study forms a basis for the biological characterization and importance of the compounds identified and creates a platform to screen many bioactive components to treat many diseases (Dhivya and Manimegalai, 2013). Kanthal *et al.* (2014) performed the GC-MS analysis of methanol extract of the whole plant of *Lactuca runcinata*. Nayak *et al.* (2014) reported the GC-MS analysis of phytocostituents of methanol rhizome extracts of some wild Zingiberaceae plants.
Pharmacological studies

Pharmacology is the biomedical science concerned with the interaction of chemical substances with living cells, tissues and organisms. It is particularly concerned with the mechanisms by which drugs counteract the manifestations of disease and affect fertility. Pharmacology is not primarily focused on the methods of synthesis or isolation of drugs or with the preparation of pharmaceutical products. Pharmacology is divided into two main subdivisions, pharmacokinetics and pharmacodynamics. Pharmacokinetics is concerned with the processes that determine the concentration of drugs in body fluids and tissues over time, including drug absorption, distribution, biotransformation (metabolism) and excretion. Pharmacodynamics is the study of the actions of drugs on target organs.

Antioxidant activity

Oxidants and Antioxidants

Free radicals are electrically charged molecules that are produced as byproducts of our own metabolism. They are continuously produced by our body’s use of oxygen such as in respiration and some cell mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution and pesticides (Li and Trush, 1994). In normal metabolism, the levels of oxidants (i.e. free radicals) and antioxidants in humans are maintained in balance, for sustaining optimal physiological conditions (Temple, 2000).

In recent times, there is an increasing interest in the role of free radical mediated damage in the etiology of human diseases. Over production of free radicals
in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA and proteins (Liu, 2002) and thus leads to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts and several forms of cancer (Halliwell, 1997). It is established that the intake of antioxidant substances reinforces defenses against free radicals.

Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl tolune (BHT) are powerful. They are proved to be toxic to humans so that they are used for industrial purposes (Safer and Al-Naghamish, 1999). The use of synthetic antioxidants has been limited because of their toxicity (Valentao et al., 2002). Therefore, it is urgent to find natural antioxidants. Plants are the basis of life on earth and are central to people's livelihoods (Albert and Kuldip, 2006). Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Antioxidants inhibit or prevent oxidation of substrates and evolve to protect cells against the damage effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxyl radical etc. (Gulcin, 2010).

*In vitro* antioxidant models

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Pietta, 2000). Therefore, studying the scavenging activity of plant extracts on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant
radicals generated endogenously during aerobic metabolism (Harsh, 2010). A single hydroxyl radical result in the formation of many molecules of lipid hydroperoxides in the cell membrane which may severely disrupts its function and leads to cell death.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 nm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical scavenging activity assessment (Eyob et al., 2008). The conversion of Fe$^{3+}$ into Fe$^{2+}$ in the presence of various fractions was calculated to determine the reducing power ability. The reducing ability of a compound generally depends on the existence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom (Umamaheswari et al., 2007).

ABTS radical scavenging activity is relatively recent one, which involves a more drastic model, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The ability in both organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity (Usha and Suriyavathana, 2012).

**Plants with antioxidant potential**

Antioxidant activities of 23 Iranian *Ocimum* accessions were studied by Javanmardi et al. (2003). Chen et al. (2004) used microplated ABTS, H$_2$O$_2$ and HRP system for evaluating total antioxidant activity of several popular vegetables and traditional Chinese herbals. *In vitro* antioxidant potentials of three local
Mediterranean food plant extracts (Cichorium intybus, Sonchus oleraceus and Papaver rhoes) were studied by Schaffer et al. (2005). Pourmorad et al. (2006) carried out a comparative study on the antioxidant potentials of some selected Iranian medicinal plant extracts. Four varieties (red, violet, white and green) of Allium cepa were studied for their total phenolic contents, antioxidant and free radical scavenging activities by Prakash et al. (2006). The antioxidant properties of 25 edible tropical plants were studied by Wong et al. (2006).

Antioxidant potential of leaves of three different species of Annona was studied by using different in vitro models like DPPH, ABTS, nitric oxide, superoxide, hydroxyl radical and lipid peroxidation (Baskar et al., 2007). Badami and Channabasavaraj (2007) studied the in vitro antioxidant activities of thirteen medicinal plants collected from Western Ghats of India. Tripathi and Kamat (2007) reported that an aqueous extract of Andrographis paniculata showed good antioxidant ability by scavenging the DPPH radical. Methanol extracts of bark, fruits and leaves of Ficus microcarpa and Caesalpinia digyna root exhibited strong scavenging effect on ABTS radical cation (Ao et al., 2008; Srinivasan et al., 2007). An aqueous extract from Choerospondias axillaris showed potent scavenging effect on DPPH (Wang et al., 2008).

The antioxidant activity of aqueous and ethanol extracts of stem of Balanites roxburghii was evaluated by various in vitro antioxidant assays (Singh et al., 2009). The antioxidant activity of methanol extract of stem bark of Gmelina arborea was studied using various in vitro assays (Patil et al., 2009). An in vitro antioxidant activity of Gymnema sylvestre leaf extract was investigated by Rachh et al. (2009). Olayinka et al. (2010) studied the in vitro antioxidant potential of aqueous extract of
Helichrysum longifolium leaf. Antioxidant potential of various extracts of stem of Saraca asoca was studied by Panchawat and Sisodia (2010).

Antioxidant potential of ethanol extract of the roots of the two varieties of Catharanthus roseus (pink flowers) and Catharanthus alba (white flowers) was evaluated by Bhutkar and Bhise (2011). Sivaprabha et al. (2011) documented the radical scavenging activity of leaves and rhizomes of Curcuma amada. The antiradical and antioxidant activities of the whole plant of Pouzolzia zeylanica in four in vitro models, including DPPH, ABTS, hydroxyl radical scavenging assays and the reducing power assay were investigated by Li et al. (2011). In vitro antioxidant activity of Polygonum barbatum leaf extract was studied by Sheela and Ramani (2011).

In vitro antioxidant activity of leaf extract of Aerva lanata was carried out by Battu and Kumar (2012). Parameshwari and Suriyavathana (2012) studied the antioxidant effects of the ethanol leaf extract of Chromolaena odorata by using different in vitro methods of assessment. In vitro antioxidant activity of Coccinia grandis root extract was investigated by Bhadauria et al. (2012). Ganga Rao et al. (2012) evaluated the antioxidant activity of methanol leaf extract of Entada pursaetha by using superoxide radical, hydroxyl radical and DPPH radical scavenging methods. In vitro antioxidant analysis of methanol root extract of Erythrina indica was performed by using DPPH, nitric oxide, superoxide assay and ferric reducing antioxidant power assay (Sre et al., 2012). Thambiraj and Paulsamy (2012) reported the in vitro antioxidant potential of methanol leaf extract of the medicinal plant Acacia caesia.
Sahoo et al. (2013) investigated antioxidant potential of methanol leaf extract of *Alpinia nigra*. *In vitro* antioxidant activity of ethanol extract of *Annona muricata* bark was evaluated by Ahalya et al. (2013). The crude methanol leaf extract of *Eurya japonica* indicated strong antioxidant activity which might helpful in preventing or slowing the progress of various oxidative stresses (Rosalind et al., 2013). Jadhav et al. (2013) determined antioxidant potential of various extracts of *Kydia calycina* leaves. The free radical scavenging potential of methanol extract of *Lagenaria siceraria* leaves was studied using *in vitro* antioxidant models (Sharma et al., 2013).

The ethanol extract of *Randia dumetorum* leaves was screened for *in vitro* antioxidant activity by oxygen radical scavenging activity such as DPPH, total antioxidant assay, superoxide metal chelation and iron reducing power activity at different concentrations (Gandhimathi and Viji Stella Bai, 2013). The *in vitro* antioxidant activity of methanol - aqueous extract of *Salacia oblonga* was analyzed through reducing power assay, H$_2$O$_2$ scavenging activity, superoxide radical scavenging activity and nitric oxide radical scavenging activity assays (Basu et al., 2013).

The antioxidant potential and phytochemical analysis of leaf galls of *Syzygium cumini* were determined by Eshwarappa et al. (2014). The methanol extract of eleven Algerian medicinal plants were investigated for their *in vitro* antioxidant activity by Benhammou et al. (2014). Onoja et al. (2014) evaluated the antioxidant effects of methanol seed extract of *Aframomum melegueta*. The antioxidant activity of leaves of *Atalantia ceylanica* was investigated using 1,1-Diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical, nitric oxide scavenging assays and ferric ion reducing power assay (Dilanka and Soysa, 2014).
Anticancer activity

Cancer

Cancer is a dreadful disease characterized by the irregular proliferation of the cells. As a cell progresses from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in cells behavior (Ashworth et al., 2011). Cancer is the second leading cause of death in all over the world (Hoyert et al., 2005). Parkin et al. (2005) reported that cancer is a major public health burden in both developed and developing countries. They mentioned that there were 10.9 million new cases, 6.7 million deaths and 24.6 million persons living with cancer around the world in 2002. It was estimated 12.7 million cancer cases and 7.6 million cancer deaths in 2008 (Ferlay et al., 2010). World Health organization (WHO) reported that it was estimated upto 13.1 million deaths in 2030 (Merel et al., 2012).

The environmental, chemical, physical, metabolic and genetic factors play a direct or indirect role in the induction and deterioration of cancers. The limited success of clinical therapies includes radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management (Dai and Mumper, 2010).

Cancer inducer

The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascitic form, it has been used as a transplantable tumor model to investigate the antitumor effects of several substances
Ascitic fluid is the direct nutritional source for tumor cells. So, a rapid increase in ascitic fluid with tumor growth may be a mean to meet the nutritional requirement of tumor cells (Rajeshwar et al., 2005).

Hull (1953) described the serial cultivation of Ehrlich ascites cells on glass for a limited period of time and Gey et al. (1954) reported some success in propagating Ehrlich cells, following intraperitoneal passage through rats. Powell (1957) observed that a protective effect exerted by explants of various animal tissues or by normal spleen monocytes was essential to promote the growth in vitro of Ehrlich and Sarcoma ascitic tumor cells. Deschner and Allen (1960) studied the Ehrlich tumor in tissue culture but were unable to demonstrate tumor formation when their cell line was inoculated into mice.

**Anticancer agents in clinical use**

There are four major structural classifications of plant derived anticancerous compounds viz., Vinca alkaloids, Epipodophylotoxin lignans, Taxane diterpenoids and Camptothecin quinoline alkaloid derivatives. Cragg and Newman (2005) documented that the isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae) introduced a new era of the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers including leukemia, lymphomas, advanced testicular cancer, breast, lung cancers and Kaposi’s sarcoma.
Pervilleine A was isolated from the roots of *Erythroxylum pervillei* (Erythroxylaceae) (Silva *et al*., 2001). Pervilleine A was selectively cytotoxic against a multidrug resistant (MDR) oral epidermoid cancer cell line (KB-V1) in the presence of the anticancer agent vinblastine (Mi *et al*., 2001). Pervilleine A is currently in preclinical development (Mi *et al*., 2003). The reliable criteria for judging the quality of any anticancer drug are prolongation of life span and its effect on hematological poietic system (Dhamija *et al*., 2013).

**Medicinal plants with potential anticancer activities**

Emerging evidence suggests that a number of plants are known to be the source of useful drugs in modern medicine and have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Sadiq *et al*., 2009; Gonzales and Valerio, 2006; Gupta *et al*., 2004 and Dahiru *et al*., 2005).

The methanol extract of *Caesalpinia bonducella* leaves was evaluated for antitumor activity against Ehrlich ascites carcinoma bearing Swiss albino mice (Gupta *et al*., 2004). Antitumor activity of methanol extract of *Bauhinia racemosa* stem bark was evaluated against Ehrlich ascites carcinoma tumor in mice (Gupta *et al*., 2004). Rajeshwar *et al.* (2005) reported the antitumor activity of *Mucuna pruriens* seeds against Ehrlich ascites carcinoma in Swiss albino mice. Zahran *et al.* (2005) studied the antitumor activity of aqueous extract of *Salix safsaf* against two types of tumors, Ehrlich ascites carcinoma cells (EACC) and acute myeloid leukemia (AML).
Dongre et al. (2007) reported the antitumor activity of methanol extract of *Hypericum hookerianum* stem against Ehrlich ascites carcinoma in Swiss albino mice. Muthuraman et al. (2008) investigated the antitumor and antioxidant potential of the ethanol extract of *Tragia plukenetii* (ETP) on Ehrlich ascites carcinoma (EAC) tumor model. The antitumor effect of ETP was evaluated by assessing *in vitro* cytotoxicity, survival time, hematological and antioxidant parameters. Oral administration of ETP increased the survival time of the EAC bearing mice.

Chitra et al. (2009) observed that ethanol extract of *Vitex negundo* enhance non viable cell counts in peritoneal exudates and decrease the viable cell count in EAC treated mice. They also reported that extract of this plant is effective against the major problem, myelosuppression and anaemia that are being encountered during chemotherapy and can bring back hemoglobin and RBC count to normal in EAC treated mice administrated with extract. Bala et al. (2010) reported the anticancer activity of *Cleome gynandra* in Swiss albino mice against Ehrlich ascites carcinoma cell line. Joshua et al. (2010) evaluated the antitumour potentials of *Amaranthus spinosus* against EAC bearing Swiss albino mice. Hassan and Abdel-Gawad (2010) documented the antitumor activity of *Zizyphus* leaves extract against Ehrlich ascites carcinoma (EAC) model in female albino mice.

Anbu et al. (2011) studied the anticancer activity of petroleum ether extract of *Abru precatorius* seed against Ehrlich ascites carcinoma in mice. Anticancer efficacy of *Cynodon dactylon* was assessed in Swiss albino mice. Mice inoculated with Ehrlich ascites carcinoma cells were administered with three doses of *Cynodon dactylon* extract viz., 100, 200 and 400mg/kg body weight orally for ten consecutive days. The results revealed that *Cynodon dactylon* extract showed significant anticancer activities.
in the tested animal models (Krishnamoorthy and Ashwini, 2011). Anticancer activity of ethanol and aqueous extracts of *Dendrophthoe falcata* was evaluated in EAC Swiss albino mice at the doses of 200 and 400 mg/kg body weight orally. Aqueous extract at both doses (200 and 400 mg/kg) and ethanol extract at 400 mg/kg dose showed potent anticancer activity (Dashora *et al.*, 2011).

Experiment performed by Kundusen *et al.* (2011) found that intraperitoneal administration of methanol extract of *Citrus maxima* at the dose levels of 200 and 400 mg/kg bodyweight increased the life span of non viable cell count and decreases the tumor volume. Antitumor activity and antioxidant status of ethanol extracts of *Brassica oleracea* var. *italica* flower was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. The ethanol extracts of *Brassica oleracea* var. *italica* flower exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice (Subramanian and Gowry, 2011). Chatterjee *et al.* (2011) recorded the antitumor activity of *Cuscuta reflexa* against EAC bearing Swiss albino mice. The administration of the extract resulted significant reduction in viable cell count and increased non viable cell count towards normal in tumor host suggested that extracts stimulate the growth and activity of immune cells by the production of interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism.

Islam *et al.* (2012) evaluated the antineoplastic activity of *Eucalyptus* extract (EuE) against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. EuE reduced tumor burden remarkably and may be considered as a potent anticancer agent. *In vitro* and *in vivo* anticancer activity of methanol extract of *Tecoma stans* flowers were investigated against Ehrlich ascites carcinoma tumor model
(Kameshwaran et al., 2012). Sakthivel et al. (2012) reported the in vivo antitumor activity of aerial parts of *Acacia nilotica* against DAL induced solid and ascitic tumor in BALB/c mice. Antitumor activity of 50% ethanol bark extract of *Magnolia grandiflora* was evaluated against tumors induced in mice using dimethyl benzanthracene and 3-methyl cholanthrene (Singh et al., 2012).

Ahuja et al. (2013) investigated the anticancer potential of the ethanol extract of *Terminalia chebula* fruits against EAC induced cancer in Swiss albino mice. They reported that high dose of *Terminalia chebula* extract (200 mg/kg body weight) significantly reduced the tumor growth which was demonstrated by increased life span of the mice and restoration of haematological parameters. Antitumor activity of methanol extracts of *Kigelia africana* leaves was evaluated against Ehrlich ascites carcinoma tumor in mice. The methanol extracts of *Kigelia africana* showed decrease in tumor size, average body weight, mean survival time thereby increasing life span of EAC tumor bearing mice. Haematological profile reverted to more or less normal levels in extracts treated mice (Sainadh et al., 2013).

*In vitro* and *in vivo* anticancer activity of hydroalcohol extract of *Ipomea carnea* leaf was studied against Ehrlich ascites carcinoma cell lines (Anand et al., 2013). The results indicated that extract of this plant possess significant antitumor activity on dose dependent manner. Periyasamy et al. (2013) designed to determine the antitumor and antioxidant properties of crude methanol extract from the leaves of *Plumeria acuminata* against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The anticancer potential of the *Vitex negundo* extract was analyzed against Ehrlich ascites carcinoma (EAC) cell line (*in vivo*). The ethanol extract of *Vitex negundo* showed decrease in tumor volume, packed cell volume and viable cell count
and increases the non viable cell count and mean survival time (MST), thereby increasing life span of EAC tumor bearing mice. Haematological profile reverted to more (or) less normal levels in extract treated mice (Kannikaparameswari and Indhumathi, 2013).

Prasad and Koch (2014) evaluated the anticancer property of the ethanol extract of *Dendrobium formosum* on Dalton’s lymphoma. Ali *et al.* (2014) investigated the *in vitro* anticancer activities of a total of 14 wild angiosperms collected in Saudi Arabia. The cytotoxic activity of each extract was assessed against human breast adenocarcinoma (MCF-7) cell lines by using the MTT assay. Among the plants screened, the potential cytotoxic activity exhibited by the extract of *Lavandula dentata* (Lamiaceae) was identified.

**Antidiabetic activity**

**Diabetes**

Diabetes mellitus is a disorder of carbohydrate metabolism in which sugars in the body are not oxidized to produce energy due to the lack of pancreatic hormone insulin.

**Diabetes inducer**

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin dependent diabetes mellitus. There is an increasing evidence that alloxan caused diabetes by rapid depletion of cells by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in
insulin release thereby a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states (Szkudelski, 2001).

Elsner et al. (2000) reported that administration of streptozotocin selectively destroys the β-cells of the islets of Langerhans. The destruction of β-cells cause the marked decrease in insulin levels (Gilman et al., 2001). STZ-induced diabetic animals may exhibit most of the diabetic problem mediated through oxidative stress (Sathishsekar and Subramanian, 2005).

**Plants with potential antidiabetic activities**

Several drugs have been used in the management of the disease. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems. The high risk of diabetes and health related complications have led to research for active antidiabetic compounds from plants (Maqsood et al., 2009).

Antihyperglycemic effect of *Tinospora crispa* extract is due to the stimulation of insulin release via modulation of beta cell Ca$^{2+}$ concentration (Noor and Ashcroft, 1998). Effect of *Ficus carica* leaf decoction, as a supplement to breakfast, was studied in insulin dependent diabetes mellitus patients (Serraclara et al., 1998). Oral administration of pulp extract of the fruit of *Syzigium cumini* to normoglycemic and STZ induced diabetic rats showed hypoglycemic activity possibly mediated by insulin secretion and inhibited insulinase activity (Grover et al., 2002). Umadevi et al. (2006) reported the antidiabetic and hyperlipidaemic effects of *Cassia auriculata* in alloxan induced diabetic rats. Chloroform, ethyl acetate and alcohol extracts of *Momordica*
*dioica* fruits were investigated for their potential antidiabetic activity in alloxan induced diabetic rats by Reddy *et al.* (2006).

The methanol leaf extract of *Costus pictus* was investigated for its antidiabetic effect in Wistar albino rats by Jothivel *et al.* (2007). Pari *et al.* (2007) investigated the insulin receptor binding effect of *Cassia auriculata* flower extract in streptozotocin induced diabetic male Wistar rats. Jayasri *et al.* (2008) carried out a study to evaluate the antidiabetic effect of *Costus pictus* leaves in normal and streptozotocin induced diabetic rats. Aqueous, alcoholic and petroleum ether extracts of leaves of *Tridax procumbens* were subjected to hypoglycemic activity in Wistar albino rats by Bhagwat *et al.* (2008). The antidiabetic activity of *Thespesia populnea* bark and leaf extracts was investigated against streptozotocin induced diabetic rats by Parthasarathy *et al.* (2009). The results of this experimental study indicated that both the extracts possess antidiabetic effect and also showed the possible mechanism due to inhibition of generation of free radical.

Ethanol and ethyl acetate extracts soluble fraction of *Swertia punicea* showed hypoglycemic effects in streptozotocin induced type 2 diabetic mice and may be beneficial to improve insulin resistance (Rao *et al.*, 2010). Aqueous leaf extract of *Aegle marmelos* showed antihyperglycemic activity in streptozotocin induced diabetic rats after 14 days treatment either by increasing utilization of glucose or by direct stimulation of glucose uptake through increased insulin secretion. Antihyperglycemic activity of ethyl ether extract at 0.25 mg/kg was reported to be the most potent active principle of *Allium sativum* which was due to increased insulin like activity (Ayodhya *et al.*, 2010). The extract of *Tribulus terrestris* significantly decreases blood glucose
level in normal and alloxan induced diabetic mice, mainly due to the increased serum insulin level (Chauhan et al., 2010).

Singh (2011) reported that aqueous extract of Eucalyptus globulus increased peripheral glucose utilization in the mouse abdominal muscle and increased insulin secretion from the clonal pancreatic beta cell line. The antidiabetic effect of Aloe vera extract was evaluated against alloxan induced diabetic rats by Rehman et al. (2011). The blood glucose level of drug treated groups of rats showed significant reduction after 30 days of treatment. It was found that water extract of A. vera has antidiabetic effect in normal and alloxan induced diabetic rats. Sundarajan et al. (2011) studied the antidiabetic activity of methanol extract of Hibiscus cannabinus leaves in streptozotocin induced diabetic rats. The results revealed that Hibiscus cannabinus leaf extract has significant antidiabetic activity, which lowered the fasting blood glucose level in streptozotocin induced diabetic rats

Mondal et al. (2012) determined the antidiabetic activity of Areca catechu leaf extract against streptozotocin induced diabetic rats. The antidiabetic activity of Anthocleista djalonensis root extract was evaluated against alloxan induced diabetic rats (Okokon et al., 2012). Saidu et al. (2012) investigated the hypoglycemic effect of aqueous Blighia sapida root bark extract (ABRE) on normoglycemic albino rats. This study indicated that consumption of the ABRE exerts significant hypoglycemic effect in normoglycemic rats. These findings support the traditional use of ABRE for controlling diabetes.

Nurdiana et al. (2013) determined the antidiabetic activity of Azadirachta excelsa extract on alloxan induced diabetic rats. The results suggested that the ethanol extract of A. excelsa possess antidiabetic activity by improving the insulin secretion
with consequent decrease in the level of plasma blood glucose and HbA1c. Ghosal and Mandal (2013) studied the antidiabetic activity of *Calamus erectus* fruit extract against streptozotocin induced diabetic rats. This study demonstrated that *C. erectus* fruit extract possess good antidiabetic potential and could improve lipid profile and oxidative stress efficiently during diabetic condition. Nagaraj (2013) reported the antihyperlipidemic activity of aqueous extract of *Fagonia cretica* whole plant in male Wistar albino rats. The aqueous extract of this plant showed significantly increased HDL levels and decreased TC, TG, HDL and LDL ratio.

The hypoglycemic and hypolipidemic effects of ethanol and aqueous extract of *Zizyphus nummularia* were evaluated by dexamethasone induced diabetic rats. Both the extracts could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus (Rajasekaran *et al.*, 2013). *In vitro* antidiabetic activity of methanol extract of *Psidium guajava* leaves was investigated by Manikandan *et al.* (2013). The antidiabetic effect of aqueous extract of *Otostegia persica* root was investigated in alloxan induced diabetic rats. The aqueous extract of this plant root significantly decreased serum glucose and HDL levels when compared to diabetic control rats (Bagherzade *et al.*, 2014). Ahalya *et al.* (2014) demonstrated the antidiabetic activity of the ethanol extract of *Annona muricata* stem in alloxan induced diabetic rats.

**Hepatoprotective activity**

**Liver disease**

Liver is a vital organ of the digestive system present in vertebrates and some other animals. It has a wide range of functions including detoxification, protein synthesis and production of biochemical necessary for digestion. The liver is
necessary for survival. Liver is the vital organ responsible for drug metabolism and appears to be a sensitive target site for substances modulating biotransformation (Ahmad et al., 2002).

Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Certain medicinal agent introduced within therapeutic ranges may injure the organ sometimes. Other chemical agents those used in herbal remedies may also induce hepatotoxicity (Boerth and Strong, 2002). Nowadays drug induced liver injury has become a major health problem. Liver diseases such as jaundice, cirrhosis and fatty liver are very common and large public health problems in the world (Balamurugan et al., 2008). Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year (Kshirsagar et al., 2011).

**Hepatotoxicity inducer**

Hepatotoxicity due to various drugs such as paracetamol, rifampicin, oral contraceptives, penicillamine, danazol, lipid lowering drugs, methyldopa, estrogen, diclofenac, tamoxifen, sulfa drugs, acetaminophen, alcohol, infections and autoimmune disorders appears to be the most common causative factor for liver diseases (Hunter, 2002; Harbans and Sharma, 2011; Goldman and Ausiello, 2004; Adewusi and Afolayan, 2010). Liver cell injury can be caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes (Saleem et al., 2010). Moreover, alcohol abuse is one of the major causes of abnormal liver findings in India.
Liver damage induced by carbon tetrachloride (CCl\textsubscript{4}) is commonly used model for the screening of hepatoprotective drugs (Slater, 1965). When rats were treated with carbon tetrachloride, it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P\textsubscript{450} dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl\textsubscript{3}) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation (Recknagel \textit{et al.}, 1976; Recknagel, 1983; DeGroot and Noll, 1986).

**Hepatoprotective agents in clinical use**

Silymarin, a flavonolignan from the seeds of 'milk thistle' (\textit{Silybum marianum}), has been widely used from ancient times because of its excellent hepatoprotective action. It is a mixture of mainly three flavonolignans, viz, silybin, silidianin and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug induced hepatitis, cirrhosis and alcoholic liver diseases. It has also been reported to be effective in certain cancers (Dixit \textit{et al.}, 2007). There is no rational therapy available for treating liver disorders so that management of liver diseases is still a challenge to the modern medicine.

**Plants with potential hepatoprotective activities**

The traditional systems of medicine have a major role in the treatment of liver ailments. Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the
management of various liver disorders in ethnomedicinal practices as well as in traditional systems of medicine in India (Bhattacharya et al., 2000).

Mondal et al. (2005) reported that methanol extract of Diospyros malabarica bark had a potent hepatoprotective activity against carbon tetrachloride induced liver damage in rats. Dash et al. (2007) reported that chloroform and methanol extracts of Ichnocarpus frutescens whole plant served as effective hepatoprotective agents in paracetamol induced liver damage in rats. Iniaghe et al. (2008) reported that the aqueous leaf extract of Acalypha racemosa served as an effective hepatoprotective agent against CCl₄ induced liver damage. Maheswari et al. (2008) investigated the hepatoprotective activity of methanol extract of leaves of Orthosiphon stamineus against paracetamol induced hepatotoxicity.

Hepatoprotective activity of hydroalcohol extract of leaves of Alocasia indica was evaluated by Mulla et al. (2009). The results showed that oral administration of hydroalcohol extract of A. indica effectively inhibited CCl₄ and paracetamol induced changes in the serum marker enzymes, cholesterol, serum protein and albumin in a dose dependent manner as compared to the normal and the standard drug silymarin treated groups. The methanol extract of leaves of Mimosa pudica at the dose of 200 mg/kg body weight was studied for the hepatoprotective effect using carbon tetrachloride induced liver damage in Wistar albino rats (Rajendran et al., 2009).

Hepatoprotective effects of different plant extracts have been studied. Some of the recent studies include hepatoprotective activity of Trianthema decandra (Balamurugan et al., 2008), Cocculus hirsutus (Thakare et al., 2009), Carica papaya (Sadeque and Begum, 2010), Carissa spinarum (Hegde and Joshi, 2010), Dodonaea viscosa (Ahmad et al., 2012), Pinus roxburghii (Khan et al., 2012), Trichodesma
*sedgwickianum* (Saboo *et al.*, 2013) and *Ipomoea staphylena* (Bag and Mumtaz, 2013).

Diethyl ether extract of the leaves of *Coccinia indica* was studied for hepatoprotective activity against carbon tetrachloride induced liver toxicity in rats (Kumar *et al.*, 2010). Hepatoprotective activity of aqueous and alcohol extracts of *Luffa acutangula* against carbon tetrachloride and rifampicin induced hepatotoxicity in rats was evaluated by Jadhav *et al.* (2010). The comparative hepatoprotective and antioxidative activity of *Phyllanthus niruri*, *Maytenus emarginata*, *Eclipta alba*, *Aloe vera*, *Solanum indicum* and *Aegle marmelos* against paracetemol induced toxicity was studied by Parmar *et al.* (2010). The hepatotoxicity was evaluated by an increase in serum AST, ALT, ALP activity and bilirubin level accompanied by significant decrease in albumin level. *Phyllanthus niruri* and *Maytenus emarginata* exhibits maximum hepatoprotective activity against paracetamol induced toxicity.

Ibrahim *et al.* (2011) investigated the hepatoprotective activity of *Boswellia serrata* extracts against paracetamol treated male rats. The results revealed that the extracts of *Boswellia serrata* have hepatoprotective activity both *in vitro* on primary hepatocytes cultures and *in vivo* model of paracetamol induced liver injury in rats. Babalola *et al.* (2011) designed to evaluate the possible hepatoprotective activity of the pretreatment with aqueous extract of the leaves of *Hyptis suaveolens* on acetaminophen induced hepatotoxicity in rabbits. The results showed that aqueous extract of the leaves of *Hyptis suaveolens* possess hepatoprotective potentials on acetaminophen induced liver damage.

Surana *et al.* (2012) studied the hepatoprotective effect of *Cassia tora* seeds in paracetamol induced hepatotoxic model. It was found that the aqueous seed extract of
this plant possess more significant hepatoprotective effect. The methanol extract of *Alstonia scholaris* stem bark was screened for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride. The methanol extract of *Alstonia scholaris* significantly decreased the biochemical parameters (Kumar *et al.*, 2012). The hepatoprotective activity of ethanol extract of *Cinnamomum* was determined against CCl₄ induced liver injury in rats (Eidi *et al.*, 2012).

Bagban *et al.* (2012) investigated hepatoprotective activity of the methanol extract of *Fagonia indica* on CCl₄ induced hepatotoxicity in albino rats. The results suggested that methanol extract of this plant at different doses showed significant hepatoprotective activity against CCl₄ induced hepatotoxicity and this might be due to the presence of flavonoids and tannins. Hepatoprotective activity of aqueous extract of *Lawsonia inermis* was evaluated against paracetamol induced hepatotoxicity in rats (Selvanayaki and Ananthi, 2012).

Yesmin *et al.* (2013) studied the hepatoprotective activity of the aqueous and n-hexane extracts of *Nigella sativa* in experimental liver damage in rats. The results revealed that hepatoprotective properties of *Nigella sativa* in liver damage of experimental rats by reducing oxidative stress are evident. Histopathological investigation and detection of active constituent, quercetin by HPLC also supported the results (Ali *et al.*, 2013).

Manikandaselvi *et al.* (2013) investigated the antihepatotoxic effect of hydroalcohol extract of leaf powder of *Azima tetracantha* and the fruit powder of *Tribulus terrestris*. The results showed that hydroalcohol extract of leaf powder of *A. tetracantha* and fruit powder of *T. terrestris* possess significant hepatoprotective
activity. Ethanol extract of Solanum trilobatum leaf and fruit were investigated for hepatoprotective activity by Pratheeba et al. (2013).

Folarin et al. (2014) investigated the comparative hepatoprotective activity of crude ethanol extract of Cuscuta australis against acetaminophen intoxication. The hepatoprotective and antioxidant activity of ethanol extract of Bauhinia hookeri against CCl₄ induced liver injury was investigated in mice (Sayed et al., 2014). The hepatoprotective activity of ethanol extract of Leea indica stem bark was evaluated against paracetamol induced hepatotoxicity in rats (Mishra et al., 2014). Dudekula et al. (2014) evaluated the ethanol extract of the whole plant of Rhus mysorensis for hepatoprotective effect against paracetamol induced liver damage in rats.

**Antifertility activity**

Several plant species have been described as antifertility agents (Lin, 1992). The practice of traditional medicine for the control of fertility in most parts of Ethiopia, India and most parts of the world is based on the uses of plant medicines for many years. Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases including for inducing infertility without proper knowledge of their function (Marles and Farnsworth, 1994). Although several herbal plants possess different types of antifertility activities such as antiimplantation, abortification, ecobolic, oestrogenic and spermicidal, a large number of medicinal plants possess some degree of toxicity.

A number of plants from Indian origin have been experimentally tested using modern techniques for their antifertility activities (Sharma et al., 2003). Maurya et al. (2004) have also given a review to provide an account of the studies carried out on
traditional plants which are used for fertility regulation. Approximately 318 different
plants are used worldwide, of which 227 plants are of Indian origin. So far, 74 plants
have been screened for their antifertility potential and 48 of them have been found to
be effective.

The methanol extract of the wood of *Quassia amara* significantly caused a
reduction in the weight of the testis, epididymis and seminal vesicle, but an increase
in that of the anterior pituitary gland. Epididymal sperm counts, serum levels of
testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were
significantly reduced when the rats were treated with the extract (Raji and Bolarinwa,
1997). The antifertility effect of triptolide and other related compounds isolated from
*Tripterygium wilfordii*, has been demonstrated in male rats. Triptolide, induces
complete infertility in the adult rats, has minimal adverse effects on the testis and acts
primarily on the epididymal sperm making triptolite an attractive lead as a post
testicular male contraceptive (Lue *et al.*, 1998).

Stem bark of *Alangium salvifolium* is used both as contraceptive and
abortifacient. Extracts of this plant possess antiprogestogenic activity (Murugan
*et al.*, 2000). Petroleum ether, benzene and ethanol extracts of *Crotalaria juncea*
seeds were administered intraperitoneally at the dose level of 25 mg/100 g body
weight to albino male mice for 30 days. The results showed decrease in the number of
spermatogonia, spermatocytes and spermatids in testis along with reduced caudal
spermatozoa (Vijaykumar *et al.*, 2003). The 50% ethanol extract of the root bark of
*Cananga odorata* administered orally at the dose of 1g/kg body weight/day for 60
days resulted in decreased epididymal sperm motility, sperm count and abnormality in
sperm morphology in male albino rats (Anitha and Indira, 2006).
Revathi et al. (2010) reported that functional sterility could be induced in male rats by whole plant ethanol extract of *Capparis aphylla* treatment, which promises to be potential male contraceptive. Effects of crude alcohol extract and decoction of whole plant of *Adiantum lunulatum* was observed on the reproductive structures of male albino rat (Bhatia et al., 2010). Effect of antifertility in aqueous leaf extract of *Andrographis paniculata* studied in male albino rats. A dose related reduction in the testicular sperm count, epididymal sperm count, motility and abnormal sperm count were observed. The results suggested that the aqueous extract of the leaves of *Andrographis paniculata* has spermicidal activity (Sathiyaraj et al., 2011).

Oral administration of alcohol extract of seeds and its fraction of *Citrus limonum* to the albino male rats showed decrease in sperm count, depending upon the duration of the treatment (Kulkarni et al., 2005). The ethanol leaf extract of *Spondias mombin* was orally administered with 250 and 500 mg/kg doses for 8 weeks. There was significant decrease in testicular and epididymal weight in the treated animals compared to the control (Asuquo et al., 2013).

Alagammal et al. (2013) studied the antifertility effect of ethanol extract of whole plant extract of *Polygala rosmarinifolia* in male albino rats. The results revealed that the ethanol extract inhibited sperm concentration, motility and testosterone which might result in a male sterility. Stalin et al. (2013) reviewed the works on antifertility of plant origin. They highlighted various plants such as *Abrus precatorius*, *Aegle marmelos*, *Curcuma longa*, *Raphanus sativus* etc. and their bioactive extracts involved in antifertility mechanism. Sundar Rajan et al. (2013) evaluated the antifertility activity of methanol extract of *Carica papaya* leaves and
*Capparis aphylla* aerial part. Umadevi et al. (2013) reviewed the several medicinal plants with potential antifertility activity.

Anitha et al. (2013) reported the antifertility effect of ethanol extract of *Cynoglossum zeylanicum* in male albino rats. Singh et al. (2013) documented a possible antifertility property of the ethanol extract of *Withania somnifera* in male albino rats. Cankaya et al. (2014) investigated the effect of *Bupleurum sulphureum* and *Cichorium intybus* plants on various male fertility parameters. Reddy et al. (2014) screened the extract of *Elytraria acaulis* for antifertility activity in male albino rats.

**Antiinflammatory activity**

**Inflammation**

Inflammation is a defensive reaction of the body against infections and injuries. Edema formation, leukocyte infiltration and granuloma formation represent typical features of inflammation (Gorzalczany et al., 2011).

**Disorders due to inflammation**

Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions such as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further
tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane (Rajendran and Lakshmi, 2008).

**Inflammation inducing models**

The xylene induced ear oedema model is useful for the evaluation of anti-inflammatory topical steroids or for non-steroidal antiphlogistic agents, especially those inhibiting phospholipase A2 (Kwang-Ho et al., 2008). Xylene induced ear oedema is an acute inflammation model which may involve inflammatory mediators such as histamine, serotonin and bradykinin. These mediators induce ear oedema by promoting vasodilation and increasing vascular permeability (Carlson, 1985). The cotton pellet-induced granuloma is an established and the most suitable method for studying the efficacy of drugs against the proliferative phase of inflammation (Parvataneni et al., 2005).

Carrageenan induced inflammation is an useful model for the estimation of antiinflammatory effect. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin and prostaglandin (Georgewill and Georgewill, 2010). The carageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal antiinflammatory agents (Rao et al., 2005). Selvam and Jachak, (2004) reported that compounds inhibiting the carageenan induced oedema are effective in inhibiting the enzyme cyclooxygenases. Greenwald (1991) reported that inhibition of oedema induced by formalin in rats is one of the most suitable test procedures to screen antiarthritic and antiinflammatory agents.
Antiinflammatory agents in clinical use

Indomethacin is a group of drugs called non-steroidal antiinflammatory drugs (NSAIDs). Indomethacin works by reducing hormones that cause inflammation and pain in the body. Indomethacin is used to treat pain or inflammation caused by many conditions such as arthritis, gout, ankylosing spondylitis, bursitis or tendinitis. Non-steroidal antiinflammatory drugs (NSAIDs), steroidal drugs and immunosuppressant drugs, which have been usually used in the relief of inflammatory diseases worldwide for a long time, are often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcer (Valiollah et al., 2009).

Recently, many natural medicines derived from plants, marine organisms, etc. were considered effective and safer for the treatment of various diseases including inflammation and pain (Su et al., 2011). The main side effect of non-steroidal antiinflammatory drugs is their ability to produce gastric lesions (Pagella et al., 1983). Saponins and flavonoids have been reported to have antiinflammatory activities (Mohammad et al., 2004; Aquila et al., 2009). On the other hand, it has been reported that free radicals are involved in the inflammatory process (Vajdovich, 2008).

Plants with potential antiinflammatory activities

The methanol extract of Alstonia macrophylla leaf was investigated for its antiinflammatory activity in carrageenan induced rat paw oedema (Arunachalam et al. 2002). Antiinflammatory activity of ethanol extract of Bouchea fluminensis leaves was demonstrated by Delaporte et al. (2002). Hajhashemi et al. (2002) studied the antiinflammatory activities of polyphenolic fraction of hydroalcohol extract and essential oil of the aerial parts of Satureja hortensis, an important Iranian folk
medicinal plant using carrageenan induced paw oedema in rats. The ethanol, chloroform and aqueous fractions of *Sideritis canariensis* var. *pannosa* were examined for their antiinflammatory and analgesic effects using several animal models (Hernandez-Perez and Rabanal, 2002).

*Mitragyna ciliata*, a widely used traditional medicinal plant to treat inflammation, hypertension, headache, rheumatism, gonorrhoea and bronchial-pulmonary diseases was investigated by Dongno *et al.* (2003) for its antiinflammatory and analgesic properties using the hexane and methanol extracts of the stem bark. Laupattarakasem *et al.* (2003) studied the antiinflammatory activity of aqueous and alcohol extracts of the leaves of *Acanthus ebracteatus*, stem bark of *Oroxylum indicum* and the stem of *Cryptolepis buchanani* and *Derris scanden*. Li *et al.* (2003) evaluated the antiinflammatory activity of ethanol extracts of nine vine plants used in the traditional Chinese medicine to treat inflammatory conditions.

The antiinflammatory activity of the aqueous extract of *Chromolaena odorata* was investigated in rats using the carrageenan induced oedema, cotton pellet granuloma and formalin induced oedema methods. The result has justified the traditional use of aqueous extract of *C. odorata* in the treatment of inflammation, since the extract has produced significant antiinflammatory activity (Owoyele *et al.*, 2005). The effect of methanol extract, petroleum ether and methanol fractions of the root bark of *Securidaca longipedunculata* on acute inflammation was evaluated by Okoli *et al.* (2006).

The antiinflammatory effects of saponin and iridoid glycosides from the flowers of *Verbascum pterocalycinum* were investigated by Akkola *et al.* (2007). Deb *et al.* (2007) investigated the antiinflammatory activity of the aqueous leaf extract
of *Eucalyptus globulus* in carrageenan induced paw oedema and cotton pellet granuloma technique in albino rats. The petroleum ether, ethyl acetate, ethanol and aqueous leaf extracts of *Calotropis gigantea* were screened by Patil *et al.* (2007) for their antiarthritic activities in albino rats. The petroleum ether, chloroform, methanol and aqueous extracts of *Sesbania sesban* leaves were investigated for their antiinflammatory activities in albino rats (Tatiya *et al.*, 2007).

The methanol extract of leaves of *Clerodendron infortunatum* was evaluated for antiinflammatory activity against the carrageenan, histamine and dextran induced rat paw edema. (Das *et al.*, 2010). The antiinflammatory effect of the aqueous fruit pulp extract of *Hunteria umbellata* was observed using the carrageenan and dextran induced rat paw edema, xylene induced ear edema and formalin induced arthritis inflammation tests. The results indicated that the aqueous extract of *H. umbellata* possess antiinflammatory activity which may be mediated by either inhibition or by blocking the release of prostaglandins and histamine, thus supporting the usage of the plant in traditional medicine (Igbe *et al.*, 2010).

Chippada *et al.* (2011) determined the *in vitro* antiinflammatory activity of methanol extract of *Centella asiatica* by HRBC membrane stabilization. The antiinflammatory property of the aqueous and alcohol extracts of the leaves of *Gendarussa vulgaris* was studied by both *in vitro* and *in vivo* methods. The alcohol extract at a concentration of 300 mg/ml showed potent activity on comparing with the standard drug diclofenac sodium (Mohamed Saleem *et al.*, 2011). Sutha *et al.* (2011) screened the ethanol leaf extract of *Erythropalum scandens* for its antiinflammatory activity in carrageenan induced paw oedema in albino rats.
Das et al. (2012) investigated the antiinflammatory property of the different parts of methanol extract of *Abroma augusta*. The result showed potent activity comparing with the standard drug, diclofenac sodium, due to the alkaloids and flavonoids present in the plant. The antiinflammatory activity of seed extract of *Entada pursaetha* was evaluated against carrageenan induced paw oedema. (Kalpanadevi et al., 2012). The whole plant ethanol extracts of *Polygala chinensis* and *Polygala javana* were evaluated for their antiinflammatory activity using carrageenan induced paw oedema by Alagammal et al. (2012). Balamurugan et al. (2012) reported the antiinflammatory activity of *Polycarpea corymbosa* against carrageenan induced paw oedema.

The antiinflammatory effects of the aqueous extract of *Acacia nilotica* pods, administered orally at doses of 50 and 100 mg/kg, were evaluated *in vivo* using various models of both acute and chronic inflammations. Xylene induced ear oedema in mice and carrageenan induced paw oedema were used to evaluate the acute effect of the plant extract. The aqueous extract of *A. nilotica* pods may contain orally effective antiinflammatory principles (Sokeng et al., 2013).

*In vitro* antiinflammatory activity of petroleum ether, ethanol and aqueous extracts of *Bombax ceiba* was evaluated by Anandarajagopal et al. (2013). Ethanol extracts showed significant response followed by aqueous extract when compared with standard, diclofenac potassium. The study suggested that the extracts possess enough potential to reduce inflammation by *in vitro*. The *in vitro* antiinflammatory activity of ethanol extract of stem of *Carmona retusa* was investigated by human red blood cell membrane stabilization method, heat induced hemolysis and proteinase
inhibitory activity. The results suggested that *Carmona retusa* can be a potential source of antiinflammatory agents (Chandrappa *et al.*, 2013).

Neha Mohan *et al.* (2013) investigated the antiinflammatory activity in ethanol extract of *Coriandrum sativum* using carrageenan induced paw oedema in albino rats. The *in vitro* antiinflammatory activity of petroleum ether extract of aerial parts of *Leucas lavandulaefolia* was evaluated by carrageenan induced paw oedema to determine the activity on acute inflammation and cotton pellet induced granuloma to determine the activity on chronic inflammation. The results suggested that petroleum ether extract of this plant possess potent antiinflammatory activity in both the model and the activity was dose dependent (Subraya and Satyanadayana, 2013).

The antiinflammatory activity of ethanol extract of *Euphorbia hirta* was assessed in lipopolysaccharide induced RAW 264.7 macrophages (Sharma *et al.*, 2014). Jami *et al.* (2014) designed to investigate the analgesic and antiinflammatory activities of the ethanol fruits extract of the *Terminalia chebula*. Sagnia *et al.* (2014) investigated the antiinflammatory activity of ethanol extract of leaves of *Cassia alata, Eleusine indica, Carica papaya, Eremomastax speciosa* and the stem bark of *Polyscias fulva*, collected in Cameroon. Gomes *et al.* (2014) evaluated the antiinflammatory effect of the ethanol extract of bark and leaves of *Cnidoscolus quercifolius* in mice using experimental models of inflammation. Ibrahim *et al.* (2014) reported the analgesic, antiinflammatory and anxiolytic activities of methanol extract of the leaves of *Sarcochlamys pulcherrima*. Ghosh *et al.* (2014) investigated the molecular mechanism of antiinflammatory action of the ethanol extract of *Dictamnus dasycarpus* leaf in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.
Antibacterial activity

The effect of plant extracts on bacteria has been studied by a large number of researchers in different parts of the world (Reddy et al., 2001; Ateb and Erdo, 2003). Agarry et al. (2005) have shown the potent antimicrobial activities of the gel and leaf of Aloe vera against a wide range of bacteria and fungi. Barberry and cranberry juice have been used to treat urinary infections while plant species such as lemon balm, garlic and tea tree are described as broad spectrum antimicrobial agents (Rios and Recio, 2005).

Mathabe et al. (2006) reported that methanol, ethanol, acetone and hot water extracts from different plant parts (leaves, roots, bark, stem and rhizome) of Indigofera daleoides, Punica granatum, Syzygium cordatum, Gymnosporia senegalensis, Ozoroa insignis, Elephantorrhiza elephantina, E. burkei, Ximenia caffra, Schotia brachypetala and Spirostachys africana showed remarkable antibacterial activity against Vibrio cholera, Escherichia coli, Staphylococcus aureus, Shigella species and Salmonella typhi.

Antimicrobial properties of leaf extract of Senna obtusifolia were investigated against both clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method (Doughari et al., 2008). The methanol leaf extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera and Ziziphus mauritiana showed significant antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis pv.malvacearum (Mahesh and Satish, 2008).
The methanol extract of forty nine different plant extracts were screened for antifungal activity, out of which forty three plants extracts exhibited varying degrees of inhibition activity against the fungi (Varaprasad et al., 2009). Senthilkumar and Reetha, (2009) reported that methanol extracts of *Aegle marmelos* and *Cassia auriculata* showed higher antibacterial activity against pathogenic bacteria.

Antibacterial activity of ethanol extract of *Acalypha wilkesiana* leaves was carried out to verify claims by the locals of its medicinal properties (Gotep et al., 2010). The extract was tested for the activity against *Staphylococcus aureus*, *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes*. Different organic and aqueous extracts of leaves of *Cassia occidentalis* were screened for their antimicrobial activity against seven human pathogenic bacterial and two fungal strains by disc diffusion assay (Arya et al., 2010). The *in vitro* antibacterial activity of ethanol, chloroform, ethyl acetate and aqueous extracts of leaves of *Plumeria rubra* has been evaluated using disc diffusion method against *Staphylococcus epidermidis* and *Escherichia coli* of bacterial strains (Baghel et al., 2010).

The ethanol extracts of leaf and flower of *Spathodea campanulata* were investigated for antimicrobial activity at 10 mg/ml concentration by using disc diffusion method against gram positive and gram negative organisms like *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas sps*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholera* (Kowti et al., 2010). Solanki (2010) described the antibacterial properties of *Cassia fistula*, *Acacia aroma*, *Azadirachta indica*, *Ziziphora clinopodioides*, *Argemone mexicana*, *Nephelium lappaceum*, *Punica granatum*, *Phyllanthus discoideus*, *Curcuma amada,*
Emblica officinalis, Nymphae odorata and Mallotus peltatus. The antibacterial activity of different plant extracts of Achillea millefolium, Ocimum basilicum, Thymus vulgaris and Urtica dioica and their phenolic phytochemical were evaluated against Paenibacillus larvae bacteria (Marghitas et al., 2011).

The antimicrobial activity of different solvent crude extracts of four medicinal plants (Ocimum sanctum, Ocimum giatissimum, Aegle marmelos and Adhathoda vasica) used in traditional Indian medicine was tested by disc diffusion method against five bacterial pathogens: Escherichia coli, Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi and klebsiella pneumonia (Prasannabalaji et al., 2012). The antimicrobial activities of aqueous, ethanol, ethyl acetate and hexane extract of eight plant species were studied. The extracts of leaves of Rosmarinus officinalis, Melissa officinalis, Urtica dioica, Laurus nobilis, Lavandula officinalis, Lavandula stoechas, Teucrium chamaedrys and Equisetum arvense were tested in vitro against two Gram positive (Bacillus cereus, Staphylococcus aureus), two Gram negative (Escherichia coli, Klebsiella pneumoniae) and Candida albicans by the microdilution method (Ceyhan et al., 2012).

Petroleum ether and methanol extracts of Cardiospermum halicacabum and Cardiospermum canescens leaves were investigated against Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes, Salmonella paratyphi, Escherichia coli, Bacillus subtilis and Klebsiella pneumoniae by using agar well diffusion method (Uma et al., 2013). Medicinal plant extracts prepared with various solvents from ten species, Murraya koenigii, Syzygium aromaticum, Piper nigrum, Ocimum tenuiforum, Laurus nobilis, Cinnamomum zeylanicum, Phyllanthus niruri, Cuminum cyminum,
Trilobatum sp. and Hibiscus rosasinensis were screened for antibacterial activity against bacterial pathogens by using disc diffusion method (Soniya et al., 2013).