Review of Literature
2. REVIEW OF LITERATURE

The Acanthaceae family under the order Scrophulariales comprises almost 250 genera with 2500 species. *Justicia* is the largest genus of Acanthaceae, with approximately 600 species that are found in pantropical and tropical regions (Durkee, 1986). Its species are widespread in tropical regions of the world (Wasshausen and Wood, 2004) and are poorly represented in temperate regions (Mabberley, 1997).

The species of *Justicia* are described as erect or scandent perennial herbs or subshrubs. Leaves present cystoliths and are petiolate with a leaf margin that is usually entire. Inflorescences are in spikes or panicles and the species rarely has solitary, terminal, or axillary flowers. The bracts and bracteoles are usually conspicuous and imbricate. The species of *Justicia* can be easily recognised by their bilabial corolla, with a posterior lip that is generally two-lobed, an anterior lip that is three lobed, two stamens, a capsule with four seeds and a basal sterile portion (Graham, 1990; Braz et al., 2002).

Though *Justicia* is the largest genus of the family Acanthaceae few species of *Justicia* had been studied extensively (36 species of approximately 600 cataloged species), with fifteen species found in the Americas, thirteen species in Asia, and eight species in Africa. Among the studies species, 31 species have ethnopharmacological or pharmacological information, 23 species were chemically investigated, and only eighteen
species were chemically and biologically studied, mainly in the last
decade. The most studies species are *Justicia adhatoda* L. *Justicia
pectoralis* Jacq., *Justicia procumbens* L., *Justicia gendarussa* Burm. F. and
*Justicia anselliana* (Nees) T. Anderson. Consequently, the phytochemical
and biological potential of other species of *Justicia* have yet to be fully
explored (Correa and Alcantara, 2011).

2.1 Pharmacognostical studies

Taxonomic identification depends largely on the exomorphology of plants,
since it is visible and convenient. But when exomorphology is found
wanting, the micromorphological details are used for identification.
Employing micromorphological features in plant identification goes back a
long way. Starch grains (Reichert, 1913; Takeoka, 1962), raphides
(Gulliver, 1886; Gibbs, 1963; Tomlinson, 1962; Kharchenko, 1928), silica
(Tomlinson, 1961), and gypsum (Brunswick, 1920) have been
conveniently employed in taxonomy. Crystals of calcium oxalate, the
cystoliths have also similar significance.

Despite the modern techniques, identification of the plant drugs by
pharmacognostic studies is more reliable. According to the World Health
Organization, the macroscopic and microscopic description of a medicinal
plant is the first step towards establishing the identity and the degree of
purity of such materials and should be carried out before any tests are
undertaken (WHO, 2002).
Very detailed anatomical details of stem, leaves, petiole, root and other parts of *J. adhatoda* have been reported (Aiyer and Kolammal, 1963). Young stem of *J. adhatoda* bears numerous glandular hairs having uni-cellular stalk and a quadricellular head. Pericycle in mature stem is thick-walled. Stele of the petiole consists of three vascular bundles of which the central one is the largest and fan shaped. Transverse section of leaf shows dorsiventral structure with two layers of palisade cells. Upper epidermal cells in surface view are more or less uniform in size, but extremely sinuous in outline. Lower surface shows variation in their size and is less wavy. Both the epidermis shows the presence of diacytic stomata. Hairs, both glandular and non-glandular, are present on each surface of the leaf (Aiyer and Kolammal, 1963; Chaudhari and Inamdar, 1984; Sharma *et al.*, 2000). Microscopically, the leaves show a palisade layer with elongated cystoliths. These cystoliths are also found in the spongy parenchyma below, while absent in the epidermal cells. The cells of the spongy layer are spread uniformly in 7 or 8 horizontal layers. Globules of oil are found dispersed throughout in the palisade and spongy layers. The epidermis shows one to three celled walled warty trichomes. Glandular trichomes are found projecting from under the surface where numerous stomata are seen (Krishnaswami and David, 1940).

Outermost layer of the root namely the phellem is comparatively thin and composed of 5 to 10 rows of thin walled rectangular to slightly
tangentially elongated cells. Phellogen is present within the phellem layer as a single row of narrow thin walled tangentially elongated cells. Next is a zone of 3 to 5 rows of thin walled tangentially elongated cells called phelloderm. Cortex in the middle is composed of large, thin walled, polygonal to tangentially elongated cells containing starch grains. Towards the periphery of the cortex lysogenously formed air spaces are present. Towards the inner region of the cortex discontinuous band of stone cells are present. Wood occupies the major part of the root and is composed of wood fibers, wood parenchyma and medullary rays. Four groups of primary xylem are present towards the centre of the wood. Uni or bi-seriate medullary rays are present extending from the centre of the root up to the zone of stone cells. The upper epidermis of the leaf consists of a layer of polygonal cells having wavy walls. Stomata are absent in the upper epidermis. A few stalkless glands with 4-celled heads are found in the depressions of the upper epidermis (Aiyer and Kolammal, 1963). Leaf anatomy of *J. adhatoda* grown under two light regimes had been studied and the biomass yield of *J. adhatoda* under different conditions was reported (Singh and Madan, 1987; Singh and Madan, 1990). Significant decrease in leaf thickness of plants grown under shade was reported, which could be attributed to a decrease in intercellular space and cell number in palisade layer (Neerakkal et al., 2001).

Patil and Patil (2011) studied foliar anatomical features of 43 species belonging to 22 genera of the family Acanthaceae and showed that
cystoliths are characteristics of Acanthaceae family. However, they are totally absent in *Acanthus spinosus* L., *Adhatoda beddomei* C.B. Clark and *Staurogyne zeylanica* O.Ktze. Sonal and Maitreyi (2011) conducted Pharmacognostic study of the root of *Justicia gendarussa* and reported that the cork of *J. gendarussa* consists of 2-3 layers, cells are radially elongated, tubular and compactly arranged. The outer cortex is compact and the inner cortex has got air chambers. Stone cells are also present in the cortex. There is distinct endodermis layer and the vascular bundles are collateral and conjoint.

Dale and Kalme (2012) conducted pharmacognostic characterization of stem and root of *Adhatoda zeylanica* Medicus (*Justicia adhatoda*). Microscopical and macroscopical examinations of the different organs and differential microchemical tests have been carried out. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values like petroleum ether-soluble extractives, alcohol-soluble extractives and water soluble extractives were calculated as well as histochemical tests and colour reactions with different chemicals were performed. Histochemical analysis showed that on the epidermis of the stem glandular and non-glandular trichomes are observed. The cortical parenchymatous cells of the stem show presence of starch, protein, alkaloids, tannin, while the pith parenchymatous cells of the stem shows presence of tannin and fat. Root shows the presence of starch, protein, fats, saponin and glycoside in the cells of the cortex (Dale and
Kalme, 2012). The macroscopy, microscopy, quantitative analysis, extractive values in ethanol and water, phytochemical screening and TLC of the leaf of *Justicia adhatoda* was investigated by Abhishek *et al.* (2014) and the reported pharmacognostic profile of *Justicia adhatoda* Linn. Leaf was helpful in sample identification, quality and purity standards.

### 2.2 Phytochemical Studies

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The interest in nature as a source of potential chemotherapeutic agents still continues. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. Higher plants contribute no less than 25% of the total (Fransworth *et al.*, 1985; Cragg and Newman, 2005). It has been established that upto 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin (Mahesh and Sathish, 2008). Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing (Wadood, *et al.*, 2013) Phytochemicals are chemical compounds that occur naturally in plants and have biological significance. Some researchers estimate that there are upto 4,000 different phytochemicals having potential to affect diseases such as cancer, stroke or metabolic syndrome. Scientists have identified thousands of them although only a small fraction of phytochemicals have been studied closely. The accumulation of phytochemicals in plants has
been studied for more than four decades and the generated knowledge has helped in realization of using desired phytochemicals.

The plants are important natural sources of medicines and pharmaceutical products (Kumar, 2004; Patwardhan et al., 2004). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro et al., 2000). Phytochemicals naturally occur in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect the plants from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah et al., 2007).

The ever increasing global interest in Ayurveda way of life has resulted not only in demand for a huge raw material of medicinal plants but also the right stage of the plant or plant part when the active principles are available in optimum quantities for herbal preparations (Chaturvedi, 2001; Shinwari, 2010).

The active principles of many drugs found in plants are secondary metabolites (Ghani, 1990). The majority of populations in many developing countries use herbal medicines either because herbal medicines are more affordable than western pharmaceuticals or because herbal medicines are more acceptable (Cunningham, 1991). Arumugasamy et al., (1993) studied the distribution of various secreting
glands of neem tree and made phytochemical analysis of the same. Phytochemical analyses of various medicinal plants found in United Arab Emirates have been screened thoroughly by Tanira et al. (1994). Tripati et al. (1996) reviewed the occurrence of secondary metabolites in piper species. Work of Anuba and Bhargava (1996) revealed the presence of various phytochemicals in as many as 22 multipurpose tree species. Coe and Anderson (1996) screened 229 medicinal plant species to assess the chemical nature of their bioactive compounds. Chaudhuri (1998) reported many non-alkaloidal constituents from the seeds of *Gloriso superba*. Phytochemical investigations of phenolic compounds and fatty acids in several medicinal plants were carried out by Lin et al., (1999), Das et al., (1999a) and Karthikeyini and Sasikumaar (2001). The presence of alkaloids, carbohydrates, phenolics, flavonoids, triterpenes, tanins, oil deposition and stearic acid has been reported by several investigators in several species of medicinal importance (Kitajima et al., 2002; Ali et al., 2003).

Medicinal properties of the *Justicia* species were investigated extensively during the past few years. *Justicia* is one of the largest and complex genus of the family Acanthaceae. Phytochemical studies on the species of this genus have shown that they are important in traditional medicine for treatment of bronchitis and asthma, urological infections, endometritis, urinary disease, cystitis, leucorrhoea and pains. The phytochemical analysis showed that phenols, tannins, alkaloids, anthraquinone,
Saponins, flavonoids and reducing sugars were found in the leaves of *J. adhatoda* (Pathak, 1970). However, the pharmacologically studied chemical component in *Justicia adhatoda* is a bitter quinazoline alkaloid, vasicine (1,2,3,9-tetrahydropyrrole (2,1-b) quinozolin-3-ol, C$_{11}$H$_{12}$N$_{2}$O) which is found in the leaves, roots, and flowers. Besides vasicine, the leaves also contain several other alkaloids (vascinone, vasicinol, adhatodine, adhatonine, adhvasinone, anisotine, and hydroxypeganine), betaine, steroids, and alkanes (Lahiri and Prahdan, 1964; Chowdhary, 1987). Bhat *et al.* (1978) also reported the chemical compounds found in *J. adhatoda* plant as essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamins C.

Phytochemical aspects like isolation and characterisation of different compounds from different parts of *J. adhatoda*, using different techniques have been studied in detail by various researchers over the years (Nakagawa and Stevens, 1873; Johne *et al.*, 1973; Gupta and Jain, 1979; Jain *et al.*, 1980; Zutshi *et al.*, 1980; Dhar *et al.*, 1981; Bhalla *et al.*, 1982; Bhartiya and Gupta, 1982; Jain and Sharma, 1982; Brain and Thapa, 1983; Chowdhary and Bhattacharyya, 1985; Gupta and Jain, 1985; Jain and Srivastava, 1986; Choudhary, 1987; Jain and Atal, 1987; Kumar *et al.*, 1987; Sing *et al.*, 1991; Wasserman and Kuo,1991; Sharma and Lal, 1992; Singh *et al.*, 1992; Muller *et al.*, 1993; Thappa *et al.*, 1996; Choudhury and Chakrabarti, 1997; Abd and Ahmed, 1998; Ahmed and Abd, 1999; Johri and Zutshi, 2000).
J. adhatoda is a primary herb in the ayurvedic system and is used in the treatment of coughs, bronchitis, asthma and symptoms of common cold (Karthikeyan et al., 2009). Different parts of J. adhatoda such as leaves, flowers and roots contain various compounds such as vasicine, vasicinine, vasicoline, vasicinone, vasicolinone, vascinolone, vasnetin, vasicol, vasicinol, vasakin, vasinone, vasicinine, adhatonine, adhavasinone, deoxyvascinone, anisotin, desmethoxyaniflorine, luteolin, quercetin, triacontane, a-amurin, kaempferol, peganine, adhatodic acid, carotene, gycosides, N-oxides of vasicine, deoxyvascin, maiontone 7-methoxyvascinone, 2-hydroxy-4-glycosyloxychalcone, 3b-D-glucoside, 3-sophoroside, β-sitosterone-D-glucoside, galactose,0-ethyl-α-D-galactopyranoside, D-galactose, triacontine, β-sitosterone-β-D-glucoside, 2,3,9-tetra hydro-5-methoxypyrolo (2,1-b) quinasolin-9, 9-acetamido-3,4-dihydropyrido (3,4-b) indole, 2-hydroxy-4-glycosyloxychalcone, 37-hydroxyhexateracont-en-15 one, 37-hydroxy hentetracontan-19-one, 3-hydroxyanisotine, 2-4-dihydroxychalcone, 9-acetamido-3,4-dihydropyrido (3,4-b) and indole, 0-ethyl-alpha-D-galactoside (Sen and Ghosh, 1925; Bhatnagar and Popli, 1966; Atal, 1980; Jain et al., 1980; Dhar et al., 1981; Bhartiya and Gupta, 1982; Jain and Sharma, 1982; Anonymous, 1985; Chawdhary and Bhattacharyya, 1985, Singh et al., 1991; Joshi, et al., 1994; Thappa et al., 1996; Choudhary and Chakrabarti, 1997; Prajapati et al., 2003).
The leaves of *A. zeylanica (J. adhatoda)* have been found to be a rich source of alkaloids of which vasicine and vasicinone are bioactive. A number of non-nitrogenous compounds have also been isolated. Sen and Ghosh (1925) isolated a solid from the leaves of the plant named as vasicine for the first time which was basic in nature. Another bronchodilator alkaloid vasicinone was later isolated from the leaves. It was identified as the autooxidation product of vasicine (Amin and Mehta, 1959; Mehta *et al*., 1963). Further, a non-nitrogenous neutral principle, vasakin (Inamdar *et al*., 1966); (+) -vascinone (Poi and Adityachaudhury, 1988); two new quinazoline alkaloids, one of which was named as adhavasinone (Chowdhury and Bhattacharyya, 1985, 1987) and two new pyrrologquinazoline alkaloids, desmethoxyaniflorine and 7-methoxyvasicinone were identified from the ethanolic extract of the leaves (Thappa *et al*., 1996).

The percentage of the alkaloids in *J. adhatoda* varies with the season. The plant has rich alkaloid content in August-October and decreases after October and reaches a minimum level in March (Pandita *et al*., 1983). The yield of the alkaloid vasicine from different samples of *J. adhatoda* in India ranged from 0.541 to 1.105 percent on dry basis. Yield as high as 2.18 per cent on dry basis has been reported from a foreign sample of which more than half was the l -form and the remainder the dl-form of the alkaloid (Anonymous, 1985). Seasonal variations and the distribution of vasicine in 5 different parts (leaves, inflorescences, petioles, stem and
roots) of *J. adhatoda* growing in Sri Lanka were studied by a TLC-UV densitometric technique. The greatest amount of vasicine was found in the inflorescences; vasicine levels in most parts of the plants were highest in July-Sept. (Arambewela *et al*., 1988). Seasonal variation of alkaloids of cultivars of *J. adhatoda* contents was reported (Pundarikakshudu and Bhavsar, 1988). Vasicinone was absent from diseased leaves of *J. adhatoda* infected with *Colletotrichum gloeosporioides* and *Fusarium oxysporum*, but no differences were observed in the phenolic compounds of healthy and diseased leaves (Parikh and Daniel, 1992). Chauhan *et al.* (1999) have reported the vasicine content in crude *J. adhatoda* leaf extracts as 0.627% and the vasicinone content as 0.060%. The concentrations of total alkaloids in leaves of 9 to 10 year old plants of *J. adhatoda* were determined throughout the year. Some variations were observed, but compared with young plants all leaves of 9 and 10 year old plants contained high concentrations of alkaloids (Rajani and Pundarikakshudu, 1996).

The medicinal properties of *J. adhatoda* are well known in India and several other countries for many years. The roots contained vasicinolone, vasicol, peganine and 2-hydroxy-4-glucosyl-oxychalcone. The flowers contained D-glucoside, kaempferol and its glucosides as well as the bioflavenoid, namely, quercetin (Rawat *et al*., 1994). The leaves are mostly used in the treatment of respiratory disorders in Ayurveda. The leaves contain an essential oil and the alkaloids quinazoline, vasicine,
vasicinone and deoxyvasicine (Shinawie, 2002). The alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity (Baquar, 1997); whereas, vasicine at low concentrations, induced bronchodilation and relaxation of the tracheal muscle. Vasicinone, the auto-oxidation product of vasicine has been reported to cause bronchodilatory effects both in vitro and in vivo (Shinawie, 2002). Gulfraz and his co-workers (2004) observed that roots of J. adhatoda contained higher concentration of protein fat and alkaloids like vasicine and vasicinone and the leaves contained higher concentration of sugar and vitamin C.

Mitra and Prasad (2010) studied the alleopatic activity of J. Adhatoda. The aqueous leaf and flower extracts showed inhibitory effects on seed germination and seedling survival of turnip. Remarkably lesser inhibitory effect of the flower extract was noted at all the doses.

HPLC determination of vasicine and vasicinone in J. adhatoda with photodiode array detection has been reported by Srivastava et al (2001). In another study (in leaf, stem and root) using High Performance Thin Layer Chromatography (HPTLC), the concentration of the alkaloids, vasicine and vasicinone was found to be more in the leaves (Das et al., 2005). Chavan et al. (2013) reported the qualitative presence of tannins, flavonoids, alkaloids, and saponins as the major phytochemicals in J. adhatoda leaf extracts by performing HPTLC.
The medicinal value of *J. adhatoda* is due to the presence of small doses of active compounds which produce physiological actions in the human and animal body. Some important bioactive compounds that have been reported in various parts of *J. adhatoda* are essential oil and quinazoline alkaloids; hence extract of *J. adhatoda* could form one of the best option for developing novel natural medicine (Dhankhar *et al.*, 2011). The wound healing and antimicrobial activity of the leaf extract of *J. adhatoda* has been reported by Subhashini *et al.* (2011). The alkaloids from *J. adhatoda* have reported excellent antibacterial activity against the most resistant bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa* and the highly pathogenic bacteria like *Salmonella typhi* (Sawant *et al.*, 2013).

The preliminary phytochemical investigations of *Justicia beddomei* leaves showed the presence of alkaloids, tannins and disaccharides (Harbone, 1988). The ethanolic *A. beddomei* extract revealed the presence of vasicine, vasicine, vasicinone, deoxyvasicinone, B-sitosterol and its glycoside (Jain and Srivastava, 1986). Srinivasa *et al.*, (2007) showed that the ethanolic extracts of *J. beddomei* leaves possess significant analgesic activity at 90 minutes of administration of test extracts and its effect is less than that of morphine sulphate (standard). The analgesic activity was evaluated in albino rats using Eddy’s hot plate method. The extract was found to produce marked analgesic effect due to the presence of alkaloids, carbohydrates etc. Another study by the same author to investigate the phytochemical constituents and *in vitro* anti-oxidant
potential of the aerial parts of *J. beddomei* showed that the ethanolic extract possess significant anthelmintic activity that can be comparable with the standard drug Piperazine citrate (Srinivasa et al., 2007).

Gottumukkala et al. (2004) isolated a new indole(3,2-b)quinoline alkaloid glycoside, jusbetonin, and three known alkaloids, namely, 10H-quinindoline, 6H-quinindoline, and 5H, 6H-quinindoline-11-one from the leaves of *Justicia betonica*. From the aerial part of *J. betonica* L., four triterpenoidal glycosides (justiciocides A-D) were also isolated (Kanchanapoom et al., 2004).

Chemical analysis of the aerial part of *J. gendarussa* afforded β-sitosterol, β-sitosterol-β-D-glycoside and aromadendrin (Bachheti et al., 2011). Investigation on *J. gendarussa* showed potent anti-inflammatory and analgesic activity (Jothimanivanan et al., 2010). Methanol extract of *J. gendarussa* can inhibit protein synthesis *in vitro*, therefore, they may be used to treat or prevent some bacterial disease caused by *Staphlococcus aureus* (Kowsalya and Sankaranarayanan, 2012). Other than the widespread conventional use of *J. gendarussa* Burm for treating various disorders, Subramanian et al. (2013) reported that the ethanolic extract of *J. gendarussa* has an effective anti-anxiety effect in mice in several animal models of anxiety like Elevated Plus Maze (EPM) and Light Dark test models (Subramanian et al., 2013).

Extensive literature survey has shown that not much work has been done on *J. wynaadensis*. One publication is the patent on the cholesterol lowering properties of *J. wynaadensis* by Subbiah et al. (2002) which
reports that the plant extract lowers cellular cholesterol and cholesteryl concentration. Their studies also have shown a novel inhibitory effect on the uptake of ox-LDL by human macrophage cell line. Medapa et al. (2011) estimated the polyphenols and flavonoids in the leaf and stem of *J. wynaadensis* and also studied catalase and peroxidase activity along with the evaluation of antioxidant property and reducing property of the plant extract and found that the reducing power of the *J. wynaadensis* leaves and stem extract were significant. GC-MS analysis of phytocomponents in the methanolic extract of *J. wynaadensis* ascertained its usage by the local community as ‘a plant possessing medicinal properties’ and 24 compounds were identified. The major constituents are Dihydrocoumarin, phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols are also estimated (Ponnamma and Manjunath, 2012).

Preliminary phytochemical screening and an acute toxicity testing of the *Justicia hypocrateriformis* plant extract were carried out by Agbor and his co-workers (2014). They reported that *J. hypocrateriformis* extract possesses antidiarrhoeal activity supported by its antioxidant potential and phytochemical constituents. Further phytochemical screening revealed the presence of phenols, tannins, flavonoids, saponins, anthraquinones, and anthocyanins in *J. hypocrateriformis*. Madhukar et al. (2014) tried to develop and validate a new, rapid, and highly sensitive ultra-performance liquid chromatography/ quadrupole-time-of-flight mass-spectrometry
(UPLC/Q-TOF-MS) method for the quantitative estimation of vasicine in the leaves and to establish in vitro cultures of *Adhatoda vasica* for production of vasicine. Adia *et al.* (2014) surveyed the documentation of plants used in malaria treatment by Prometra Herbalists in Uganda and in their investigation they found that *Justicia betonica* L. were highly cited as being used in malaria treatment.

2.3 Molecular Studies

2.3.1 DNA Barcoding

DNA barcoding involves the generation of DNA sequencing data from particular genetic regions in an organism and the use of these sequence data to identify or “barcode” that organism and distinguish it from other species (Schori and Showalter, 2011). It is a method of identifying an organism based on sequence data from one to several regions. Barcoding works by matching sequence data from a query sample (an unknown specimen) to a reference sequence (from a voucher specimen).

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism’s DNA to identify it as belonging to a particular species (Herbert *et al.*, 2003). It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a pre-existing classification.

The use of nucleotide sequence variation to investigate evolutionary relationships is not a new concept. The limitations inherent in morphology
based identification systems and the dwindling pool of taxonomists signal the need for a new approach to taxon recognition. Microgenomic identification systems, which permit life’s discrimination through the analysis of a small segment of the genome, represent one extremely promising approach to the diagnosis of biological diversity (Hebert et al., 2003). This concept has already gained broad acceptance among those working with the least morphologically tractable groups, such as viruses, bacteria and protists (Nanney, 1982; Pace, 1997; Allander et al., 2001). However, the problems inherent in morphological taxonomy are general enough to merit the extension of this approach to all life. In fact, there are growing numbers of cases in which DNA based identification systems have been applied to higher organisms (Brown et al., 1999; Bucklin et al., 1999; Trewick 2000; Vincent et al., 2000).

DNA sequence databases like GenBank contain many sequences that are not tied to vouchered specimens (for example, herbarium specimens, cultured cell lines, or sometimes images). Herbert et al. (2003) proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library would provide a new master key for identifying species, one whose power will rise with increased taxon coverage and with faster, cheaper sequencing. Woese (2005) used sequence difference in ribosomal RNA (rRNA) to discover archaea, which in turn led to the redrawing of the evolutionary tree and molecular markers have been successfully used in molecular systematics. Molecular
barcoding provides a standardised method for this process through the use of a short DNA sequence from a particular region of the genome to provide a ‘barcode’ for identifying the species.

Anvarkhan et al. (2013) conducted experiment to identify three species of genus *Allium* using five barcode regions including *ndhJ*, *rpoC1*, *rpoB*, *YCF5* and *rbcl* and reported that *ndhJ* is the most effective barcoding locus for the tested *Allium* species and the barcode locus of *YCF5* had the highest sequencing percentage with 93.33%.

Several chloroplast gene regions are typically used as plant barcodes, with muturase K (*matK*) and ribulose 1,5-biphosphate carboxylase/oxygenase large subunit (*rbcL*) considered core barcodes (Hollingsworth et al., 2009). Herbert et al. (2003) and his co-workers reported that the mitochondrial gene cytochrome oxidase I (COI) can serve as the core of a global bio-identification system for animals. However, finding a plant equivalent has proved difficult. Different research groups/research consortia from systematic community came out with various suggestions. The majority preference of the CBOL (Consortium for the Barcode of Life) Plant Woking group recommended a core-barcode consisting of portions of two plastid coding regions, *rbcl*+*matK*, to be supplemented with additional markers as required (CBOL Plant Working Group, 2009). The choice of *rbcl*+*matK*, as a core barcode was based on the straight forward recovery of the *rbcl* region and the discriminatory power of the *matK* region. *matK* is one of the most
rapidly evolving coding sections of the plastid genome and is perhaps the closest plant analogue to the COI animal barcode. But, \textit{matK} can be difficult to PCR amplify using existing primer sets. In contrast, the barcode region of \textit{rbcL} is easy to amplify, sequence, and align in most plants and provides a useful backbone to the barcode dataset (Hollingsworth \textit{et al.}, 2011).

Plant DNA barcoding has multiple applications which include identifying plant leaves even when flowers or fruit are not available, identifying insect larvae, identifying the diet of an animal based on its stomach contents and identifying products in commerce (Kress \textit{et al.}, 2005; Soininen \textit{et al.}, 2009). Barcodes are sometimes used to identify unknown species or to assess whether species should be combined or separated (Lambert \textit{et al.}, 2005). Lambert and his co-workers (2005) also examined the possibility of using DNA barcoding to assess the past diversity of Earth’s biota. DNA barcoding provides insights into species-level taxonomy and contributes towards the taxonomic process of defining and delimiting species and also to assist in the process of identifying unknown specimens to known species (Hollingsworth \textit{et al.}, 2011). It has also been used for ecological surveys (Dick and Kress, 2009), cryptic taxon identification (Lahaye \textit{et al.}, 2008) and confirmation of medicinal plant samples (Xue and Li, 2011).
2.3.2 RAPD Analysis

DNA marker systems have been effectively used for genetic variation analysis (Lee, 1995). These markers can identify many genetic loci simultaneously with an excellent coverage of an entire genome, are phenotypically neutral, and can be applied at any development stage (Jones et al., 1997a). Furthermore, molecular markers are not subject to environmental change, making them especially informative and superior to traditional methods of genotyping such as the use of morphological traits and biochemical markers (Tanksley et al., 1989; Messmer et al., 1993; Melchinger et al., 1994).

Molecular marker techniques include restriction fragment length polymorphisms (RFLPs); simple sequence repeats (SSRs) or microsatellites, random amplification of polymorphic DNA (RAPDs) and amplified fragment length polymorphisms (AFLPs). These techniques differ in their principles and applications and generate different amounts of data points (Das et al., 1999).

RAPD is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence (Williams et al., 1990). These primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphisms function as genetic markers and can be used to construct genetic maps. Since most of the RAPD markers are dominant, it is not possible to distinguish whether the amplified DNA segment is heterozygous (two different copies) or homozygous...
(two identical copies) at a particular locus. In rare cases, co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, may be detected (Williams et al., 1990).

The basic technique of RAPD involves (i) extraction of highly pure DNA, (ii) addition of single arbitrary primer, (iii) polymerase chain reaction (PCR), (iv) separation of fragments by gel electrophoresis, (v) visualization of RAPD-PCR fragments after ethidium bromide staining under UV light and (vi) determination of fragment size comparing with known molecular marker with the help of gel analysis software. It is important to note that RAPD technique requires maintaining strictly consistent reaction conditions in order to achieve reproducible profiles. In practice, band profiles can be difficult to reproduce between (and even within) laboratories, if personnel, equipment or conditions are changed (Karp et al., 1997). Despite these limitations, the enormous attraction of this technique is that there is no requirement for DNA probes or sequence information for primer design. The procedure involves no blotting or hybridizing steps. The technique is quick, simple and efficient and requires only the purchase of a thermocycling machine and agarose gel apparatus and relevant chemicals, which are available as commercial kits (e.g., Ready-To-Go RAPD analysis beads; GE Healthcare, Buckinghamshire, UK). Another advantage is the requirement for only small amounts of DNA (10–100 µg per reaction) (Karp et al., 1997).
The RAPD markers have been used to detect genomic variations within and between varieties of sweet potato. A total of 160 primers were tested and eight showed consistent amplified band patterns among the plants with variations within and between varieties (Lin et al., 2009) of sweet potato. Genetic diversity was evaluated by RAPD markers and morpho-agronomic characters for a total of 42 accessions of Barberton daisy (Gerbera jamesonii) employing a set of 12 primer pairs (da-Mata et al., 2009). Germplasm accessions of 80 Plantago species were studied using RAPD with the help of 20 random primers (Singh et al., 2009). Lately, RAPD has been used for estimation of genetic diversity in various endangered plant species (Zheng et al., 2008, Liu et al., 2007, Wang et al., 2005 and Lu et al., 2006). Genetically more reliable DNA markers of RAPD and ISSR were utilised to study the genetic relationships among J. gendarussa Burm. F. collected from four locations in Tamil Nadu and the phenogram was constructed using data obtained by UPGMA method to disclose its alterations that could have occurred during evolution and the results exhibited that apart from geographical factors, ecological factors could also be responsible for genetic diversity in plant species (Kalaiwani and Fathima, 2013). Kumar et al. (2014) used DNA based dominant molecular marker techniques, RAPD and ISSR (inter-simple sequence repeat) to unravel the genetic variability and relationships across thirty two wild accessions of J. adhatoda L. Molecular markers were also used as a tool in bacterial taxonomy (Wilson, 1995).
2.4 Pharmacological Studies

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. In recent years, antimicrobial properties of medicinal plants are being reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The literature survey revealed that *J. adhatoda* has been widely studied for its pharmacological activities and regarded as Universal Panacea in Ayurvedic medicines and finds its position as a versatile plant having a wide spectrum of medicinal activities. Dhar *et al.* (1968) reported that the ethanolic extract of *A. vasica* exhibited hypoglycaemic activity in rats. Modak and Rao (1996) found that when non-nitrogenous principle of the leaves in suspension form administered orally at the dose of 25 mg/kg, lowered the blood sugar level of rabbits for a short period of time.

The uterotonic activity of vasicine obtained from *A. zeylanica* was studied in detail both by *in vitro* and *in vivo* methods employing the uteri under different hormonal influences and of different species of animals. Vasicine also showed uterotonic activity in human myometrium strip and abortifacient activity in guinea pigs in a preliminary investigation. Vasicinone however, was devoid of uterotonic activity (Gupta *et al.*, 1977a).

Vasicinone was found to have a potent bronchodilatory activity in both *in vitro* and *in vivo* studies. In isolated tracheal chain of guinea pig, vasicinone (100-800 ug/ml) produced a dose dependent relaxation of
tracheal smooth muscles. The bronchoconstrictions induced by histamine and carbachol were also inhibited by vasicinone (2.5 to 20 mg/kg i.v.). It possessed antianaphylactic activity in both in vitro and in vivo studies in rats as was evidenced by its inhibitory activity on the release of histamine (Bhide et al., 1974). On comparing with vasicine, it was found that vasicinone was a bronchodilator, weak cardiac stimulant and a potent antianaphylactic agent while vasicine was a bronchoconstrictor, cardiac depressant and devoid of antianaphylactic activity. The study also revealed that a minor change in the structure of vasicine induced vast changes in its pharmacological properties (Bhide et al., 1976).

In a screening study of anti-fertility activity of J. adhatoda, after administration of extract of leaves either in mice or in rats, no effects on the pregnancy were recorded (Bhaduri et al., 1968). But the animals treated with about 100 mg/kg of different J. adhatoda extracts did not show any implantation sites (Prakash et al., 1985). The effect of J. adhatoda leaf extract on early gestation was studied. There was no effect on the maternal body weight or any other parameter recorded in the form of statistically significant differences between the treated and control animals. Analysis of J. adhatoda leaf extract showed that it contained the vasicine ranges from 0.0541 to 1.105% (Bhaduri et al., 1968). Seasonal variation in vasicine content in Adhatoda species grown under different north Indian plain conditions has also been reported (Bagchi et al., 2003).
*J. adhatoda* being a rich source of nitrogen is grown as green manure in rice fields and also in tobacco and tea gardens. When grown in rice fields, it acted as an aquatic weedicide. The weedicidal properties have been attributed to the volatile principle present in the plant (Anonymous, 1985). The leaves are used as an insecticide in the same way as tobacco leaves. An infusion of the leaves is used against white ants and the red Spiders of tea. The leaves are used to cover fruits and are reported to prevent the growth of mould as a fungicide. Antiviral properties of *J. adhatoda* have also been reported (Singh and Singh, 1972). Stem bark extract of *J. adhatoda* possesses antiviral activity against potato virus X (Saha and Kalyanasundaram, 1962). Several researchers investigated the various pharmacological activities of Vasicine and vasicinone isolated from *J. adhatoda*. Synergetic effect of vasicinone on vasicine in the bronchiodilation, as well as an increase in ciliary movements has been reported by Gupta *et al.* (1977). Another study by the same researcher revealed that Vasicine and vasicinone mixed in 1:1 ratio showed more bronchodilatory activity and antagonism against histamine induced bronchoconstriction as compared to vasicine alone (Gupta *et al.*, 1977).

*A. vasica* is used to treat asthma, cough, bronchitis, fever, diarrhoea, dysentery, haemoptysis, rheumatic and painful swellings, tuberculosis and infective hepatitis (Vakil *et al.*, 1974; Sivarajan and Balachandran, 1994). Leaves, flowers and roots of this plant are used in herbal drugs against cancer (Kulkarni, 1998; Pandey, 2002) and tuberculosis (Gupta and Chopra, 1954).
Fresh flowers are bound over the eyes in ophthalmia (Nadkarni, 2001). Roots-antiviral, Leaf- antispasmodic, Root and Leaf are hypoglycaemic (Modak and Rao, 1996). Alkaloid vasicinone is bronchoconstrictor (Amin and Mehta, 1959; Mehta et al., 1963) and vasicine is a respiratory stimulant (Chandel et al., 1996). Effect of *A. vasica* extract on the central nervous system has been reported by Debelmas and Hache (1976). Insect antifertility and antifeedant allelochemics in *Adhatoda vasica* were also reported (Saxena and Tikku, 1986). The pharmacokinetics of the plant is also reported by Amla et al. (1987). The use of the plant against joint pain, swellings on joints and neck and inflammation of eyes were also investigated (Anuraaga, 1999). Vasicine hydrochloride possesses significant thrombopoetic action in animals and potential use in the management of hemorrhagic disorders (Atal, 1980). The wood of *A. zeylanica* is used for gunpowder charcoal and as fuel for brick-burning. Beads and rosaries are made from the wood. The pollen grains of *A. vasica* are suspected to cause allergy in susceptible persons (Anonymous, 1985). ‘Vasaka’ in combination of *Aloe indica* has been reported to be useful in patients with cough and cold (Swarge and Kulkarni, 1990). *A. zeylanica* is one of the constituents of a herbomineral preparation ‘svasa kuthararasa’ and an Ayurvedic preparation ‘shereeshadi kashaya’ (*Albizia lebbeck, Solanum xanthocarpum, Glycyrrhiza glabra* and *A. zeylanica*) which have been reported as useful in patients suffering from bronchial asthma in a project study at a hospital in Varanasi (Chandra et al., 1996).
It was also observed that the cardiac depressant effect manifested by vasicine was corrected by vasicinone. Vasicine showed bronchodilatory activity both *in vitro* and *in vivo*. Vasicinone showed bronchodilatory activity *in vitro* but bronchoconstrictory activity *in vivo*; it is probably biotransformed *in vivo*, causing bronchoconstriction. L-forms of vasicine and vasicinone are more active than their racemic form (Anonymous, 1985). The alkaloids from *J. adhatoda* were found to offer pronounced protection against allergen-induced bronchial obstruction in guinea pigs when administered at the dosage of 10 mg/ml of aerosol (Dorch and Wagner, 1991). Anaphylactic activity and control of postpartum haemorrhage of vasicinone has been reported (Anonymous, 1985). *J. adhatoda* is reported to have anti-inflammatory activity (Chakraborty and Brantner, 2001; Rajput *et al.*, 2004). The alkaloid fraction from *J. adhatoda* showed potent anti-inflammatory activity at a dose of 50 µg/pellet equivalent to that of hydrocortisone while the MeOH extract and the other fractions showed less activity (Chakraborty and Brantner, 2001). Antitussive activity of *J. adhatoda* extract is also reported (Dhuley, 1999). Antifeedant and ovipositional deterrent to tea mosquito bug (*Helopeltis theivora*) of *J. adhatoda* has been reported (Deka *et al.*, 1999, Guleria, 2000; Deka *et al.*, 2001). Effect of *J. adhatoda* on larval hatching and reducing the population of *Meloidogyne incognita* has been reported (Masood *et al.*, 1985; Pandey, 1997). Allelopathic effect of *J. adhatoda* is also reported (Ayaz and
Hussain, 1989). Bronchodilatory and anti-allergic effects of *J. adhatoda* have been reported (Chatterjee, 1999; Geppe and Kaprushkina, 1999). Activity against the pulse beetle, *Callosobruchus maculatus* (Fabricius) infesting cowpea seed have been reported (Bhaduri and Ram, 1985). Antifeedant and anthelmintic potential of *J. adhatoda* is also reported (Mathew et al., 1998; D' Cruz et al., 1980). *J. adhatoda* has activity against pharyngitis (Farooq and Pathak, 1998). Effect of aqueous extract of *J. adhatoda* on longevity and fecundity of tea mosquito bug (Deka et al., 1998) have also been reported. Toxicity of water soluble leaf extracts of *J. adhatoda* against larvae and egg masses of three *Meloidogyne* species has also been reported (Hussaini et al., 1996). Activity against *Sclerotium rolfsii* causing stem rot in tube rose (Das et al., 1997; Pani and Patra, 1997), rice bug, rice stem borers and tiger beetles (Pandey et al., 1995) and foliar disease in mulberry (Kumar and Vijayan, 1999) have been reported. Leaf extract of *J. adhatoda* has insecticidal activity (Sundararajan and Kumuthakavalli, 2000; Hiremath et al., 1997; Srivastava et al., 1996). Both non-abortive (Burgos et al., 1997) and abortive activity of *J. adhatoda* leaf extract has been reported (Nath et al., 1992, Zutshi et al., 1980; Gupta et al., 1978). Wound healing activity of *J. adhatoda* has been reported (Bhargava et al., 1988; Zama and Singh, 1988). Root and leaf extracts inhibited growth of *X. campestris* pv. *vignicola* (Thammaiah et al., 1995). Effectiveness of extract against powdery mildew (*Phyllactinia corylela*), leaf spot
(Pseudocercospora mori) and leaf rust (Cerotelium fici) of mulberry (Biswas et al., 1995), the Ailanthus web worm (Ahmad et al., 1991) and pre and post-harvest diseases of sponge-gourd fruits (Ahmed and Prasad, 1995) were also reported. Effectiveness of herbal smoke prepared using the leaves of J. adhatoda against Armigeres subalbatus and Culex uinquefasciatus was reported (Pandian and Manoharan, 1995). The expectorant and bronchodilatory activity (Joshi et al., 1994) and activity in nymphal mortality of Poekilocerus pictus (Gupta and Gupta, 1993) have also been reported in J. adhatoda. Reaction of feeding the cotton leafworm, Spodoptera littoralis on J. adhatoda has been reported (Hegazy et al., 1992).

J. adhatoda is also accredited with proven action against reduction of gingival inflammation (Muller et al., 1993; Patel and Bhatt, 1984). Antibacterial activity against E.coli, Sarcina lutea, Micrococcus pyogenes var. aureus and Bacillus megaterium have been reported (Anonymous, 1985, Prasad et al., 1999; Brantner and Chakraborty, 1998; Mishra and Tewari, 1990). Toxicity of extract of J. adhatoda against Alternaria brassicae has been reported (Ram, 1997). The methanolic extract of J. adhatoda was evaluated for anti-inflammatory activity by the modified hen’s egg chrioallantoic membrane test and it gave positive results (Chakrabarty and Brantner, 2001). Another study revealed that A. vasica leaf extract has modulatory influence against gamma irradiation in Swiss
albino mice (Kumar et al., 2005). Vinthapooshan and Sundar (2011) suggested that the extract of J. adhatoda positively modulates the immunity of the host.

Sequential extraction of J. gendarussa in various solvents (n-hexane, benzene, ethyl acetate, chloroform, acetone, ethanol and water) confirmed that all of these extracts at 50µg/ml, inhibited lymphocyte proliferation (Arokiyaraj, 2007). Although the exact mechanism of this effect is not known it may be mediated by the interaction between the active components of the extracts and cell-surface molecules or growth factors involving mitogen activation, and it is possible that identification and elucidation of the active constituents in J. gendarussa may provide useful leads to the development of new and effective immunosuppressant drugs.

The ethanolic extracts of J. gendarussa showed significant anti-arthritic activity that was statistically similar to that of aspirin (Paval et al., 2009). Krishna et al. (2009) studied antioxidant and CCl₄ induced Hepatotoxicity properties of leaf extract of J. gendarussa and reported that it bore moderate antioxidant and hepatoprotective activity. Uddin and his co-workers (2011) reported the isolation of stigmasterol, lupeol and 16-hydroxylupeol from the methanol extract of J. gendarussa and its antioxidant, antimicrobial and cytotoxic properties of various extractives of the whole plant for the first time. In the antimicrobial screening, the methanolic crude extract as well as its petroleum ether and carbon
tetrachloride soluble fractions revealed mild inhibitory activity with average zone of inhibition of 7-10 mm each as compared to standard (43-45mm) exhibited by ciprofloxacin.

Recent investigations also suggest the antibacterial and antifungal properties of *Justicia*. He *et al.* (2012) isolated a new compound, JR6 from *Justicia procumbens* and reported that it remarkably inhibited growth in human bladder cancer EJ cells by decreasing cell proliferation, reduced the SOD activity, increased the content of reactive oxygen species (ROS), and induced apoptosis. Rashmi and Linu (2012) reported that the *J. adhatoda* extract possesses potential antibacterial and antifungal activity. Saha and his co-workers (2012) confirm the significant anthelmintic activities of leaves and stem extract of *J. gendarussa*. Chavan *et al.* (2013) reported that the methanolic extract of *Justicia adhatoda* was the most active antiviral agent against Herpes Simplex Virus-2 (HSV-2) and aqueous extract against HSV-1. These methanolic and aqueous extracts may contain alkaloids and its derivatives as potent molecular targets against the HSV.

Gangabavani and Ravishankar (2013) studied analgesic and anti-inflammatory effects of ethanolic extract of *J. betonica* in different experimental models of pain and inflammation. The analgesic activity was carried out using Eddy’s hot plate method in rats and acetic acid induced writhing in mice. The anti-inflammatory activity was carried out using carrageenan-induced paw oedema in rats and HRBC (human red blood
(in-vitro test) and they reported that the ethanolic extract of *J. betonica* shows a significant Analgesic and Anti-inflammatory activities.

The effectiveness of aqueous extracts of various medicinal plants in detoxification of aflatoxin B1 (AFB1) was tested in vitro by thin-layer chromatography and enzyme-linked immunosorbent assay (ELISA). Among the different plant extracts, the leaf extract of *Adhatoda vasica* Nees showed the maximum degradation of AFB1 (≥ 98%) after incubation for 24h at 37°C (Vijayanandraj *et al.*, 2014).

Inhibition of Acetylcholinesterase (AChE) is still considered as the main therapeutic strategy against Alzheimer’s disease (AD). Many plant derived phytochemicals have shown AChE inhibitory activity in addition to the currently approved drugs for loss of memory. Calderon *et al.* (2013) isolated five compounds viz, Olean-12-en-3β-24 diol, aurantamide, aurantiamide acetate, 2α,3β-dihydroxy-olean-12-en-28-oic acid, and quindoline from the dichloromethane extract of the stems of *Justicia secunda* and monitored variations in TLC profiles of both the isolated and the other non-identified compounds in *Justicia refractifolia* and *Justicia graciliflora*. The compound classes, phenolic and olefinic amides, feruloyltyramine amides, 2,5-diaryl-3,4-dimethyltetrahydrofuranoid lignans, peptide alkaloids, phenylalanine derivatives, conjugated ynone, indolquinoline alkaloids, triterpenes and pigments, were tentatively identified based on the LC–DAD–APCI–MS analysis. The most frequently
encountered compound among the species was auranamide while the distribution of quindoline was limited to \textit{J. secunda}. They also determined the acetylcholinesterase inhibitory activity of these isolated compounds.

The methanolic extracts of plants used in traditional ayurvedic system of medicine of India for improving the memory and cognitive function were screened for AChE inhibition and antioxidant activity by Mathew and Subramanian (2014). Of the 20 plant materials tested, the methanolic extracts from fruits of \textit{Emblica officinalis}, rhizome of \textit{Nardostachys jatamansi}, flower of \textit{Nelumbo nucifera}, fruit of \textit{Punica granatum}, root of \textit{Rauvolfia serpentina} and fruit of \textit{Terminalia chebula} were selected as promising candidates as sources of potent AChE inhibitors as well as antioxidants.

\textbf{2.4.1 \textit{In-vitro} cytotoxic potential}

The potential role of various plants in cancer therapy as a direct anti-cancer agent, chemopreventive agent and radiosensitiser or immunity enhancer is studied by various investigators from time to time and many plant species have shown significant anti-cancer activity. Infact the anti-cancer property of a number of plants are yet to be explored. Little work has been done in \textit{Justicia} species in this regard.

Pahwa \textit{et al.} (1987) used the Vasicine obtained from \textit{A. vasica} for chronic toxicity studies in rats and monkeys for six months, where the mortality and body weight of treated animals were found comparable to control.
No abnormality was found in the major organs while carrying out their autopsy and histopathological examination indicating the relatively nontoxic nature of vasicine hydrochloride. Elamgovan et al. (1994) evaluated \textit{in vitro} anti-cancer effects of bioflavonoids obtained from \textit{Citrus} species viz. quercelon, catechin, lutelin and rutin against human carcinoma of larynx (Hep-2) and sarcoma 180 (S-180) cell lines. The result is that luteolin and quercetin inhibited the proliferation of the cells. Luteolin caused depletion of glutathione in the cells and a decline in DNA synthesis, as seen by 3H thymidine uptake studies, thus demonstrating its anticancer potential. The anti-tumour effect of the crude extract of \textit{Centella asiatica} as well as its partially purified fraction was studied in both \textit{in vitro} short and long term chemosensitivity test systems and \textit{in vivo} tumour models (Babu et al., 1995). The purified fraction inhibited the proliferation of transformed cell lines of Ehrlich Ascites Carcinoma (EAC) tumour cells and Daltons Lymphoma Ascites (DLA) tumour cells more significantly than the crude extract. It also suppressed the multiplication of mouse lung fibroblast cells in long term culture. The study further showed that the in vivo administration of both extracts retarded the development of solid and ascites tumours and increased the lifespan of the tumour bearing mice.

The methanol eluted fraction of the petroleum ether extract of the root bark of \textit{Salacia oblonga} (Subramoniam et al., 1996) showed 100\% cytotoxicity on \textit{Ehrlich ascites} tumour cells 86. Fresh root suspension of
Janakia araylpathera exhibited strong anti-tumour effects in mice challenged with Ehrlich ascites Carcinoma (EAC) cells. It prolonged the survival of all mice and protected a number of mice from tumour growth, probably by enhancing the activity of immune system. Devi et al. (1996) isolated Withaferin A, a steroidal lactone from the roots of Withania somnifera, the applicability of this drug as a radiosensitizer in cancer therapy was evaluated. Banerjee et al. (1996) have studied the modulatory effects of the alcoholic extracts of leaves of Ocimum sanctum on various enzyme levels in the liver, lung and stomach of mouse. Goel et al. (1998) studied the effect of aqueous extract of Podophyllum hexandrum, an herb from the Himalayas. It has shown significant anti-tumour effect when drug was tested in strain ‘A’ mice carrying solid tumours developed by transplanting Ehrlich ascites Carcinoma (EAC) cells. Radioprotective effects were also seen when the drug was administered to mice before whole body lethal irradiation of 10 Gy.

Six species used traditionally in Malaysia for treatment of cancer was studied against several cell lines to reveal their cytotoxicity effects (Lee and Houghton, 2005). The isolation of the Vinca alkaloids, vinblastine and vincristine from Catharanthus roseus introduced a new era of the use of plant material as anti-cancer agents (Cragg and Newman, 2005). Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast, lung
and Kaposi’s sarcoma. Fiore et al. (2006) demonstrated antiproliferative activity for *Salvia dominica* against colorectal adenocarcinoma, choriocarcinoma, prostate adenocarcinoma and endometrium adenocarcinoma cell lines. Two novel alkaloids, *schischkinii* and *montamine* have been isolated from the seeds of *Centaurea schischkinii* and *Centaurea montana* (Shoeb et al., 2005; 2006). Both the alkaloids exhibited significant cytotoxicity against human colon cancer cell line. Antimutagenic and antioxidant status have also been attributed for *A. zeylanica*. It exerts antioxidant effect against lipid peroxide and xanthine oxidase induced oxidation (Jahangir et al., 2006).

Identification of organic compounds from plants is of clinical significance because of the effect that they might have in patients with haematopoietic disorders. Caceres et al. (2001) studied the effect of the plant extract of *Justicia spicigera* (Acanthaceae) in different haematopoietic cells: human leukaemic cell lines, umbilical cord blood cells, and mouse bone marrow cells. By examining colony formation and performing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay it was shown that the plant extract of *Justicia spicigera* contains cytotoxic factors for leukaemic cells and has no proliferative activity on normal haematopoietic progenitor cells. The cytotoxic activity of *Justicia gendarussa* methanolic leaf extracts was evaluated by testing against various human cancer cell lines (HT-29, HeLa and BxPC-3) using MTT assay. The results showed that methanolic leaf extracts were very
toxic against BxPC-3 and HeLa cells with IC$_{50}$ values of 16µg/ml and 5µg/ml, respectively. Locklear et al. (2010) reported the estrogen and progestagen agonist effects of Justicia pectoralis in competitive Estrogen Receptor (ER) and Progesterone Receptor (PR) binding assays. They reported that the methanolic extracts of J. pectoralis from Costa Rica enhanced the expression of both ER and PR-regulated genes, indicating a plausible mechanism of action for this plant in the treatment of menopause or PMS. Although J. pectoralis extract showed significant estrogen/progesterone agonist activities in vitro, it did not significantly alter the proliferation of MCF-7 breast cancer cells at concentrations upto 20µg/ml.

Marathakam and co-workers (2012) carried out Investigations of the phytochemical constituents and in-vitro antioxidant potential of the aerial parts of Justicia beddomei and reported the presence of bioactive components especially phenolics and flavonoids. The phenolic and flavonoid content was found to be highest in methanolic extract and lowest in petroleum ether extract. Six different quinazoline alkaloids (vasicoline, vasicolinone, vasicinone, vasicine, adhatodine and anisotine) were found in the leaf of Justicia adhatoda that can be used as anti-tuberculosis agents. These alkaloids inhibit the activity of mtFabH thereby preventing the initial step of fatty acid biosynthesis and can be effective against M. tuberculosis (Jha et al, 2012). It is also reported
that *J. gendarussa* leaf extracts have potential cytotoxic activity on human cancer cell lines particularly BxPC-3 cells (Ayob *et al.*, 2013).

Prabavathy and Vallinachiar (2013) studied the cytotoxic potential of *J. beddomei*. The MTT assay of the extracts was carried out on A549 lung adenocarcinoma cells and they reported that the endophytic *Aspergillus* of *J. beddomei* had an IC 50 of 22.73 µg/ml, while the plant extract had an IC 50 of 6.25 µg/ml. The study indicates the presence of anticancer compounds in the plant and its endophytic *Aspergillus* sp. The endophyte was shown to possess bioactivity three times than that of the host plant. Three new lignans, Pronaphthalide A, Procumbiene, and Procumbenoside J, along with a novel natural product Juspurpudin, and twelve other known lignans were isolated from *Justicia procumbens*. All compounds were evaluated for their in vitro cytotoxic activity against Human LoVo and BGC-823 cell lines except for compound, Procumbiene, and eight of them were found to possess potent cytotoxicity (Jin *et al.*, 2014). Chavan and Chowdhary (2014) reported that both methanolic and aqueous extracts of *J. adhatoda* leaves were non-cytotoxic to influenza virus in the concentration range of 10mg/ml to 0.01mg/ml.