Introduction
1. INTRODUCTION

Nature always acts as a great source of salvation for human beings by providing different remedies from its plants, animals and other sources to cure all ailments of mankind (Dubey et al., 2004). The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. Medicinal plants provide affordable means of health care for poor and marginalized people and they are also a potential source of many therapeutic active compounds. Millions of rural and urban households in India use medicinal plants as an important therapeutic aid for alleviating human ailments with increasing realization of the health hazards and toxicity associated with the indiscriminative use of synthetic drugs and antibiotics. Regardless of the availability of a wealth of synthetic drugs, plants remain even in the present century an integral part of the health care in different countries, especially in the developing countries like India. The universal role of plants in the treatment of diseases was well established by their employment in all important systems of medicine. Yet there are many plants which have been unexplored in the field of medicine or science (Reddy et al., 2010).

Natural products have long been a thriving source for the discovery of new drugs because of their chemical diversity. With increased use of herbal remedies, traditionally used medicinal plants are receiving attention from
scientific and pharmaceutical communities. The newer work on medicinal plants is mostly the rediscovery of traditional effects at cellular and molecular level (Shailasree et al., 2012). The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Medicinal and aromatic plants are viewed as a possible bridge between sustainable development, affordable health care and conservation of the vital biodiversity (King, 1992). Plants represent a huge storehouse of drugs: they produce more than 10,000 different compounds to protect themselves from predators. These compounds are potential drugs (King, 1992; Izuakor, 2005). Furthermore, an increasing reliance on the use of medicinal plants in the industrialised societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants, as well as from traditionally used rural herbal remedies (UNESCO, 1994). In the modern world more than 30% of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998). Ethnobotanical information from all over the world has led to the discovery of approximately 120 plant derived drugs which account for about 25 percent of all prescription drugs consumed per year in North America (Cox and Balick, 1994).

The biogeographic position of India is so unique that all known types of ecosystems ranging from coldest place like the Nubra Valley with – 57° C,
dry cold deserts of Ladakh, temperate and Alpine and subtropical regions of the North-West and trans-Himalayas, rain forests, wet evergreen humid tropics of Western Ghats, arid and semi-arid conditions of Peninsular India, dry desert conditions of Rajasthan and Gujarat to the tidal mangroves of the Sunderban shows beyond doubt that India is rich in all the three levels of biodiversity—such as species diversity, genetic diversity and habitat diversity (Barbhuiya et al., 2009). There are about 426 biomes representing different habitat diversity that gave rise to one of the richest centres in the world for plant genetic resources. The total number of flowering plant species, although only 17,000, the intraspecific variability found in them makes it one of the highest in the world. Out of this seventeen thousand plants species available, the classic systems of medicines like Ayurveda, Siddha and Unani make use of only about 2000 plants in various medicinal formulations (Singh, 2005).

India has one of the richest plant medical traditions in the world. There are estimated to be around 25,000 effective plant based formulations. The ethanopharmacology and traditional system of medicine are re-emerging to offer an attractive discovery engine. Medicinal plants and their products are a source of many potent and newer powerful herbal drugs (Srivastava et al., 1996). Due to the toxic and adverse side effects of synthetic drugs traditional herbal medicine has the potential as a source of new bioactive molecules (Malik and Khan, 2010).
In India the Western Ghats are a major biodiversity hotspot along the Western coast covering an area of 159,000 Sq.Km. It is a niche for 4500-15,000 plant species (Mayers et al., 2000). Several of them are endemic to this region and many of them have been identified to have potential medicinal value. Due to over exploitation several species are also categorized as threatened (Gadgil, 1996; Daniel, 1997). Out of an estimated 17,000 higher plant species occurring in India, more than 1000 species are used over several centuries in the traditional systems of medicine viz. Ayurveda, Siddha and Unani. Ayurveda has long been the main system of health care in India, although western medicine has become more widespread, especially in urban areas. The traditional medicines are derived from medicinal plants, mineral and organic matter. The herbal drugs are prepared from medicinal plants only. In rural India, 70% of the population depend on the traditional type of medicine ‘the Ayurveda’. Kerala with its abundance of luxuriant flora is synonymous with Ayurveda and is possibly the only state in the country where Ayurveda continues to be practiced in its purest form.

Medicinal plants play an important role in human life to combat diseases since time immemorial. The earliest mention of the medicinal use of plants has been found in the Rig Veda which was written between 4000 and 6000 B.C. All five traditional systems of medicine- Ayurveda, Siddha, Unani, Tibetan and Homeopathy, mention about 2000 plant species of medicinal value. Terrestrial plants have been used as medicines in Egypt,
China, India, and Greece since ancient times and an impressive number of modern drugs have been developed from them. The first written record on the medicinal uses of plants appeared in about 2600 B.C from the Sumerians and Akkaidians (Samuelsson, 1999). The ‘Ebers Papyrus’, the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 B.C. The Chinese ‘Materia Medica’, which describes more than 600 medicinal plants have been well documented with the first record dating from 1100 B.C (Cragg et. al., 1994). Greeks have also contributed substantially to the rational development of herbal drugs. The WHO estimates that approximately 80% of the world’s inhabitants rely on traditional medicine for their primary health care (Fransworth, et al., 1985). Tribals in India even now depend largely on the surrounding plants/forests for their day to day needs. General public, academic and government interest in traditional medicine is growing rapidly due to the increased side effects, adverse drug reactions and cost factor of the modern system of medicine and hence most of the people depend on the alternative system of medicine. Ayurveda is an important form of alternative medicine that is widely available in India.

Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. There is a growing focus on the importance of medicinal plants and traditional systems in solving the health care problems of the world. Medicinal plants
either through systematic screening programmes or by serendipity possess an important position in drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence, despite the advantages of the synthetic and combinational chemistry as well as molecular modelling, medicinal plants remain an important source for new drugs and new drug leads (Newman et al., 2000, 2003).

It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin. Hence, during the last few decades there has been an interest in the study of medicinal plants and their long established use in different countries. Of the 877 new chemical entities (NCES) introduced between 1981 and 2002, nearly half (49%) are natural products or semi synthetic products, semi-synthetic natural products analogues, synthetic compounds based on natural products. However, today it is compulsory to acquire scientific testimony as to whether it is reasonable to use a plant or its active principles.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attacks from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long term health when consumed by humans and can be used effectively to treat human disease. Due to unscientific, unsustainable and discriminative collection practices followed, availability of medicinal plants
in its natural home has been depleted over the year. Some of the species have even become scarce due to over exploitation. This has further led to extinction of many valuable medicinal plants. Many others are endangered. Therefore efforts are necessary for the reintroduction of medicinal plants and eco-restoration to enhance availability of medicinal plants. Many of the medicinal plants required by the trade are gathered mainly from the wild growth thus depleting the vegetation of its valuable medicinal plant wealth. This should be prevented and herbal gardens and gene banks covering important medicinal plants should be established to conserve them.

The Acanthaceae family is an important source of therapeutic drugs and the ethnopharmacological knowledge of this family requires immediate documentation as several of its species are near extinction. *Justicia* is the largest genus of Acanthaceae, with approximately 600 species. Several species of *Justicia* are widely used in folk medicine for the treatment of respiratory and gastrointestinal diseases as well as inflammation, including applications in rheumatism and arthritis. This is due to the presence of quinazoline compounds such as Vasicine, Vasicinone and Vasicinol. Vasicine and Vasicinone are well-known bronchodilators, respiratory stimulant, hypotensive and abortifacient. The plants are also utilized for their effects on the central nervous system as hallucinogens, somniferous agents, sedatives, depressors, and treatments for epilepsy and other mental disorders. Other species are popularly used in the
treatment of headache and fever, cancer, diabetes, and HIV. Whole plant and aerial parts are usually used in folk medicine. Extracts made from the leaves are mostly used, followed by those extracts made from the root and some species are used as mixtures.

Different species of the genus *Justicia* are believed to be effective in treating various ailments like bronchial asthma, chest disease and urinary disorders. *J. adhatoda* and *J. beddomei* are the genuine sources of the drug 'vasa' in Ayurveda. Non availability of genuine drug in sufficient quantities may lead to substitution or adulteration with the available plants either from the same genus or from different genera. Morphological characters of plants have been used extensively for diagnostic/identification purposes and they are still indispensable to the taxonomists. However, external morphological study alone is not adequate and other branches of study are of considerable value in proper assessment of the systematic status, especially in the case of a medicinal plant where the crude drug (dried, chopped or powdered) may not give sufficient morphological features for the correct taxonomic judgement. Recent researches have shown that contributions to systematics can come from any branch of botany. Vegetative anatomy, floral anatomy, palynology and embryology have played a significant role in plant taxonomy. In the present investigation, morphological analyses were supplemented with data from other branches of science. Different species of *Justicia* (*J. adhatoda, J. beddomei, J. betonica, J. gendarussa, J. santapauui* and
J. wynaadensis) which have confusing morphological features in dried form have been selected for developing identification characters based on various parameters. Resource base of the selected plants were also taken into consideration. Plants which have good resource base and chance of adulteration and substitution only were considered for the present study. Six species of Justicia viz, J. adhatoda, J. beddomei, J. betonica, J. gendarussa, J. santapau and J. wynaadensis were selected for the present investigation. A brief description and the known medicinal properties and the uses of the selected species are given below:

1. **Justicia adhatoda L.**

Synonyms: Adhatoda vasica Nees, Adhatoda zeylanica Medic

Justicia adhatoda of the Acanthaceae family is a well-known plant drug in Ayurvedic and Unani medicine and the plant has been used in the indigenous system of medicine in India for more than 2000 years (Atal, 1980). It is commonly known as Malabar nut tree and local names in some areas are Vasaka (Sanskrit), Arusha (Hindi), Adadodai (Tamil), and Valiya adalotakam (Malayalam).

Justicia adhatoda is a sub herbaceous perennial shrub, found throughout the year in plains and sub-Himalayan tracts of India, ascending upto 1200 m altitude. It is a stiff, evergreen, much-branched shrub with a strong, unpleasant odour, 1.2-6 m tall. Leaves opposite, elliptic-lanceolate or ovate-lanceolate, margins entire, apex acute, 5-13 cm long, hairy, light
green above, dark beneath, leathery. Flowers are large, white with red or yellow-barred throats, borne in compact, axillary, pedunculate spikes with large bracts. Fruits (capsules) are clavate, longitudinally channelled, 1.9-2.2 cm long and 0.8 cm wide, pubescent; Seeds globular. The plant flowers from August to October (Sasidharan, 2013).

*Justicia adhatoda* is a very valuable Ayurvedic medicinal plant used to treat cold, cough, asthma, and tuberculosis (Dhankhar *et al.*, 2011). Its main action is expectorant and antispasmodic (bronchodialator) (Karthikeyan *et al.*, 2009). The extract of roots and leaves of *J.* *adhatoda* are commonly used by rural population against diabetes, cough and certain liver disorders (Sivarajan and Balachandran, 1994). The leaves, roots and flowers of *J.* *adhatoda* also called vasa or vasaka were used extensively in traditional Indian medicine for thousands of years to treat respiratory disorders such as asthma. *J.* *adhatoda* is considered useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders. In areas where the plant grows wild, local populations commonly use the shrub to treat coughs, colds, and asthma. The leaves are also used to treat skin infections, fever, and inflammation. The alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity; whereas, vasicine, at low concentrations, induced bronchodilation and relaxation of the tracheal muscle. The plant has antispasmodic, antihelminthic, insecticidal and parasiticidal properties (Gulfraz *et al.*, 2004).
2. *Justicia beddomei* (Clarke) Bennet.

Synonym: *Adhatoda beddomei*

The plant species is endemic to India occurring in the Travancore of South Western Ghats, Valparai (South Arcot), Akkamalai (Coimbatore) and Mahendragiri (Kanyakumari Dist.). The plant is very often confused with *Justicia adhatoda*.

*Justicia beddomei* is a glabrous shrub that grows well in the shade and moist areas. The plant is cultivated in Kerala and Tamil Nadu for its medicinal use. The leaf is simple, entire, wavy, ovate lanceolate, attenuate at base, apex acuminate, 6 to 14 cm long, 3 to 4.5 mm broad, midrib prominent at the lower surface, slightly grooved on the upper surface, lateral veins 6 to 10 pairs arising at an angle of 45 to 60°, running parallel to each other, somewhat glaborous but minutely puberulous on the veins. Odour is not characteristic, taste slightly bitter. Flowers small, in short heads, up to 2.5 cm diameter; bracts ovate, up to 1.2 cm long, obscurely 5-ribbed. Calyx 5-partite, the lobes imbricate, the 2 lowest often subconnate. Corolla-tube is short; limb 2-lipped, the upper lip galeate subentire, the lower spreading, 3-lobed. Stamens 2, near the top of the corolla-tube; anthers 2-celled, the cells minutely apiculate at base. Disc cup shaped. Ovary is 2-celled; ovules 2 in each cell; style filiform; stigma entire. Fruit a clavate capsule with a long solid base; seeds 1 or 2, suborbicular, compressed, rugose (Sasidharan, 2013).
The root, leaves and flowers of *J. beddomei* are used to prepare juice and decoction to treat fever, intrinsic haemorrhage, cough, asthma, consumption, obesity, oedema, skin diseases, vomiting, piles, pox, retention of urine, diseases of mouth and as a rejuvenative. Decotions are used for fever and cough and asthma. The Juice of *J. beddomei* mixed with honey alleviates fever, cough, wasting, jaundice, kapha and pitta (10-20 ml). Leaves are also good for irritable cough and for blood mixed diarrhoea and especially in haemoptysis. Flowers are used in ophthalmia. The roots along with the leaf juice are used in phthisis, cough, haemoptysis and asthma (Warrier *et al.*, 1993 and Asolkar *et al.*, 2000).

3. *Justicia betonica* L.

Synonyms: *Adhatoda betonica* (L) Nees, *Justicia pseudobetonica* Roth.,

*Justicia ochroleuca* BLume., *Betonica frutescens* Bont.

Common Names: Squirrel Tail, White Shrimp Plant, Paper Plume.

*J. betonica* Linn. is a glabrous shrub that can grow up to 2 m in height. The stem is terete, tumid and purple above nodes. Leaves are oval-oblong-lanceolate, 6-17.5 X 2.2-4 cm, from an acute base tapering into petiole, subacuminate. Spikes are terminal, 6.5-23.5 cm long, simple, occasionally branched; peduncles 3-8.5 cm; bracts 4-ranked, imbricate, broadly ovate, 1.4-2 X 0.7-1.2 cm, acute at apex, usually scarious at margins, prominently 3-nerves. Bracteoles are elliptic, 1.2-1.6 X 0.4-0.6 cm; Calyx –lobes linear – lanceolate, 3.5 X 1-1.5 mm, densely bristy without.
Corolla infundibular; tube 5-8 mm long, greenish white; limp pinkish mauve; upper lip broadly ovate, 6-7 mm long, faintly hooded; lower one ovate oblong, 4-5.5 X 7-9 mm long, lobes oval-oblong; spur of lower anther – thecae 1-1.25 mm long. Capsules ovoidly clavate, 10.16 x 5-6 mm, bristly, with a short, solid base; seeds orbicular, 4-8 mm broad, subcompressed, rugulose – tuberculate (Khare, 2007).

Its natural habitat includes India and tropical east Africa. It is sometimes called the white shrimp plant or squirrel's tail. Its blooms are held in slim upright spires and are comprised of papery, green-veined white bracts enclosing pale pink flowers. The plant flowers in February - March.

*J. betonica* is regarded as a useful medicinal plant and used in folklore medicine. The Kenyans make use of the plant in the treatment of diarrhoea, orchitis, paralysis, headache, bruises, to relax muscles, to lower cholesterol, to reduce stomach gas, to increase urination and as a cough remedy (Pascaline et al., 2008). In India inflorescence is given orally to treat vomiting and constipation. The Indian and Sri Lankan community apply poultice made from crushed leaves of the plant over abscesses to provide relief from pain and swelling (Khare, 2007). In Uganda *J. betonica* is used in HIV treatment (Lamorde et al., 2008). It is used as antimalarial, antibacterial, antiviral agent and to treat livestock helminthosis. It is also used in antiplatelet aggregation induced by Adrenaline. In Ethiopia roots are used to treat snake bite in their cattle (Bekalo et al., 2009). The Indians use the inflorescence as a hair wash
Decoction of the whole plant is used by Lou tribe of Tanzania to provide relief of stomach ache (Kokwaro and Luo, 1998).

4. *Justicia gendarussa* Burm. F.

Synonyms: *Gendarussa vulgaris*, *Adhatoda subserrata*

Local names of the plants are: *Bhutakeshi*, *Krishnanirgundi* (Sanskrit); *Nili nargandi*, (Hindi); *Vadaikutti* (Tamil); *Vada-kodi* (Malayalam).

*J. gendarussa* is an evergreen shrub, 2-4 ft.high, found throughout the greater part of India and Andaman Islands. Leaves 2.5-5.0 inch long, lanceolate or linear lanceolate, glabrous; flowers small, white with pink or purple spots inside, in terminal or axillary spikes; capsules 0.5 inch long, clavate, glabrous, containing four seeds (Sasidharan, 2013).

*J. gendarussa* is commonly known as willow-leaved *Justicia* or Nili-Nirgundi. It is a shade loving, quick growing, evergreen shrub found throughout India and also in all Asian countries like Malaysia, Indonesia, Srilanka and Bangladesh (Ratnasooriya *et al.*, 2007). The plant is known for its medicinal properties in the Indian traditional system of medicine. In traditional medicine the leaves of the plant are recommended to treat a number of ailments such as fever, hemiplegia, rheumatism, arthritis, muscle pain, lumbago, headache and earache (Ahmad and Holdsworth, 2003). The people of Mt. Yinggeling, Hainan Islands in China, use leaves of *J. gendarussa* to cure injuries (Zheng and Xing 2009).
*J. gendarussa* is reputed for its beneficial effects in respiratory disorders like cough, cold, bronchitis, throat infections, pulmonary infections and allergic disorders like bronchial asthma. It has anodyne, diaphoretic, diuretic, antiphlogistic, antispasmodic, carminative, emetic, febrifuge and laxative properties. The leaves and roots are acrid, bitter, thermogenic, emmenagogue, antiperiodic and insecticidal. They are useful in the treatment of chronic rheumatism, cephalagia, hemicephalagia, facial paralysis, ostalgia, hemicranias, cough, bronchitis, arthritis, dysmenorrhea, amenorrhea, internal haemorrhages, intermittent fevers, ascites and debility (Warrier *et al.*, 1993). Fresh leaves are used topically to treat oedema of beriberi and rheumatism. Dried leaves are used as insecticide. The plant is used in traditional medicinal practices for chronic rheumatism, inflamations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever (Mrunthunjaya, 2007).

5. *Justicia santapaui* Bennet.

*Justicia santapaui* Bennet is an unresolved name

**Synonyms:** *Justicia montana, Justicia andersonii*

*J. santapaui* is a shrub, branches terete or obtusely 4-angled, glabrous. Leaves 25-40 x 7-10 cm, oblanceolate, apex acute, base attenuate, glabrous, nerves 8 pairs, prominent; petiole 5-6 cm long. Spike 20 cm long, axillary, peduncled, simple or branched; bracts and bracteoles similar, 5 x 1.5 mm, oblong, pubescent. Flowers lax; calyx lobes 9 mm
long, lanceolate, puberulus; corolla white with pink spots, villous at the base between the filaments; ovary densely hairy. Capsule 25 mm long, clavate, glabrous; seeds 4, rugose. Flowering in January-April. The plant species is distributed in the evergreen forests of the Western Ghats and endemic to the Southern Western Ghats (Sasidharan, 2013).


Synonyms: *adhatoda wynaadensis, Ecbolium wynadense*

*J. wynaadensis* is a subshrub, with slender stem 2-3 m long with distant nodes terete, smooth. Oppositely arranged leaves, 5-10 cm long, are elliptic-lance shaped, long-pointed, base narrow, with 6-8 pairs of veins. Leaves are carried on 1-2 cm long stalks. Flowers are borne in pairs on drooping spikes 5-10 cm long, axillary and terminal, slender; rachis pubescent, sometimes branched. Flowers in distant pairs; bracts 3 x 1 mm, hairy; corolla 12 mm long, throat hairy; ovary glabrous. Capsule 17 mm long, pubescent; seeds obovoid, oblique, minutely rugulose, and dark brown in colour. *J. wynaadensis* is found in the Western Ghats. The plants usually bear flowers during December to February (Sasidharan, 2013).

*J. wynaadensis* is reported to be endemic to the regions of the Western Ghats, from South Canara, Coorg (*Kodagu*) to Wynaad, East Nilgiris and South Malabar Hills in South India, up to 3000 ft in evergreen forests and on waste lands (Gamble, 1967). A survey among the local populace revealed that, the plant under study locally called Maddhu thoppu is
believed to acquire the medicinal property during the Hindu calendar month of Kataka or Adi (July to August). The plant is believed to have maximum medicinal property when harvested on the 17\textsuperscript{th} of this month (first week of August). The juice from the stem and leaves of this plant is extracted either by soaking in water or boiling in water. The deep purple coloured extract thus obtained is consumed, generally as a sweet dish by the local community. Traditionally the extract of this plant is consumed by the local community during the monsoons by incorporating it into a dessert, as it is said to possess maximum medicinal properties during the season and is said to keep them healthy throughout the year (Ponnamma and Manjunath, 2012). The use of this plant by the Kurichiar tribes of Tirunelli forest, Wayanad, Kerala as an external application over rheumatic swellings has been reported (Udayan et al., 2008). In Dakshina Kannada a sweet dish is made out of the leaves of Justicia wynaadensis (‘aati soppu’) and is consumed during the monsoon season. The leaves of J.wynaadensis are believed to imbibe 18 medicinal principles useful to improve human health (Bhagya et al., 2013).

In recent years considerable interest has been generated for the use of new phytomedicine and their possible pharmacological value. Most of the pharmaceutical industries are highly dependent on the area and wild population for the supply of raw materials for extraction of medicinally important compounds, and hence the demand for medicinal plants is increasing. Since, plants are non-narcotic, have no side effects, are
easily available at affordable prices they become the only source of health
care to the poor.

There are 25 million species of plants, identified on a worldwide basis, out
of which 20,000 have been documented and only 5000 of them have been
phytochemically studied. Approximately, 119 pre natural compounds
extracted from the higher plants are used in medicine throughout the
world. Majority of these are safe with less or no side effects.

The present study was undertaken for pharmacognostical, phytochemical,
molecular and in vitro pharmacological investigation of various species of
Justicia occurring in Kerala and Tamil Nadu.

1.1 Pharmacognostical Studies

In plants most of the taxonomic evidence is usually drawn from
exomorphology of plants, because of its easily visible and convenient
nature. Moreover there is high degree of coincidence between the
expressed phenotypic characters and the genotype of the taxon. It is only
when the exomorphic characters are inadequate; the micromorphological
ones are employed for identification. Use of micromorphological features in
plant classification has a long history. The importance of micromorphological
features for the taxonomic consideration of different groups of plants is
now well established (Ramayya, 1972; Tomlinson, 1979; Ogundipe and
Akinrinlade, 1998; Parveen et al., 2000). Micromorphological parameters
of different plant parts have been used as aids in the taxonomical
recognition of species (Kathiresan et al., 2011). The foliar epidermis is one of the most noteworthy taxonomic characters from a biosystematic point of view, and taxonomic studies of a number of families are conducted on the basis of the leaf epidermis (Bhatia, 1984; Jones, 1986). Anatomical adaptations of different parts of a plant differ dramatically between species. Anatomical differences in stomatal density, leaf thickness, epidermal thickness, and palisade mesophyll thickness between different species have been described (Wylie, 1954; Jackson, 1967; Carpenter and Smith, 1975; Givnish, 1988; Lee et al., 1990). Despite the modern techniques, identification of the plant drugs by pharmacognostic studies is more reliable.

Histochemical methods have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of secondary metabolites. These methods, in combination with various microscopic imaging techniques, can be utilized in the study to compare different species. Micromorphological studies using SEM and anatomical and histochemical studies using various stains will be useful for developing species specific markers for identification of different species.

1.2 Phytochemical Investigations

There are 25 million species of plants identified, out of which 20,000 have been documented and only 5000 of them have been phytochemically studied. Approximately, 119 pure natural compounds extracted from the
higher plants are used in medicines throughout the world. Majority of these are safe with less or no side effects. Medicinal plants are subjected to phytochemical studies because phytochemical information on a species of medicinal plant is the essential basis for fine chemical analysis to be followed by in vitro and clinical studies. Since every species of the medicinal plant contains more than one active compound it is necessary to know this composition before other studies are undertaken. A phytochemical survey would provide information on the distribution of certain chemicals in different species to offer a wider choice of material for the work of other scientists (Rao et al., 1985).

Among the chemical methods of plant examination, chromatographic analysis plays a very important role and it has been introduced to all the modern pharmacopoeias. Because of numerous advantages of the chromatographic methods, they comprise an integral part of the medicinal plant analysis. Thin Layer Chromatography (TLC) is a principal separation technique in plant chemistry. Comparative quantification of major secondary metabolites in various parts of the same plant and different species will provide insight into the possibilities of plant part substitution and use of alternate source plants respectively for future industrial use to implement sustainable harvesting and decreasing pressure on species of high demand. This can be used as a method for checking substitution and adulteration which in turn will result in the improvement of the quality and efficacy of the drug.
Antioxidants or anti-oxidative agents, reduce the effect of dangerous oxidants or free radicles formed in the body. The anti-oxidants bind together with the harmful free radicles, decreasing their destructive power and help to repair the damage already sustained by the cells. Therefore the importance for the search for natural anti-oxidant has greatly increased in the recent years (Jayaprakasha et al., 2002). Phenolics are the largest group of phytochemicals that account for most of the anti-oxidant activity in plants or plant products and flavonoids are the naturally occurring phenolic compounds. The genus *Justicia* is a rich source of naturally occurring anti-oxidants phenolics and flavonoids. Alkaloids are nitrogen containing compounds with enormous potential in the pharmaceutical industry. The plant derived alkaloids currently in clinical use include the analgesics morphine and codeine, the anti-cancer agents vinblastine and taxol. Other important alkaloids of plant origin include caffeine, nicotine, cocaine etc. The total alkaloid content in the different plant parts can help in species delimitation.

**1.3 Molecular Studies**

*rbcL*-PCR based DNA barcoding techniques provide an advanced technique for plant identification. DNA barcoding is a method of identifying an organism based on sequence data from one to several gene regions. Barcoding works by matching sequence data from an unknown specimen to a reference sequence. The goal of DNA barcoding is conceptually simple: Find one or a few regions of DNA that will distinguish
among the majority of the world’s species, and sequence these from sample sets to produce a large scale reference library of life on earth (Herbert et al., 2003). This approach can then be used as a tool for species identification and to help in the discovery of new species (Herbert et al., 2004). DNA barcoding is proving to be an exciting and powerful tool for identifying and verifying plant specimens. It has the potential to uniquely identify medicinal plants and provide quality control and standardization of the plant material supplied to the pharmaceutical industry (Schori and Showalter, 2011).

The molecular approach has proved itself as an increasingly valuable tool in the identification of plant species (Morell et al., 1995). In this field one of the most successful techniques is random amplified polymorphic DNA (RAPD) (Welsh and Mac Clelland, 1990) which has two advantages: it allows random sampling of markers over whole genomic DNA and does not require any previous information on the genome of the organism under investigation. RAPD fingerprints have recently been used widely to estimate genetic and taxonomic relationships in plants. It is extremely useful for studying the relationship between various species. RAPD markers can give precision in the systematic study of plants especially when the conventional system of classification and identification are inconclusive.
1.4 In-vitro 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. Possibly, the largest amount of in vitro testing has taken place to date in the search of novel anticancer agents. The most common way in which, this is done is cytotoxic methods carried out using one or more mammalian cell lines, grown under conditions where they actively grow and undergo mitotic division. The rate of multiplication and growth is measured indirectly by indicators of growth, e.g. formation of a colour and the rate of proliferation of a cancer cell line in the presence and absence of the test substance is compared, usually after a specified time. Selectivity can be assessed and the addition of normal cell lines to the battery enables selectivity between cancer cell lines and normal cell lines to be determined. This gives an indication of potential usefulness in a clinical setting, for which a selectivity of at least two orders of magnitude in favour of the cancer cell lines being the more susceptible is required (Suffness and Pezzuto, 1991).

The aim of the pharmaceutical industry is to develop novel drug entities for the treatment of disease. Such drugs require specificity of action and are aimed at a particular subset of receptors. Although natural products
continue to supply banks of compounds for new screens, the focus of industry is currently on combinatorial synthesis for new drug development. It must not be forgotten that natural products which result from millennia of biosynthetic pathways modified by evolution have a well-established track record as medicinal agents and present a wide range of structural diversity (Phillipson, 2001). Biological screening of herbal drugs is an important aspect of drug standardisation. The selected species are traditionally known for bronchodilator activities. *In vitro* pharmacological studies might lead to a world of new activities not explored so far.

**The objectives of the present study are the following:**

- To develop pharmacognostical and phytochemical markers for the identification of selected *Justicia* spp.
- To develop DNA databank using *rbcL*-PCR molecular technique.
- Molecular fingerprinting of selected *Justicia* spp. using RAPD analysis
- *In vitro* pharmacological screening of selected *Justicia* spp. for the evaluation of cytotoxicity and acetylcholinesterase (AChE) inhibition activities.