6. SUMMARY

Studies on taxonomy, ethnobotany, and genetic diversity are imperative for plants with traditional medicinal value. Throughout the ages, human beings have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines (Cragg and Newman, 2005). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The use of plant derived products in disease management is an important breakthrough in the history of humankind. The use of plants as medicines is as old as human civilization itself and out of about 258,650 species of higher plants reported from the world; more than 10% are used to cure ailing communities (Shinwari, 2010).

Traditional medicine has been practised for many centuries by a substantial proportion of the Indian population. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased world-wide in the recent past. Plant extract represents a continuous effort to find new compounds with the potential to act against various diseases. Approximately 20% of the plants found in the world have been evaluated for their pharmacological or biological efficacy, and a substantial number of new antibiotics that are introduced in the market are obtained from either natural or semi synthetic resources.
The selected species of the genus *Justicia* had been used in folk remedies over the years and is reported to have a broad range of therapeutic effects including antiviral, antifungal, analgesic, anti-inflammatory, and bronchodilatory and immunostimulant activity. Due to over exploitation many species are in danger of extinction and hence there is an urgent need for documenting the knowledge regarding these species. The micro economy related to it opens the chance for adulteration and substitutions.

### 6.1 Pharmacognostical investigations

Preliminary morphological screening of the selected six species of *Justicia* showed similarities and differences. Close morphological similarity was observed between *J. adhatoda*, *J. beddomei*.

Microscopic investigation of the leaves and roots of the six species of *Justicia* showed similarities and differences alike. All the species possess diacytic stomata but there are differences in the nature of epidermal cells and epidermal hairs. Anatomical characteristics of the leaf are of varying types, however, *J. adhatoda*, *J. beddomei* and *J. betonica* showed similarities on a number of features. Except in *J. adhatoda* all other species showed cuticular wax and silica deposits. Anatomical details of the root also showed a similarity between *J. adhatoda* and *J. beddomei*. *J. santapau* deviated from the other species in having a thick and continuous ring of stone cells in the cortical region of the root. Histochemical staining revealed the presence of lignin. Starch grains are rarely present in all the species but tannin and oil depositions are absent.
in all the selected species. These pharmacognostic parameters could be useful in the identification of authentic species of *Justicia*.

### 6.2 Phytochemical Screening

Phytochemical analysis conducted on the plant part extracts of the selected species of *Justicia* revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Major class of phytochemicals such as phenolic, flavonoids and alkaloids were estimated spectrophotometrically and it is observed that the leaves are the richest source of these compounds. Variation is observed only in case of *J. santapaui* where the methanolic extract of the root gave maximum value in TFC. Analysis of the plant part extracts revealed the rich presence of phytochemicals such as phenols, flavonoids and alkaloids and their quantification proved that it is species specific and plant part specific. Hence it could be useful as supplementary information for the taxonomists to identify the species and plant parts.

TLC profiles from the petroleum ether, Chloroform and the methanolic extracts of the leaves, stem and the roots of selected *Justicia* species has given species specific bands. Under 254nm the petroleum ether extracts of the stem and the leaf of *J. adhatoda* and *J. beddomei* showed similarities. The roots of *J. santapaui* and *J. wynaadensis* showed maximum similarities in their banding pattern under 366nm. The chloroform extract of the root of all the species gave a common band with $R_f$ 0.86 under UV 254nm. Among the methanolic extract the leaf of
**J. betonica** showed a green fluorescence at R$_{f}$ 0.76 under UV 254nm. Chemical features of plants may be used for classifying, and identifying species, but only a fraction of these have been used to any great extent. The phytochemical profiles of the selected species of *Justicia* can provide supplementary and complementary inputs when other kinds of characterisation methods give occasional unambiguous results.

### 6.3 Molecular Studies

Chemotaxonomy is traditionally restricted to comprise fatty acids, proteins, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences. Authentication at the DNA level provides more reliability because DNA is a stable macromolecule that is not affected by external factors and is found in all cells. Barcoding of the six species of *Justicia* using *rbcL*-PCR technique provided a consensus sequence data of the selected species which is deposited in the NCBI GenBank. *Justicia* showed poor availability of *matK* sequence in GenBank and the present study added *rbcL* sequence of *J. wayanadensis, J. beddomi, J. betonica* and *J. santapaui* in the GenBank. This data was used for constructing the phylogenetic tree to establish evolutionary relationships among the species. A better and more reliable taxonomic strategy was obtained on BLAST analysis and tree analysis.

Molecular techniques like RAPD provide an efficient means to produce a large number of molecular markers which could be employed to examine
the genetic relatedness among genus and species. RAPD markers can be used in the plant systematics when there is confusion in the conventional classification and identification system. This approach is extremely useful to study the relationship between various species. In the present study the similarity indices revealed that *J. adhatoda* is closer to *J. beddomi* while *J. adhatoda* and *J. santapau* are the most distant from each other at genetic level. Future research using nuclear DNA fingerprints and other molecular markers such as ITS and mitochondrial DNA to study molecular systematics will contribute to a more accurate classification of the genus *Justicia*.

### 6.4. *In vitro* Pharmacological investigations

Medicinal values of plants have assumed an important dimension in the past few decades. Plants produce a very diverse group of secondary metabolites with antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the ageing process. The organic compounds present in the medicinal plants provide definite physiological action on the human body. These compounds are synthesized by the secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with compound functions in the plant.

Acetocholinesterase inhibitory properties of the selected species of *Justice* were tested using the Ellman colorimetric method. Significant inhibition of the enzyme at 25 mg/ml was observed for the methanolic extracts from
*J. adhatoda* (48.8%). However, the cholinergic activities may not be very relevant to the treatment of Alzheimer’s disease since the percentage of activity is mild in most of the species studied. Among the species investigated *J. adhatoda* showed maximum activity and with respect to the parts used the stem showed comparatively better activity followed by root and leaf and it is dose dependent. Variations are observed in *J. gendarussa* where roots showed higher activity than the leaf and the stem. In *J. santapauui* the order is leaf >stem >root. The IC$_{50}$ value for all the species is above 25 mg/ml and hence the anti-AChE activity of selected *Justicia* species is evaluated as very low. In fact reports are not available regarding the use of *Justicia* species as cognitive enhancers in the traditional medicine systems and the present work forms one of the first of its kind.

Most of the medicinal plants usually show some type of cytotoxicity. However few plants have been significantly investigated for cytotoxic activity. The selected *Justicia* species were evaluated for antitumour potentials using trypan blue assay in DLA cells with five different concentrations. Of the investigated plants *J. adhatoda* was found to be more potent than other species. The best cytotoxic activity was observed with the methanolic extract from the leaf of *J. gendarussa* (35% at 200 µg/ml) and the percentage cytotoxicity is dose dependant in all the plant parts and the species. The IC$_{50}$ value for all the species is above 200 µg/ml. Though this work presents only mild activity of the selected
species of *Justicia* it was insightful and it is clear that there is much toxological work yet to be done with the genus *Justicia*.

Morphological characters of the plants have been used for identification purposes and they still stand as the best practice. Sometimes external morphology is not conclusive for plant identification especially when the crude drug cannot give sufficient morphological features. Hence the taxonomists try to collect information from other branches of science to help identification. Phytochemical profiles and molecular fingerprints usually supplement the morphological and pharmacognostical characters for plant identification. Pharmacological screening is also an added advantage for proper species identification and future studies.

There is a one-in-four chance that a drug used from any pharmacy has an active ingredient derived from a plant (Balick and Cox, 1996). Indeed, the international consumer market for medicinal herbs and botanicals is estimated to be at about US $18 billion (van Wyk and Wink, 2004). Hence, in our technological age, plants continue to play a significant role both medically and economically.

The present work was an attempt to correlate pharmacognostical, phytochemical, pharmacological and genetic data to help the correct identification of the species and the plant parts when it is available afresh, dried or in crude drug format.