INTRODUCTION
Chapter I

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Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Early humans recognized their dependence on nature for both health and wellness. Physical evidence of the use of herbal remedies has been found from some 60,000 years ago (Wickramasinghe and Bandaranayake, 2002). The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus (Bensky and Gamble, 1993). Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history.

Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non active. They evolved which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well defined herbal pharmacopeias. Traditional medicine evolved over centuries, depending on local flora, culture, and religion (Cassileth, 1998). In the twentieth century, much of the pharmacopeia of scientific medicines was derived from the traditional herbal knowledge of native people. This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world.
Recently, there has been a shift in universal trend from synthetic to herbal medicine, which we can say ‘Return to Nature’. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. People are attracted to herbal therapies for many reasons, the most important reason being that, like our ancestors, we believe they will help us to live healthier lives. Herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products. As such, they represent a substantial proportion of the global drug market (WHO, 1990; WHO, 1999; Roberts et al., 1997).

The world market for herbal medicine including herbal products and raw materials has been estimated to have an annual growth rate between 5 and 15%. Total global herbal drug market is estimated as US $ 62 billion and is expected to grow US $ 1 to 5 trillion by the year 2050. India has a great wealth of traditional knowledge and wisdom. Ayurveda contributes Rs 3500 crores (US $813 million) annually to the internal market. The Indian medicinal plants-based industry is growing at the rate of 7–15% annually. The value of medicinal plants-related trade in India is estimated about Rs. 5000 crores per annum. Global trend leading to increased demands of medicinal plants for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other products is an opportunity sector for Indian trade and commerce (Kalpana Joshi et al., 2004).

Nature has bestowed our country with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world. Countries with ancient civilizations such as China, India, South America, Egypt, etc. are still using several plant remedies for various conditions. In this regard India has a unique position in
the world, where a number of recognized indigenous systems of medicine viz., Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are utilized for the health care of people. No doubt that the herbal drugs are popular among rural and urban community of India (Alok Sharma et al., 2008). The reason for the popularity and acceptability is the belief that all natural products are safe. As herbal wave sweeping over society is on the rise, many of the major pharmaceutical companies have renewed their strategies in favor of herbal drugs so the time seems to ripe for botanicals of better quality (Bensky and Gamble, 1993).

Need of Standardization

Commercial production of herbal medicines and their trade are the fast growing sector of industry today due to increasing demand of medicinal plants; the supply line is adversely affected leading to the adulteration and substitution for genuine drugs (Sagar Bhanu et al., 2005).

Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. Herbal medicines are very different from well-defined synthetic drugs. For example, the availability and quality of the raw materials are often problematic; the active principles are frequently unknown; and standardization, stability, and quality control are feasible but not easy (Bhutani, 2003). Medicinal plant materials are characterized accordingly by sensory microscopic and macroscopic characteristics. Taking into consideration the variation in sources of crude drugs and their chemical nature, they are standardized by using different techniques including the method of estimation of chief active constituent (Thatte, 2003).

Authenticity, purity and assay are the important aspects of the standardization and quality control. As the name implies authenticity relates to proving that the material
corresponds to right identity. Quality control of botanicals starts right from identification of plant. Every herbal formulation must be standardized as per World Health Organization’s (WHO) guidelines. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal drug medicines (Sagar et al., 2005). Pharmacognosy, with all of its tools and expertise, plays an integral role in this process, not only for ensuring the authenticity, purity, and consistency of the materials being used and investigated, but also for developing biological models for determining efficacy (Roy and John, 2005).

Pharmacognosy

Pharmacognosy is an important link between pharmacology and medicinal chemistry. The basic knowledge of biochemistry and chemical engineering is essential for the development of collection, processing and storage technology of crude extracts. Pharmacology is essential for understanding the action of drugs on animals and human system. Pharmacognosy is a vital link between Ayurvedic and Allopathic systems of medicine.

WHO has developed several guidelines for carrying out standardization procedures of raw herbal products, which basically include pharmacognostical, physico-chemical, pharmacological and toxicological methods to standardize herbal materials or products. Microscopic and macroscopic standards could be drawn out where a plant can be differentiated from another plant which may look similar in external appearance. Currently, genetic fingerprinting and the use of analytical quality control equipments like HPLC and HPTLC are performed on a large scale for standardization and identification of herbal drugs. Phytochemistry has evolved as a major branch of Pharmacognosy in developing markers for the purpose of identification and standardization (Kumar, 2007).
Pharmacognostic studies are required to determine the comparative morphoanatomical and micrographic features for identification and quality control purpose. Several microscopic parameters, including phloem fibers, trichomes and foliar architecture have also been considered for this study. The aim of Pharmacognosy is to analyze comparative morphoanatomical and micrographic features which might provide assistance in the identification, analysis and standardization of medicinal plants. Ultimately, Pharmacognosy provides the quality standard for identification of medicinal plants.

Traditionally, Pharmacognosy mainly addressed quality related issues using routine botanical and organoleptic parameters of crude drugs. Pharmacognosy became more interdisciplinary because of subsequent advances in analytical chemistry. These developments added emphasis on chemoprofiling assisted characterization with chromatographic and spectroscopic techniques. The new Pharmacognosy includes all aspects of drug development and discovery, where biotechnology driven applications will play an important role (Kalpana Joshi et al., 2004).

**Phytochemistry**

The identification of biologically active compounds is an essential requirement for quality control and dose determination of plant-based drugs. A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. Those compounds which are responsible for the medicinal activity of the herb are the secondary metabolites. For example, alkaloids which are nitrogenous principles of organic compounds combine with acids to form crystalline salts. In addition, herbs may contain saponins, resins, oleoresins, lactones and volatile oils. Complete phytochemical investigations of most of the medicinally important herbs of India have not
been carried out so far. This would be beneficial in standardization and dose
determination of herbal drugs. Further, there should be a quality control test for the entire
preparation to ensure the quality of the drug (Dubey et al., 2004).

The beneficial medicinal effects of plant materials typically result from the
combinations of secondary products present in the plant. The medicinal actions of plants
are unique to a particular plant species or group is consistent with this concept as the
combinations of secondary products in a particular plant are often taxonomically distinct
(Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids,
proteins, heme, chlorophyll and nucleic acids, which are common to all plants and are
involved in the primary metabolic processes of building and maintaining plant cells
(Kaufman et al., 1999; Wink, 1999). Secondary products have both a defensive role
against herbivory, pathogen attack and inter-plant competition and an attractant role
toward beneficial organisms such as pollinators or symbionts (Kaufman et al., 1999;
Wink and Schimmer, 1999). Plant secondary products also have protective actions in
relation to abiotic stresses such as those associated with changes in temperature, water
status, light levels, UV exposure and mineral nutrients (Kaufman et al., 1999).
Furthermore, a recent work has indicated the potential roles of secondary products at the
cellular level as plant growth regulators, modulators of gene expression, and in signal
transduction (Kaufman et al., 1999).

Although the secondary products can have a variety of functions in plants, it is
likely that their ecological function may have some bearing on potential medicinal effects
for humans. For example, secondary products involved in plant defense through
cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in
humans. Likewise, secondary products involved in defense against herbivores through
neurotoxin activity could have beneficial effects in humans (i.e. as antidepressants, sedatives, muscle relaxants, or anesthetics) through their action on the central nervous system. To promote the ecological survival of plants, structures of secondary products have evolved to interact with molecular targets affecting the cells, tissues, and physiological functions in competing microorganisms, plants, and animals (Wink and Schimmer, 1999). In this respect, some secondary products of plants may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites (e.g. central nervous system, endocrine system, etc.) (Kaufman et al., 1999).

**Antibacterial studies**

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), and viruses (antiviral activity). The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials (Das et al., 1999). Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics are generally considered to be low.

Infectious diseases are the prime cause of death world-wide and they account for approximately 50% of death in tropical countries (Iwu et al., 1999). This may be due to poverty and the increasing incidence of multiple drug resistance. Bacterial resistance to almost all antibacterial agents has been reported (Truiti et al., 2003) and this resistance is largely due to indiscriminate use of antimicrobial drugs for the treatment of infectious diseases (Afolayan and Ahero, 2006). Furthermore some antibiotics have serious undesirable side effects which limit their applications, it is necessary to develop new
antimicrobial agents that are very effective with minimal side effects and higher plants represent a potential source of novel antibiotic prototypes (Mauceer-Grimes et al., 1996; Afolayan, 2003).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Almagboul et al., 1985). Many plants have been screened scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. So, it is worth to study plants and plant products for activity against resistant bacteria.

**Tissue Culture**

The unscrupulous collection of medicinal plants from wild habitats by traders has threatened the very existence of valuable medicinal plant resources. Due to biopiracy and over exploitation, some of the ethnomedicinal plants are becoming rare. Reserves of medicinal plants are diminishing and are in danger of extinction due to unfriendly harvesting techniques, loss of growth habitat and their unmonitored trades. Low seed set, poor seed viability, high dormancy and low percentage of seed germination are some of the problems in propagation of some medicinal plants. The production of crude drugs is subject to the vagaries of the climate, crop disease, varying methods of collection and drying which influence quality, and to the inherent variation of active constituents arising from plants of the same species having different genetic characteristics. To overcome this problem, recently, one of the rapidly expanding areas of Pharmacognosy has involved the application of tissue culture of plant cells, tissues and organs in the study of medicinal plants. This includes development of commercial production of expensive bio-
medicaments, discovery of new metabolites, selection of superior strains of medicinal plants and improvement of medicinal plant species by genetic engineering.

Much work has been done on well known Siddha medicinal plants to exhibit their potential as therapeutic agents. Effects of various plant extracts in disease therapy has encouraged workers to look for the extracts of roots, seeds, leaves, stems and bark of many plants with a view to harness their constituents for the treatment of various diseases. Standardization profiles of herbal drugs are not available for most drugs. Keeping the above mentioned areas of plant science research; this study is an attempt to establish the standardization parameters for complete Pharmacognostic evaluation of selected plant species of Fabaceae.

The Fabaceae family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill et al., 1981; Judd et al., 1999). The Fabaceae are herbs or shrubs, rarely trees, distributed nearly equally over tropical and subtropical regions of both hemispheres. They abound in Africa and India. Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of Fabaceae are unexplored which deserve attention and research. Indigofera aspalathoides Vahl ex DC., Indigofera viscosa Lam., Tephrosia spinosa (L. f) Pers. and Tephrosia villosa (L.) Pers. are such plants which have not been explored extensively by the scientific world so far.

The genus Indigofera comprises around 700 species that are distributed geographically in tropical regions (Bakasso, 2008). The plant Indigofera aspalathoides Vahl ex DC. (Leguminosae) is commonly known as ‘Shivanarvembu’ in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoots are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous
affections (Kirtikar and Basu, 1975). The leaves are also applied to abscesses. The whole plant is used in edematous tumors and the ashes are used to treat dandruff (The Wealth of India, 1959). The methanol extract of *Indigofera aspalathoides* also possesses hepatoprotective activity (Gupta et al., 2004). The stem is traditionally used for various skin disorders and cancers (Rajkapoor et al., 2004). Whole plant powder or potion is used for joint pains (Murugasa Mudaliyar, 1988). Powdered barks mixed with coconut oil are applied on the affected parts cautiously for six months to cure leprosy (Jeeva et al., 2007). The ash of the whole plant is added with coconut oil and applied topically to treat psoriasis (Revathi and Parimelazhagan, 2010). Yoganarasimhan (2000) reported that the whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases. Shivanarvembu Kuli Tailam is prescribed for chronic weeping eczema in children, frequently occurring scalp on lower limbs, insect bites, leprosy, chronic ulcers and boils (Anonymous, 1984, 1992).

*Indigofera viscosa* Lam. is a herb distributed in hill slopes of southern peninsular India. The macerate of the crushed whole plant is used as rectal application, twice a week for one week, to stop diarrhea (Kusamba Chifundera, 2001).

The genus *Tephrosia* is a pantropical taxa with about four hundred species distributed throughout the world (Gillett, 1971). About twenty four species of *Tephrosia* were recorded in India (Gamble and Fischer, 1918; Saldanha and Singh, 1984). Most of the *Tephrosia* species are herbs to under shrubs and grow as weeds. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer properties (Sarin Jagat 1976; Chen Yuh-Lin, 1978; Bentley et al., 1987). *Tephrosia spinosa* (L. f) Pers. is commonly known as Mullu Kolingi in Tamil. Decoction of roots is given for rheumatism,
indigestion, diarrhea and fevers (Yoganarasimhan, 2000; Useful Plants of India, 2000). The whole plant is used to treat asthma, ulcer, diarrhea, swellings and leucorrhoea (Murugesa Medaliar, 1988). Bark decoction is used to cure enlargement of spleen (Sadasiva Pillai, 1978).

*Tephrosia villosa* (L.) Pers. is an erect white tomentose viscid under shrub distributed in dry lands. The plant is commonly called as Poonai Kolinji in Tamil. Fresh roots are considered as hypoglycemic (Yoganarasimhan, 2000). Leaf juice is used for dropsy (Yoganarasimhan, 2000; Rahmatullah Qureshi et al., 2010) and roots are used in preparation of toothpaste (Jayaweera, 1980 – 1982). Root, leaves and bark are used as anthelmintic and antipyretic and also cure diseases of liver, spleen, heart, blood and leprosy (Varaprasad Bobbarala et al. 2009). Root powders are used to cure stomachache (Giday et al., 2009). Juice of the leaves is given in dropsy and also useful in diabetes (Chopra et al., 1956).

Traditional medicines are an indomitable arena of Pharmacognosy. To integrate and work with globalization, traditional medicine must reassess and open itself in its diagnostic and therapeutic approach. It will thus influence productivity, and progress as well as enhance its therapeutic efficiency and competitiveness. Now-a-days, routine Pharmacognosy has changed into a highly demanding interdisciplinary research. Hence, for the better quality control and standardization processes, the present study attempts to bring out not only the Pharmacognostical and Antibacterial potentials of the selected plants but also Gas Chromatographic Screening of bioactive compounds and micropropagation protocol of the plants are undertaken.
The main objectives of the present investigations are

- to study the Pharmacognostical characters of the chosen plant species,
- to carry out the preliminary phytochemical screening and bio-chemical studies of various extracts of the plant species chosen for study,
- isolation and identification of chemical composition of the crude extract by using GC/MS.
- to screen the plants for their antibacterial activity against selected microorganisms,
- to develop a suitable protocol for in-vitro regeneration of chosen plant species.

The findings are aimed at filling up the lacuna in scientific authentification of the selected plants for commercial preparation of validated drugs.