Chapter I

INTRODUCTION

Man uses plants in different ways according to his needs, particularly as food and medicine. Among the entire flora, 35,000 to 70,000 species have been used for medicinal purposes (Ponnu, et al., 2003). India is one of the richest floristic regions of the world and the country has been a source of plants and their products since antiquity.

With its varied climatic zones, India possesses rich biodiversity of the world. More than 45,000 plant species occur in the country (Vasisht and Kumar, 2003). Medicinal and aromatic plants are used in different systems of medicines including Ayurveda, Unani, Siddha and Homeopathy which are in practice and use in India. Nearly 2500 species of plants are used in these systems. Besides the usage of plants in these systems, they are also made use in tribal and folk medicine. Around 7500 species of plants are used in the tribal and folk medicine. Many medicinal plants provide modern drugs. More than 90% of the medicinal plants are collected from the wild (Vasisht and Kumar, 2003).

According to World Health Organisation (WHO), 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs for their primary health care need (Britto and Mahesh, 2007). Major part of the traditional healing involves the use of plant extracts containing active constituents (Ahmad, et al., 1998). Natural products are a source of synthetic and herbal medicine (Singh and Singh, 2001). They are non toxic, having no side effects and easily available at affordable prices (Britto and Mahesh, 2007). Therefore demand for medicinal plant is increasing.
in both developing and developed countries. WHO reports that the traditional medicinal plant sector occupied an important position in the socio-cultural, spiritual and medicinal area of rural and tribal families.

Majority of the world population, especially in developing countries, depend on traditional system of medicine as a remedy for a number of diseases. Many plants are used mainly as herbal preparations, in different systems of medicine in different countries (Ahmad, et al., 1998).

In India 90% of the prescriptions contain plant products (Britto and Mahesh, 2007). According to the National Prescription Audit of the United States for the year 1993, out of the 99 compounds analyzed, which constitute the 150 most prescribed drugs in that year, around 55% were either directly from the natural products or had structures based on natural product pharmacophores. These facts point to the importance and potential of plants as a primary source of drugs. In cancer therapy, 67% of the effective drugs are of natural origin. These include plant derived agents like vinblastine and vincristine isolated from Madagascar periwinkle, Catharanthus roseus (L.) G. Don.; taxol from the bark of pacific yew tree, Taxus brevifolia; podophyllotoxin from the roots of Podophyllum and camptothecin from the bark of Camptotheca acuminata (Cragg, et al., 2006). Majority of the anti-cancer agents are still extracted from plants rather than synthesizing chemically on commercial scale (Wink, et al., 2005). Of the 520 new drugs approved between 1983 and 1994, 39% were natural products or derived from natural products and 60%-80% of antibacterial and anticancer drugs were also derived from natural products (Cragg, et al., 1997).

Traditional healers use indigenous medicinal plants to treat many of the ailments. They commonly use the decoction or crude extract of plant or plant parts. The therapeutic effect of plant extracts can be
attributed to a particular “active principle” or “chemical entity”. Studies on the chemical constituents and their role in clinical effectiveness have been worked on. The Pharmaceutical industries all over the world are in search of new drugs from natural sources.

Ayurveda is the main system of healing in India. *Charaka Samhita* written by Charaka around 100 A.D., describes 341 plant medicines and also medicines of animal and mineral origin. *Rauwolfia serpentina*(L.) Benth. ex Kurz. is listed in this earliest Ayurvedic medicinal text and has been used from that time onwards to treat mental illness and insomnia. The roots of *R. serpentina* (L.) Benth. ex Kurz. contain numerous alkaloids (Vinod and Shekhawat, 2005). Other traditions of medicine share common roots with Ayurveda, which is the oldest surviving medical tradition in the world (Chevallier, 2001). The raw materials for Ayurvedic medicines are achieved from plants in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. Ancient Indian scholars – Charak, Sushruta and Vagbatta and several others have given detailed description of Indian medicinal plants in *Atharvaveda* (Chatterjee, 2000). Ayurveda, the Indian system of traditional medicine, uses dry powder or crude extracts of medicinal plants to treat various diseases including cancer (Jagetia and Baliga, 2006). The plants are selected on the basis of medicinal folklore reports and their repeated use in Indian traditional system of medicine. Vast majority of medicines in this system are from plants.

The medicinal value of plants lies in the chemical components that produce a definite physiological action on the human body. The most important of these bio-active compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga, *et al.*, 2003). The ability of a herbal medicine to affect body system depends on the chemical constituents that it contains. An active principle or chemical component in a plant is responsible for a particular clinical effectiveness and can completely replace the plant extract. Plants
contain thousands of chemical constituents that interact in complex ways. In some, all the constituents interact in complex ways to produce the therapeutic effect of the medicine.

For a medicine to be effective, the exact plant which is having the attributed clinical effectiveness should be used. A guarantee for the cure can only be given when the exact plant of the right quality is used. The correct identity of the raw material, the plant, is important. Any plant other than the specific plant cannot give the attributed clinical effectiveness. Therefore collection of the exact herb is a very important step in any formulation. The exact identity of the plant is ascertained by taxonomic studies. Misidentification of the plant will result in different medicinal effect from the expected. Correct botanical identification of plants is an important and necessary step in any medicinal formulation. For this purpose taxonomic methodology based on morphological characteristics are employed. Traditionally, taxonomy was used for mere plant identification. But in the modern concept, it includes informations from all relevant fields of biological sciences (Singh, 2000). New approaches in recent years give a greater reliance on phytochemical information—the Chemotaxonomy. Phytochemistry helps in separating and identifying the chemical content or active principle responsible for the clinical effectiveness. For the preparation of a plant based drug one should depend on taxonomy to identify and authenticate the herb and phytochemistry for isolation and identification of the active principle. Chemical screening of the plant will reveal the major as well as the minor chemical constituents. Both taxonomy and phytochemistry are inevitable for the preparation of crude or purified plant drug. The taxonomic literatures will help us to provide the exact identity and botanical name of the plant according to the rules of International Code for Botanical Nomenclature (ICBN). It also provides a list of
characteristics of the species and their arrangement and position in the plant kingdom.

1.1. Taxonomy

Taxonomy is basically concerned with the classification of organisms. Taxonomy has a unique position in Biology. Early man used plants for all primary requirements like food, clothing, housing and medicine. Man learnt to identify, describe, name and classify food, clothes and all objects that influence their life. Even today man relies directly on plants for many uses. Man has been classifying plants since the advent of civilization. Taxonomy was recognized as a formal subject only in 1813 by A.P. de Candole, as a combination of taxis (arrangement) and nomos (rules or laws) (Singh, 2000).

Plant Taxonomy or Systematic Botany is the study of plants with respect to their identification, nomenclature, description, classification and phylogeny. Classification is an arrangement of plants into groups on the basis of similarities. For an ideal system of classification, all the following procedures like identification, description, nomenclature and phylogeny should be carried out. Identification is recognizing an unknown specimen with an already known taxon and assigning a correct rank and position in an extant classification. The description of a taxon involves listing its features by recording appropriate character states. Nomenclature deals with the determination of a correct name for a taxon using rules and recommendations of the ICBN. Phylogeny is the study of the genealogy and evolutionary history of a taxonomic group. Genealogy is the study of ancestral relationships and lineages.

The modern trend in plant classification uses the studies from comparative anatomy, embryology, cytogenetics, phytochemistry, and palynology etc. New approaches are: a) study of chemical constitution - phytochemical information (Chemotaxonomy),
b) studies on ultrastructure and micromorphology, c) statistical analysis of available data (Taxometric) and d) phylogenetic data to construct phylogenetic relationship (Cladistics)( Singh, 2000).

1.1.1. Phytochemistry in relation to taxonomy

Chemical systematics is the study of chemical variation in different organisms. The chemical features of plants are used in developing classification for solving taxonomic problems. This approach of taxonomy is called chemotaxonomy. Chemotaxonomy is used in all groups of plants. From early 1960 onwards, the phytochemical characters have been used in taxonomy (Waterman, 1998).

Recently the development of new and powerful analytical techniques contributed much in the field of Phytochemistry. Various studies confirm that phytochemical characters correlate quite well with other plant characters. Chemotaxonomy is a major source of new characters and information. The chemical characters are more important when they show a high degree of correlation with other features.

The plants classified by taste, colour, smell etc. were the practice of ancient people. Modern methods of chemical identification of plant products like chromatography and spectroscopy, gained important status in phytochemistry. The taxonomical studies in relation to chemistry involve the study of the distribution of chemical compounds in related families of plants. The compounds exist in individual parts of plants such as bark, wood, leaves, roots etc. With the development of improved techniques for studying biological molecules, especially proteins and nucleic acids, the knowledge in phytochemistry has greatly increased.

Chemical information is used to improve classification. Man classified plants as edible and inedible which is based on their chemical differences. A large variety of chemical compounds are
found in plants. The biosynthetic pathways responsible for these compounds also differ from one taxonomic group to another. The distribution of these compounds and their biosynthetic pathways correspond well with the existing taxonomic arrangements based on more traditional criteria such as morphology. In some cases, chemical data contradicted existing hypothesis. This necessitates a re-examination of the problem. Chemical data provide informations in situations where other forms of data are insufficient (Singh, 2000). The distribution of serological characteristics of seed proteins with primulaceae is studied and the results obtained are in accordance with the proposed subdivisions of the family made on morphological basis (John, 1978). The leaf phenolics of the leaves of the 11 species Fouquieriaceae studied reveal that there is no variation among the species in the phenolic distribution. All species contained ellagic acid, isoquercitrin, rutin, caffeic acid and scopolin (Scogin, 1978). The taxonomy of *Primula* species has been in dispute due to the high morphologic variability and several hybridizations. Three morphological features of the trichome, size and dimensional ratio of stalk, neck and gland head were studied. These three trichome elements are found to be typical for each species of Primula – *Primula auricula, Primula daonensis* and *Primula hirsuta*. The morphological characters treat the 3 species differently. The studied flavonoids are different in the three species. Three different flavanoid profiles are obtained. Pytochemical investigations of the flavonoid composition of leaves are taxonomic markers. Thus both the morphological and taxonomic markers support the separation of the three species (Fico et al., 2007).

Apocynaceae members show anti-leukemia activity and this is attributed to the frequent occurrence of indole alkaloids as major anti-tumor active constituents in many of the genera, *Catharanthus*, *Rauwolfia*, *Tabernaemontana*, *Thevetia*, *Alstonia*, *Cerbera*, *Holarrhena*, *Chilocarpus*, *Kopsia*, *Hunteria* (Cragg et al., 2006). Many of the
medicinally important plants such as *Alstonia scholaris* (L.) R. Br., *Catharanthus pusillus* (Murray.) G. Don., *Catharanthus roseus* (L.) G. Don, *Cerbera odollam* Gaertn., *Holarrhena pubescens* (Buch-Ham.) Wall. ex Don, *Plumeria alba* L., *Plumeria rubra* L., *Rauwolfia serpentina* (L.) Benth. ex Kurz., *Rauwolfia tetraphylla* L., *Tabernaemontana alternifolia* L., *Tabernaemontana divaricata* (L.) R. Br., *Wrightia tinctoria* (Roxb.) R. Br. belong to the family Apocynaceae. The plants are used in many of the crude as well as purified drugs in both traditional and modern systems of medicines. Hence there is a need for standardizing the raw drug. Taxonomy and Phytochemistry are the next important parameters by which standardization is done. There still exists a lacuna in the field of Apocynaceae members.

George Bentham (1800 – 1884) and sir Joseph Dalton Hooker (1817 – 1911) jointly published the *Genera plantarum* in 1862-1883 in three volumes. Apocynaceae is included in the cohort Gentianales of the series Bicarpellatae in their work. Bicarpellatae is placed in the sub-class Gamopetalae of the class Dicotyledons.

### 1.2. Phytochemistry

Phytochemistry deals with the chemical structure of a number of organic substances accumulated by plants. The biosynthetic turnover and metabolism, the natural distribution and the biological function of all the organic substances in the plants are dealt with in this branch. The phytochemical investigation of a plant involves authentication and extraction of the plant material, separation and isolation of the plant constituents, characterization of the isolated compounds, investigation of the biosynthetic pathways to particular compounds and quantitative evaluations (Harborne, 1973)

Early scientists in the field of phytochemistry couldn’t investigate a number of compounds in plants because of the lack of instruments
and techniques. In the nineteenth century, progress in this field was rapid. In 1803 narcotine, the first alkaloid was isolated; morphine, strychnine, emetine and many other followed. The studies of the chemical nature of fats and fixed oils were also carried out. In the middle of twentieth century, the chemistry of the natural product became the thrust area and a number of compounds were isolated and their structures were determined. Natural product Chemists later turned their attention to the biosynthetic pathways found in plants. All new techniques of separation and analyses available at that time were used by phytochemists (Evans, 2002).

1.2.1. Phytochemistry and medicinal plants

Since prehistoric times, Man has turned to plants for fulfilling their basic needs- food, clothing and shelter. Plants have been the earliest source of medicine and still constitute the chief components for traditional and modern medicines. Archeological evidences indicate the use of plants for healing. Digs at the Shanidar cave in northern Iraq revealed the 60,000 year old grave of a Neanderthal medical practitioner. Arrayed around his body were the remains of eight species of flowers, several of which are still used in these days for medical purposes by inhabitants of that region (Dwyer et al., 1986).

Medicinal and aromatic plants play an important role in the socio-cultural, spiritual and health-care needs of rural and tribal people of the emerging and developing countries. In many developing countries, a large section of the population still relies on traditional medicine to meet their health-care needs. More and more people in the developing countries have turned to alternative therapies and herbal remedies, resulting in an increase in the demand of medicinal plants and their products in those parts of the world (Vasisht and Kumar, 2003).

Humans depend mainly on plants to treat majority of illness. Minor problems such as coughs and colds to life-threatening diseases such
as tuberculosis and malaria are treated by plant based drugs. Now herbal remedies are coming back into prominence. Herbal medicine often complements conventional treatments, providing safe well-tolerated remedies for chronic diseases. The ability of a herbal medicine to affect body systems depends on the chemical constituents that it contains. Plants contain thousands of chemical constituents, which interact in complex ways to produce the therapeutic effect. Researches in isolated plant constituents have also paved the way to the development of many of the important and useful drugs. Tubocurarine - the most powerful muscle relaxant, derived from curare – *Chondrodendron tomentosum* and the strongest pain killer, morphine from opium poppy – *Papaver somniferum*. Many of the anaesthetics like cocaine obtained from coca - *Erythroxylum coca* are derived from plants. Today biomedicine still relies on plants rather than the laboratory, for at least 25% of its medicines. Many of such most effective drugs are quinine with anti-malarial properties derived from *Cinchona*; digoxin from *Digitalis lanata* for heart cure and ephedrine with cough relieving properties from *Ephedra* (Chevallier *et al.*, 2001).

Spectroscopic techniques such as nuclear magnetic resonance spectroscopy, mass spectrometry or X-ray crystallography are all used in determining the structure of the isolated active principle. The isolation of the natural products such as digoxin, morphine and quinine has resulted in replacing the plant extracts. It is supposed that any plant with chemical effect must contain an active principle that can replace the plant extract but in many cases it is the crude extract that exhibits the therapeutic effect (Phillipson, 1995).

Now, in this century, with all the available techniques of separation, isolation and determination of the structure of the plant constituents, there is a great need for research in this direction. This approach will definitely lead the isolation of active principle and thereby evolving new clinical agents to cure many of the diseases.
In general, herbal drugs are creating a number of problems in the global drug market. The enormous increase in the demand of herbal drugs in recent years revealed the extent of depletion of natural resources, lack of standardized protocol and the authenticity of the raw materials used. The inadequate supply of authenticated plant material is the reason for adulteration and substitution. For the accuracy, precision and consistency of formulations along with desired therapeutic effect, quality assurance of raw materials is necessary. This is achieved by phytochemical studies which include HPTLC chemoprofiling.

1.2.2. Biochemical Systematics or Chemotaxonomy

One of the most rapidly developing fields in phytochemistry is the biochemical systematics or chemotaxonomy. As early as in 1960, phytochemical characters have started to attract the plant taxonomists. The new and advanced analytical techniques have developed and thus facilitated the screening of large number of taxa in a very short time. These phytochemical data thus gathered are used in plant taxonomy (Waterman, 1998).

Phytochemical characters of taxonomic significance are grouped into three viz: i) primary constituents like proteins, nucleic acid, chlorophyll and polysaccharides; ii) secondary constituents like alkaloids, flavonoids, phenols, sterols etc. and iii) miscellaneous substances.

The phytochemical investigation of a plant may thus involve 1) authentication and extraction of the plant material, 2) separation and isolation of the chemical constituents, 3) characterization of the isolated chemical compound, 4) investigation of the biosynthetic pathways to particular compound, 5) quantitative evaluation, and 6) the pharmaceutical assessment of the separated compounds.
1.2.3. Authentication of the plant material

The plant material should be properly authenticated. The plant should be identified and authenticated by an acknowledged authority or by a taxonomic expert. The plant material can be ascertained by visiting botanical gardens and matching the botanical profiles with living plants, comparing it with the preserved specimens in the herbaria and museums. Photographs of the plant can also aid in this field of authentication. In phytochemical research, it is now a common practice to deposit a voucher-specimen of the plant in a recognized herbarium.

1.2.4. Extraction

The authenticated plant material can be subjected to extraction. The extraction method depends on the nature of the plant material and the components to be isolated. Dried materials are usually powdered before extraction. Fresh plant parts (leaves, stem, root) are macerated with various solvents such as water, alcohol, petroleum, ether and chloroform. Extraction may be performed by repeated maceration with agitation and percolation or by continuous extraction which is done in soxhlet extractor (Harborne. 1973).

1.2.5. Separation and isolation of constituents

The most difficult operation in phytochemistry is the isolation and separation of plant constituents. The available methodologies are sublimation, distillation, fractional liberation, fractional crystallization and adsorption chromatography (Evans, 2002).

1.2.6. Thin Layer Chromatography (TLC)

Thin layer chromatography is a technique by which the complex mixture can be resolved into individual compounds. It is based on both qualitative and quantitative analysis. Various types of thin layer
chromatographic techniques are in practice. They are ascending, descending, two dimensional and circular chromatography.

Of the many chromatographic methods presently available, thin layer chromatography (TLC) is widely used for the rapid analysis of drugs and drug preparations especially herbals (Evans, 2002).

1.2.7. High Performance Thin Layer Chromatography (HPTLC)

HPTLC is a powerful tool for qualitative and quantitative analysis of the herbal drugs. It is a sophisticated and automated form of TLC and it uses the technique in more optimized way. HPTLC is the instrumental version of TLC which is a versatile separation technique. It can handle several samples of divergent nature and composition, supporting several analyses at a time. It can speed up the work and allows the doing of many things at a time, usually not possible with other analytical techniques. The sample fractions are scanned with a light beam in the visible or ultraviolet (U.V) range long or short of the spectrum.

1.2.8. Methods of identification of plant constituents

After isolating and purifying, the plant constituent has to be identified. First the class of compound should be determined and then the particular substance within the class is found out. The class of compound can be concluded by studying its response to color tests, its solubility and Rf properties and its UV spectral characteristics. Complete identification within the class depends on studying other properties like melting point (for solids), boiling point (for liquids), optical rotation (for optically active compounds), Rf value etc. A known plant compound can be identified by ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) methods. The present study has undertaken ultraviolet and visible spectroscopy and mass spectroscopy to identify the constituents separated.
1.2.9. Mass spectroscopy

In mass spectrometry a mass spectrum is obtained by converting the compounds of a sample into rapidly moving ions (generally positive ions). These ions are resolved on the basis of their mass to charge ratio. The spectrum produced, known as mass spectrum gives important information about various masses produced and also about their relative abundances (Yadhav, 2003).

In the present study mass spectrum analysis was done by Q1 MS scan in a LC-MSMS system of applied Biosystems (API 2000 model) using turbo-ion spray ionization mode. Reserpine is scanned in positive ion mode in a molecular weight range of 100 to 700.

1.3. Scope of the present investigation

Plants are rich sources of potent drugs to cure mankind of diseases. Man has always relied entirely on plants to treat all kinds of diseases. Today herbal remedies are coming back into prominence.

Many plants are well known for their medicinal effects. For eg. *Aloe vera* was known to Cleopatra as a healing for skin ailment. But it is only now the active constituents responsible for their medicinal actions have been isolated and observed.

In this globalized world the alternative systems of medicine like Ayurveda, Unani, Siddha, Homeopathy and Neuropathy are all in existence, and in this era, safety, efficacy and quality control of these medicines are important. To achieve accuracy, precision and consistency of formulation along with desired therapeutic effect, quality assurance of raw material is necessary.

The raw material at source should be properly identified and certified. Identification, conformation and authentication are the
methods adopted for quality control that include investigation into adulteration, substitution and the presence of foreign matter. This is achieved by macroscopic and microscopic analyses, and also through chromatographic studies.

Usually plant materials are collected by unskilled labourers. They recognize it by local name and it is difficult for them to differentiate the species botanically. This is an important cause of adulteration and substitution because a single vernacular name is given for many different species or many different species are known by a single name. The correct material can be determined by taxonomic studies.

1.3.1. Authentication

Authentication is one of the important steps in collecting the raw material for a medicine. The detailed taxonomic identification with internationally accepted nomenclature is an integral part of authentication.

1.3.2. Adulteration / substitution

Due to popularity of herbal drugs, adulteration and substitution have become common. The depletion of natural sources may be one of the reasons. Lack of correct identification may also be the cause. Pharmaceutical companies are getting plant materials from traders, who in turn get these from untrained people from rural and forest areas. These are the reasons for wide-spread adulteration and substitution.

Quality is vital for herbal medicine. That means, ensuring the herbs and herbal materials used are of good quality, properly grown, well dried and correctly processed. The exact plant should be used for the right clinical effect. The important step for any herbal formulation is to obtain the exact plant material.
The available literature reveal that the family Apocynaceae consists of several members that are of medicinal importance and many of them are used in the preparation of drugs. Therefore there is a need for preparing a protocol for checking the exactness of the herb. Many of the species have not been studied in this approach and the present study is aimed at getting an insight into these aspects. The present investigation comprises 11 medicinal and related species belonging to the seven genera of the family Apocynaceae. The focus area of this research is taxonomy and phytochemistry of 11 plants belonging to family Apocynaceae with a view to provide the correct Botanical identity and to explore the chemoprofile.

**1.4. Objectives of the present investigation**

1) Identification and authentication of the 11 plants under study which include taxonomic description, phenology and distribution; medicinal uses and preparation of herbarium.

2) Determination of the ash value and the concentration of selected inorganic elements.

3) Preliminary phytochemical analysis for the detection of secondary metabolites and preparation of chemoprofile by HPTLC.

4) Detection of reserpine in the leaves and stems using LC-MSMS

5) Preparation of dendrogram by cluster analysis of HPTLC data and thereby analyzing the relationship between the species studied