Chapter 1

General Introduction

The order Myrtales is a comparatively large taxon of dicotyledonous plants. Bentham and Hooker (1862-1883) placed this order in Series Calyciflorae of Polypetalae. It is characterized by transition from perigyny to epigyny, stem tissues containing intraxylary phloem, leaves more often opposite than alternate, cyclic flowers and a hypanthium. The plants belonging to this order are found in tropical as well as temperate countries. The six families included in this order by Bentham and Hooker were Rhizophoraceae, Combretaceae, Myrtaceae, Melastomataceae, Lythraceae and Onagraceae.

Engler and Prantl (1887-1899) added three more families, Punicaceae, Lecythidaceae and Alangiaceae into their suborder Myrtineae of order Myrtiflorae. Hutchinson (1973) kept 10 families to this order by adding Barringtoniaceae, Asteranthaceae and Sonneratiaceae to this order. The Barringtoniaceae and Asteranthaceae were splinter groups separated from Lecythidaceae. Sonneratiaceae was a group taken out from Lythraceae. The families removed were Alangiaceae (kept in Araliales) and Onagraceae (kept in Onagrales).

The Myrtales of Cronquist (1981) was slightly different. He removed Rhizophoraceae and kept the same in a unifamilial order Rhizophorales. The Alangiaceae was shifted to the order Cornales and both the orders Rhizophorales and Cornales were kept alongwith Myrtales in subclass Rosidae. The family Lecythidaceae was placed in the order Lecythidales kept in a primitive subclass Dilleniidae. He added families such as Penaeaceae, Crypteroniaceae, Thymeleaceae, Trapaceae and Oliniaceae also into this order. The families circumscribed by him were 12 which contained more than 9000 species. About three-fourths of the species belonged to two large families the Melastomataceae (4000 spp.) and the Myrtaceae (3000 spp.). Another four families the Onagraceae, Combretaceae, Lythraceae and Thymeleaceae had 400 to 650 spp. each. The remaining 6 families within this order were Sonneratiaceae, Lythraceae, Penaeaceae, Crypteroniaceae, Thymeleaceae, Trapaceae, Myrtaceae, Punicaceae, Onagraceae, Oliniaceae, Melastomataceae and Combretaceae.

Myrtales of Takhtajan (1980) contained four suborders Myrtineae, Haloragineae, Rhizophorineae and Lecythidineae. All the suborders except Myrtineae lack internal phloem and are unifamilial containing families Haloragaceae, Rhizophoraceae and Lecythidaceae respectively. The Myrtineae contained 11 families such as Crypteroniaceae, Lythraceae, Sonneratiaceae, Punicaceae, Melastomataceae, Oliniaceae, Penaeaceae, Myrtaceae, Combretaceae, Onagraceae and Trapaceae.

Kubitzki (1990) included the family Rhynchocalycaceae, a splinter family of Crypteroniaceae due to bisexual flowers; flowers polygamodioecious in the latter family. The remaining 11 families in this order were the same as that of Cronquist. The Rhizophoraceae were included in a unifamilial order Rhizophorales and placed nearby. The Alangiaceae were kept in Cornales. The family Lecythidaceae was considered primitive and kept in another unifamilial order Lecythidales kept away in Dilleniidae near Theales.

Thorne (2000) divided the order Myrtales to three suborders. The first suborder Melastomatineae contained Penaeaceae, Oliniaceae, Rhynchocalycaceae, Alzateaceae, Crypteroniaceae, Melastomataceae and Memecylaceae, the last family being a splinter group of the Melastomataceae. The second suborder Myrtineae contained Myrtaceae, Onagraceae and Vochysiaceae. The third suborder Lythrineae included Lythraceae, Onagraceae and Combretaceae. The family Punicaceae had been reduced to a subfamily Punicoideae and included in the Lythraceae. The Alangiaceae were kept in Cornales and Rhizophoraceae in Geraniales. Dahlgren (1988) also treated Rhizophoraceae in Geraniales.

A glance through the different classificatory schemes on the order Myrtales indicates that no two classifications are alike. This reflects that the relationships among the families are not well defined. Though not discussed above, there exist many differences in the intrafamilial classification also and these will be discussed in the respective chapters on families). The present investigation, therefore, is an attempt to find out the chemical interrelationships existing among the taxa included within with a view to reduce the taxonomic confusion existing among them. Such an attempt falls into a well-known discipline of Taxonomy: **Chemosystematics.**

Chemosystematics

Chemosystematics (Chemotaxonomy or Chemical plant taxonomy), in which chemical characters are used as aids in taxonomy, owes its origin to the earliest classification of plant kingdom where the algae are grouped based on their color (pigments), storage wall and cell wall. Though Helen Abott predicted, as early as in 1886, that the study on chemical principles of plants from a purely botanical view would become a new field of research, McNair (1929) who screened 300 oils, fats and waxes occurring in 83 families and arranged them in relation to taxonomy may be hailed as the pioneer in this field. Manske's (1944) works on alkaloids and Mirov's (1961) work on volatile oils are other landmarks in the history of chemical plant taxonomy. But it is with the simultaneous release of three books 'Chemotaxonomie der Pflanzen' (Hegnauer, 1962-65), 'Biochemical Systematics' (Alston and Turner, 1963) and 'Chemical Plant Taxonomy' (Swain, 1963), this branch of taxonomy is recognized as a legitimate branch of taxonomy.

Swain (1963) defines chemotaxonomy as "the investigation of the distribution of chemical compounds or groups of biosynthetically related compounds, in series of related or supposedly related plants". Since the chemical compounds are intermediates between the genes and their morphological expressions, the chemical characters are sometimes considered more important than the characters from other disciplines. The cryptic nature of the chemical characters, in that they are 'hidden' from the eyes of the 'classical' taxonomist also favoured giving them undue weightage in some treatments. But the critical studies conducted in recent years proved that the chemical characters also are susceptible to all the drawbacks such as parallelism, convergence, reduction, environmental modification and inconsistency, as other taxonomic evidences and, therefore, the present day taxonomists give those equal weightages at par with evidences from other disciplines.

Any chemical compound present in the plant at any stage of growth is a potential taxonomic marker which may be used at higher or lower levels of taxonomic hierarchy. Thus the presence of chlorophyll delimits the green plants from fungi while betacyanins circumscribe the Centrospermae. This means that none of the chemical markers can be dismissed because of its wide or narrow distribution. But a good chemical character must

be 'variable, stable, unambiguous and not easily, if at all, changeable'. The presence of high incidence of contrasting characters, the ease of determination and low correlation are added advantages of a chemical marker. A high correlation between characters would be of great phylogenetic interest. The chemical characters have an added advantage over morphological characters in that they can be exactly described in terms of definite structural and configurational formulae.

Most of the chemical characters are attempted at ordering them into phylogenetic sequences. Though the absence of fossil record may seem an obvious disadvantage, the knowledge of biosynthetic routes definitely gives the chance to judge whether a character is early or late in evolution. An organism having a long biosynthetic sequence is believed to be more advanced than an organism with a short sequence. Thus if a sequence A→ B→ C→ D is recognized, then it is assumed that an organism with A has fewer enzymes than one with D and the latter is considered more advanced than the former. As an alternative to this, if a compound is shown to be statistically correlated in its occurrence with another character already known to be advanced, then the first character also is considered advanced (Sporne, 1956).

The chemical characters available for a chemotaxonomist are legion. Davis and Heywood (1967) classified them into three groups (1) directly visible substances, (2) serology, electrophoresis of proteins and DNA/RNA analyses and (3) plant products.

Directly visible substances present in plants are starch grains, raphides and other crystals. The form, structure and the mechanism of formation of starch grains were used by the earlier taxonomists. Recently Czaja (1978) conducted an exhaustive study on the behaviour of starch grains in polarized light and their swelling pattern when placed in water and warmed. Raphides were used as a taxonomic character in Orchidaceae, Scitaminae, Rubiaceae, etc. (Gibbs, 1963). Crystals of calcium oxalate, silica and gypsum were used in the classification of *Allium*, Poaceae and Tamaricaceae respectively (Davis and Heywood, 1967).

Serology, which employs the specificity of antigen-antibody reaction, was extensively used in 1930's and 1940's to classify higher plants. But the difficulty in distinguishing the different antigen-antibody systems in liquid media prompted the development of Ouchterlony and Elek double diffusion method. Electrophoresis of proteins, peptide

finger print data and the amino acid sequences are some other ways of utilizing proteins in chemotaxonomy. **DNA homology** i.e. the extent to which two DNA molecules pair, is looked upon as the similarity index between the source organisms from which the two molecules come from. This work is extended to DNA-RNA homology where the extent of duplex formation between RNA and single-stranded DNA is taken as the affinity index (Alston and Turner, 1966). Of late the gene sequences are used in Taxonomy, but this is now a separate branch known as **Molecular Taxonomy**.

Plant products include all the plant metabolites stored in plants for a longer period of time. They are classified into two groups, the primary and secondary metabolites. The **primary metabolites** include sugars, polysaccharides, amino acids, peptides, fatty acids and fats. The qualitative analysis of these compounds is more significant than the quantitative, since the quantity may vary according to the environmental conditions. Some of the sugars used in taxonomy are (1) glycolose- found in the free state in the leaves of Caprifoliaceae members, (2) apiose- a cell wall constituent of many aquatic monocotyledons, (3) sorbitol- in the fruits of Rosaceae and (4) dulcitol- in Celastraceae. Non-protein amino acids having a restricted distribution in plant kingdom are also effectively used in classification. Sulphur-containing amino acids and azetidine-2-carboxylic acid in Liliaceae are examples of their utility. Among the fatty acids, erucic acid in Brassicaceae, petroselinic acid in Apiaceae and chaulmoogric acid in Flacourtiaceae are examples of characteristic components of plant groups.

The **secondary metabolites** are all those compounds produced by the plant but are not ultimately involved in basic energy transfer or assimilatory activity of all tissues (Alston and Irwin, 1961). They are synthesized by the secondary metabolic pathways which are almost independent of the primary metabolic pathways. The functions of most of these plants are obscure. Earlier, these compounds were considered 'excretory products', 'waste products' or 'side products' and Heslop-Harrison calls them 'luxury diversifications' which reflect the chemical virtuosity of the plant concerned. All these compounds are genetically controlled and probably are fixed at a low selection pressure. But studies in the recent past indicate that most of the secondary metabolites have a positive role in plants, in that, most of them are defensive in nature, greatly important for

survival of plants in this planet. Some of the secondary metabolites effectively used in the present study are as follows:

Flavonoids

Flavonoids by far, are the group of natural products which is the most sought after by chemotaxonomists. This group includes all the C_6-C_3-C_6 compounds related to a flavone skeleton. The different classes of flavonoids are distinguished by the oxidation level of the C_3 fragment and the various members of each class are recognized by the number and positions of the hydroxyl groups, substitutions and glycosylation. These compounds are preferred over most of the other low molecular weight constituents like terpenes and alkaloids, for their universal distribution in vascular plants (that also in all organs), structural diversity and stability during extraction procedures and the ease and rapidness of identification. Even herbarium specimens can be screened for these compounds. The facilities required for the isolation and characterization of the common flavonoids are paper chromatographic set ups and a spectrophotometer.

In one of the most critical studies on the reliability of flavonoids as taxonomic markers, McClure and Alston examined the pattern of flavonoid chemistry in *Spirodela* grown in as many as 58 different culture media. The different treatments included addition of various growth hormones like auxins and gibberellic acids. In almost every instance the flavonoid pattern remained unchanged and this conclusively proves that the formation of flavonoids is concluded completely by intrinsic factors (Alston and Turner, 1966).

All the classes of flavonoids have been effectively utilized in taxonomy. But the most useful characters are those which are visibly colored or those which exhibit fluorescence in ultraviolet light, such as anthocyanidins, flavonols, flavones, chalcones and aurones. Proanthocyanidins (leucoanthocyanins) are also included here because they liberate anthocyanidins which are easily detectable when treated with acid. Most of the flavonoids occur in plants as their glycosides and so a methanolic extraction followed by an aqueous extraction of the residue (left after distilling off the methanol) would separate out most of these compounds in water. These compounds may either be chromatographed or hydrolysed to determine the aglycone. The aglycones are few in number and are easily identified with the help of Rf value (or Rst value, when a standard is used), color in UV

light, fluorescence in UV light, change in fluorescence with ammonia fumes, stability in chromatograms after the paper/plates are sprayed with sodium carbonate solution, color development after ferric chloride spray and UV spectra. Once the basic skeleton is known, further characterization is easy because many reagents are available now, which react with the hydroxy or methoxy groups at specific locations and effect hypsochromic or bathochromic shifts in the methanolic spectra. The identification of compounds is confirmed by co-chromatography with the standards (in six solvent systems) isolated from the known plant sources or procured from the commercial sources.

One of the advantages of working on flavonoids is that this data can be interpreted in terms of phylogeny. The chemogenetic series of flavonoids, analogous to morphogenetic series, is identified from the biosynthetic studies and from the correlation of flavonoid structures with progressive specialization in plants. Harborne (1967) and Swain (1975) have proposed more or less similar evolutionary schemes for flavonoids. Based on this as well as the correlation studies, it is concluded that 3-oxygenation, more hydroxyl groups and simple glycosylation in anthocyanidins, presence of proanthocyanidins (in leaf) and myricetin, absence of methylation, more hydroxyl groups, hydroxylation at 6-position in flavonols and C-acylation are primitive features in angiosperms. 3-Deoxygenation, O-methylation, absence of proanthocyanidins (in leaf) and myricetin, presence of flavones, their O-methylation, complex hydroxylation, 6-oxygenation and 2'-hydroxylation, aurones and absence of C-glycosylation, biflavonyls and flavonones are considered advanced. By considering the reduction trends, which involve reverse trends down the biosynthetic series, the presence of seemingly primitive features (according to above dicta) in certain groups, which are otherwise advanced, may be considered highly advanced. These reduction trends may culminate in the ultimate loss of flavonoid skeleton from plants (Gornall and Bohm, 1978).

'Bioflavonoids' are a group of flavonoids exhibiting pharmacological properties, especially 'Vitamin P' activity. 'Vitamin P' refers to a group of compounds which are known to be the 'permeability factors' which increase the capillary resistance and thereby used to treat subcutaneous capillary bleeding. Rutin (3- rutinoside of quercetin), its methylated derivatives and flavanones from *Citrus* fruits formed the principal components of Vitamin P. The interest on physiological effects of flavonoids resulted in a

spurt on the research on these compounds and consequently more than 200 preparations were in use (Meyers, *et al.*, 1972). It is experimentally established that flavonoids with free hydroxyl groups at the 3', 4'- positions exert beneficial physiological effects on the capillaries through (1) chelating metals and thus sparing ascorbate from oxidation, (2) prolonging epinephrine action by the inhibition of O-methyl transferase and (3) stimulating the pituitary-adrenal axis (De Eds, 1968). Srinivasan *et al.*, (1971) presented evidence that flavonoids play another important role in circulatory system by acting on the aggregation of erythrocytes. Most of the flavonoids occur as water soluble glycosides in plants. A single flavonoid aglycone may occur, in a plant, in several glycosidic combinations and for this reason it is considered better to examine the aglycones in hydrolysed plant extracts (Harborne, 1984).

Phenolic acids

The term phenolic acid includes both the benzoic and cinnamic acids and they are a group widely distributed in plant kingdom. The common benzoic acids, *p*-hydroxy benzoic, vanillic and syringic acids are normally derived from p-coumaroyl, coniferyl and sinapyl residues present in lignin and so located in all angiosperms. Gymnosperm lignin lacks syringyl residues. Gentisic and protocatechuic acids are two other phenolic acids common in higher plants. Salicylic and *o*-pyrocatechuic acids are frequently met with in Ericaceae. Salicylic acid occurs as its methyl ester in the oil of wintergreen (*Gaultheria procumbens*). Salicylic acid and its derivatives inhibit cyclooxygenase, a key enzyme of prostaglandin biosynthesis and therefore used in cardiac problems. Gallic and digallic acids along with the dimeric ellagic acid are the phenolic components of gallo- and ellagitannins. Hexahydroxydiphenic acid is another component homologous with ellagic acid found in tannins. These acids are said to be absent in lower groups of plants. Some phenolic acids with unusual hydroxylation patterns such as 2-hydroxy-4-methoxy, 2-hydroxy-5-methoxy and 3, 5-dimethoxy acids are seen in *Primula officinalis*. Similarly 2-hydroxy-6-methoxy benzoic acid and 2,4-dihydroxy benzoic acid are reported from *Gloriosa superba* whereas 2-hydroxy-6-methoxy benzoic acid is present in *Colchicum autumnale*. Aldehydes such as *p*-hydroxy benzaldehyde, vanillin and salicaldehyde

(corresponding to the common phenolic acids *p*-hydroxy benzoic, vanillic and salicylic acid) also are seen in many plants.

Quinones

Quinones, the aromatic diketones, form the largest class of natural colouring matters and are formed by the polyketide pathway. Of the total 800 and more compounds known, about 50% occur in higher plants and of the rest, a large percentage are known from fungi. Though quinones are the sole colouring pigments of fungi, in higher plants they play a subsidiary role, being mostly confined to the bark or underground portions and if present in other parts, make very little contribution to the colour, due to the masking by other pigments. Colourless quinones and their derivatives also exist in plants possessing different physiological roles. Quinones may be benzo, naphtho or anthraquinones depending on the mono-, bi- or tri-cyclic ring systems they possess.

Saponins

Saponins are the glycosides in which an oligosaccharide consisting of 2-5 sugar units and one glucuronic acid molecule, is linked to C_3 position of the aglycones, are soluble in water and like soaps, produce stable froth (emulsions) when shaken with water. They are characteristically toxic to fish and haemolyse RBC. Sapogenins, the aglycones, may be either a triterpene like β-amyrin or a steroid possessing a spiroketals side chain like sarasapogenin. A series of saponins may have different sugar components but same sapogenin.

Saponins are found widespread in higher plants, having been detected in well over 70 plant families and 3000 species. Steroid saponins are frequent in monocot families, whereas triterpene saponins are predominant in dicots. Gymnosperms are virtually free of saponins.

Alkaloids:

Alkaloids are basic plant products possessing a nitrogen-containing heterocyclic ring system and showing marked pharmacological activity. The term 'alkaloid' means 'alkali like substance', and they form the largest class of secondary plant substances; at

present numbering more than 12,000. They form a very heterogeneous group exhibiting a wide range of chemical structures and properties.

All alkaloids, as a rule, occur in plants only, though a few of them are seen in animals too. Almost all of them can be synthesized chemically. Present studies indicate a rather restricted distribution of alkaloids amongst plants. Among Cryptogams they occur in some fungi like *Claviceps* and *Amanita* and some ferns. *Cephalotaxus, Ephedra* and *Cycas* are the Gymnosperms showing alkaloids. Among Angiosperms, monocots generally have a lesser incidence of alkaloids, seen in the Amaryllidaceae, Liliaceae, Stemonaceae, Arecaceae, Poaceae and Orchidaceae. The Ranunculaceae, Menispermaceae, Berberidaceae, Papaveraceae, Rutaceae, Rhamnaceae, Fabaceae, Rubiaceae, Asteraceae, Apocynaceae, Asclepiadaceae, Loganiaceae and Solanaceae, include most of the alkaloid-yielding plants. Usually alkaloids with complex structures are characteristic of specific plant families, *e.g.*, benzyl isoquinolines in Ranales, I upines in Fabaceae, tropanes in Solanaceae, ergot alkaloids in Convolvulaceae and colchicine in the Liliaceae. Certain interrelationships appear exist between alkaloids and volatile terpenes in that they tend to be mutually exclusive.

Economic importance of Myrtales

From an economic point of view, the order Myrtales provides a good number of useful plants (a detailed discussion on this is available in chapters dealing with individual families). Some of the very important products from this order are

1) **Timbers** like Red gum, Karri, Blue gum and Mallet bark from various species of *Eucalyptus* (Myrtaceae)
2) **Fruits** like Grumichama and Pitanga from *Eugenia* sp., Guava from *Psidium*, Jambolan, Rose apple and mountain apple from *Syzygium* (Myrtaceae) and Pomegranate from *Punica* (Punicaceae).
3) **Volatile oils** like Allspice, Bay, Blue gum and clove from species of *Pimenta, Eucalyptus* and *Syzygium* (Myrtaceae)
4) **Tannin** sources from *Eucalyptus* species (Myrtaceae) and *Rhizophora* (Rhizophoraceae).

5) **Gums** like gum ghatti, and tannin sources from *Anogeissus* and *Terminalia* sp. (Combretaceae)
6) **Dyes** like henna from *Lawsonia* (Lythraceae).
7) **Nuts** like Brazil nuts and Paradise nuts from *Bertholletia* and *Lecythis* (Lecythidaceae)
8) A large number of **medicinal plants** like *Terminalias*, *Woodfordia*, *Syzygium* etc.

The present Ph.D. programme

Taking into consideration both these issues, the present Ph. D. programme is planned in such a way that both the taxonomic puzzle within the order and problems in the area of medicinal plants are addressed into. Therefore for **chemotaxonomical studies,** 55 plants belonging to these families have been subjected to a detailed analysis for their flavonoids, phenolic acids, alkaloids, quinones and other markers in leaves as well as stems and based on the pattern of distribution of these compounds in the taxa in question, the various relationships at inter- and intrafamilial levels are identified. The plants selected belong to Myrtaceae (17), Combretaceae (13), Lythraceae (7), Melastomataceae (9), Rhizophoraceae (3), Punicaceae (1), Onagraceae(1), Lecythidaceae (3) and Alangiaceae (1).

Since **medicinal plants** belonging to the families within this order are poorly studied for their constituents, biomarkers (both chemical and pharmacognostic) and for value-addition, 15 plants are taken up for detailed studies on above-said parameters. Besides the specific parts used commonly in medicine, other plant parts used by many a rural folk for medicinal purposes also are subjected to chemical and pharmacognostic studies. In chemical studies, emphasis is given to antioxidant polyphenols which are poorly studied in a conventional phytochemical treatment.

The medicinal plants taken up for studies are the following:

1. *Alangium salvifolium* Wang.
2. *Ammania baccifera* Linn.
3. *Barringtonia acutangula* Gaert.

4. *Careya arborea* Roxb.
5. *Combretum ovalifolium* Roxb.
6. *Lagerstroemia flos-reginae* Roxb.
7. *Lawsonia inermis* Linn.
8. *Ludwigia octovalvis* Roxb.
9. *Melastoma malabathricum* Linn.
10. *Pimenta dioica* Merr.
11. *Psidium guajava* Linn.(Red fleshed fruit)
12. *Syzygium malaccense* Miq.
13. *Terminalia arjuna* W. & A.
14. *Terminalia chebula* Retz.
15. *Woodfordia floribunda* Salisb.

The objectives of the Ph.D programme are the following:

1. To study the chemical inter-relationships among various taxa so that a logical grouping of the various taxa can be arrived at.
2. To study the chemical relationship of the Rhizophoraceae and to assign a taxonomic position to this family.
3. To assess the identity of the Punicaceae from the Lythraceae in which it was included.
4. To find out the chemical affinity of the Lecythidaceae to the Myrtales in general and to the Myrtaceae in particular.
5. To find out the affinities of the Alangiaceae to the Myrtales into which it was included earlier and Cornales in which it is included presently.
6. To assess the taxonomic validity of the two subfamilies Leptospermoideae and Myrtoideae within the Myrtaceae.
7. To locate chemical characters and biomarkers in various parts of medicinal plants which will help in finding out the active components and the genuineness of a drug.
8. To locate the pharmacognostic features and biomarkers useful for quality control analysis.

9. To study the chemical diversity of the medicinal plants which are poorly studied.
10. Bioprospecting of taxa allied to the medicinal plants to locate substitute sources of alkaloids, bioflavonoids and combretastatins.

The **thesis** is arranged in the following manner. Since two aspects i.e. chemotaxonomy of the families and detailed studies on the medicinal plants within these families are taken up, the thesis is divided into two parts; the first part dealing with the former subject and the second part dealing with the latter topic. After the general introduction, the second chapter embodies the methodology adapted for chemical and pharmacognostic analyses. The major families studied i. e. Myrtaceae, Combretaceae, Lythraceae, Melastomataceae and Rhizophoraceae form individual chapters. The smaller families like Punicaceae, Onagraceae, Lecythidaceae & Alangiaceae are included in the next chapter. In the second part the medicinal plants studied are grouped on the basis of families in which they belong. Thus the plants belonging to Myrtaceae, Combretaceae and Lythraceae form individual chapters and the plants of remaining families are included in the next chapter. The last chapter contains the summary and conclusions and the highlights of the present investigation. The Appendix includes the list and reprints of the articles published during the course of the present work.