Chapter 16

Summary and Highlights

The order Myrtales is a comparatively large taxon of dicotyledonous plants. The six families included in this order by Bentham and Hooker were Rhizophoraceae, Combretaceae, Myrtaceae, Melastomaceae, Lythraceae and Onagraceae. The plants belonging to these taxa were treated differently by Engler and Prantl (1887-1899) who added three more families, Punicaceae, Lecythidaceae and Alangiaceae; Hutchinson (1973) who added Barringtoniaceae, Asteranthaceae and Sonneratiaceae and removed Alangiaceae and Onagraceae; Cronquist (in 1981 removed Rhizophoraceae to Rhizophorales, Alangiaceae to Cornales, Lecythidaceae to Lecythidales and added Penaeaceae, Crypteroniaceae, Thymeleaceae, Trapaceae and Oliniaceae also into this order); Takhtajan (in 1980 divided to four suborders Myrtineae, Haloragineae, Rhizophorineae and Lecythidineae); Kubitzki (in 1990 included one more family, the Rhynchocalycaceae) and Thorne (2000) who divided the order Myrtales to suborders Melastomatineae (containing Penaeaceae, Oliniaceae, Rhynchocalycaceae, Alzateaceae, Crypteroniaceae, Melastomataceae and Memecylaceae), suborder Myrtineae (containing Myrtaceae, Onagraceae and Vochysiaceae), suborder Lythrineae (including Lythraceae and Combretaceae). Dahlgren (1988) included Rhizophoraceae in Geraniales. The causes of the confusion on this taxon are the poorly -defined relationships among the families and the many differences in the intrafamilial classifications. The present investigation, therefore, is an attempt to find out the **chemical interrelationships** existing among the taxa included within, with a view to ease the taxonomic confusion existing among them. This approach, known as the Chemosystematic treatment is used widely to solve many taxonomic problems.

Chemosystematics, in which chemical characters are used as aids in taxonomy, is a legitimate branch of Taxonomy and is used very effectively in solving taxonomic riddles and tracing out the phylogeny of the taxa. The classificatory schemes proposed by Cronquist and Dahlgren are almost entirely on the chemical characters. Though almost all the chemical compounds present in the plant at any stage of growth are used as taxonomic markers at higher or lower levels of taxonomic hierarchy, it is the **Plant products** (including all the plant metabolites stored in plants for a longer period of time) which are preferred by many. Among these compounds it is the **flavonoids** which are often used by a chemotaxonomist due to variability, stability, unambiguity and the ease of determination and low correlation. The knowledge of biosynthetic pathway helps ordering them into a phylogenetic sequence. These compounds exhibit a number of pharmacological properties also and are some of the best antioxidants.

From an economic point of view, the order Myrtales provides a good number of important plants such as timbers (Red gum, Karri, Blue gum and Mallet bark), Fruits (Grumichama, Pitanga, guava, Jambolan, Rose apple, Pomegranate and mountain apple), volatile oils (Allspice, Bay, Blue gum and clove), Tannin sources (*Eucalyptus, Rhizophora),* gums (gum ghatti), nuts (Brazil nuts and Paradise nuts) and a large number of medicinal plants (*Terminalia*s, *Woodfordia, Syzygium).* The medicinal plants belonging to the families within this order are poorly studied for their constituents, biomarkers (both chemical and pharmacognostic) and for value-addition.

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 using standard methods and based on the pattern of distribution of these compounds in the different taxa, the various relationships at inter- and intrafamilial levels are identified. In addition fifteen **medicinal plants** belonging to this order are taken up for detailed studies on their constituents, biomarkers (both chemical and pharmacognostic) and for value-addition. Besides the specific parts used as drugs, 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.

Chemotaxonomical studies

The first family **Myrtaceae**, where 17 members were screened, was found to be very rich in flavonols such as Kaempferol, quercetin, myricetin and gossypetin. The presence of gossypetin, which is otherwise rare in plant kingdom, is highly interesting. The higher prevalence of highly hydroxylated flavonols such as quercetin and myricetin along with their methoxylated derivatives, gallic acids and quinones and near absence of flavones and total absence of glycoflavones are the characters binding all the plants screened in this family. Between the two sub families, Myrtoideae and Leptospermoideae, there is a clear cut distinction in that gossypetin (and its methoxy derivatives) and a higher variety of phenolic acids are present in the former and their absence in the latter. The two varieties of *Syzygium malaccense* screened also show chemical differences. The same is the case with the two varieties of *Psidium guajava.*

The **Combretaceae**, where 13 plants were studied are similar to the Myrtaceae in having the same flavonols especially gossypetin and therefore are closely allied to the latter family. The presence of combretastatins and biflavones are the distinguishing features of this family which, incidentally, keeps this family as an advanced member in the order.

The **Lythraceae**, where 7 members were analysed for their chemotaxonomic markers, contained both flavones and flavonols and thus are similar to the other two families mentioned above. The absence of gossypetin, characteristic to the other two families, makes this family chemically slightly different from them. Therefore the separation of this family in a suborder Lythrineae is supported. Within the family, the two tribes Ammanieae and Lythreae are found to be chemically distinct.

The nine members of the family **Melastomataceae** are found to possess both flavonols and flavones. The flavonols encountered were kaempferol, quercetin and its methoxylated derivatives, quercetagetin and myricetin. The presence of the very same flavonols especially gossypetin, indicates that the Melastomataceae are similar to the

Myrtaceae and Combretaceae. The chemical data delineate the subfamily Memecyloideae clearly from the other subfamily, Melastomatoideae.

All the three members of the **Rhizophoraceae** were found to be very rich in flavones and flavonols. The distinct flavonols of the Myrtales, the gossypetin and its methoxy derivatives are visibly absent here. Therefore this family may not be at home with the other families of the Myrtales such as Myrtaceae, Combretaceae, etc. The absence of the isoquinoline alkaloids of the Cornales and iridoids keeps this family away from the Cornales also. Therefore the phenolic profile favour the treatment of Rhizophoraceae in a unifamilial order Rhizophorales closer to the Myrtales. The shifting of this family to the Geraniales as practiced by Thorne (2000) does not get any chemical support because tannins are unheard in any of the member families of the Geraniales.

The **Alangiaceae** are very distinct in possessing *o*-coumaric and protocatechuic acids. This family does not contain gossypetin, the flavonol characteristic to the Myrtales. Instead, it contains isoquinoline alkaloids which are not seen in Myrtales. All these chemical differences support the shifting of this family to another order Cornales by all recent taxonomists.

The family **Lecythidaceae** is similar to the rest of the Myrtales in containing gossypetin, its derivatives and other flavonols. Therefore the exclusion of this family from the Myrtales does not get any chemical support. But the differences in other characters such as lack of internal phloem and in having alternate leaves and bitegmic tenuinucellar ovules indicate that this family possesses distinct identity and therefore it may be treated as an order Lecythidales as practised by Takhtajan (1980). The Lecythidales with their clear disc, calyptrate calyx, inferior ovary, peculiar fruits and arillate seeds are entirely different from the Theales or Malvales. The triterpenoids saponins of the Lecythidaceae also are not common in these orders. Therefore the shifting of Lecythidaceae (or Lecythidales) near to Theales and Malvales cannot be justified.

The **Punicaceae** are distinct in having flavones in leaves and flavonols in the stem. Presence of alkaloids of pyridine type in both stems and leaves is another distinctive feature of this family. These chemical characters do not support including Punicaceae in Lythraceae as a subfamily Punicoideae as recommended by Thorne (2000). Lythraceae are characterized by quinones and the alkaloids of *Punica* are not reported in any member of Lythraceae. The Punicaceae can be considered as an advanced family of the Myrtales.

The **Onagraceae** with quercetin and gallic acid, the latter in large amounts, fits very well with the other families of the Myrtales.

Based on the chemical data, the order **Myrtales** can be considered consisting of two suborders **Myrtineae** and **Lythrineae**. The former suborder contains Myrtaceae, Combretaceae, Melastomataceae, Onagraceae and Punicaceae and the latter contains a single family Lythraceae. The Rhizophoraceae and Lecythidaceae are kept in separate, but closely placed orders **Rhizophorales** and **Lecythidales**. The Alangiaceae are kept away in the Order **Cornales.**

In addition to the contributions to the taxonomic studies, the present investigation unearthed 47 plants as rich sources of bioflavonoids and three new sources of combretastatins, which can be commercially tapped for the said compounds.

Medicinal plants

The work on the medicinal plants of Myrtales provided a large amount of data useful in understanding the multifarious activities of these plants and in quality control procedures. The phytochemical studies revealed the fact that all these plants as well as their various parts are very rich in phytochemicals such as flavonols, flavones and anthocyanidins. Flavonols are the most common flavonoids. The flavonols located are kaempferol, quercetin, myricetin and gossypetin and their various methoxylated its derivatives. Flavones are located in only one plant. Almost all these compounds are physiologically active bioflavonoids. Tannins especially proanthocyanins also are widely distributed in these plants. Quinones, another group of antioxidant phenolics also were fairly common. Alkaloids were rather rare, located in only two plants.

The phenolics reported above are found to exert a multitude of pharmacological properties. Of late, Flavonoids which are found to be the most common components of the medicinal plants screened here are found to have profound health benefits. **Quercetin** appeared to have many beneficial effects on human health including cardiovascular protection, anti-cancer activity, antiulcer effects, antiallergic activity, cataract prevention and antiviral and anti-inflammatory effects (Miller, 1996) and also inhibits lipid peroxidation *in vitro*. **Kaempferol** had a stimulatory effect on alkaline phosphatase activity in MG-63 human osteoblasts through ERK and estrogen receptor pathway. It was also shown to inhibit proliferation and increase mediator content in human leukemic mast cells. The activities of phenolic acids, which are found to be omnipresent, as well as those of quinones also are well recognised.

The present project resulted in finding out the biomarkers, chemical diversity and antioxidant compounds of all the parts of the plants selected. The biomarkers are extremely useful in identifying the genuineness of the drug and also to find out adulteration. The keys prepared using the chemical markers useful in identifying the various pants of the drugs provides ample proof for this claim. Here two keys are prepared; one for the leaf drugs and second for the stem drugs.

Similar to the phytochemical markers, the **pharmacognostic characters** also are found to be of great use in identifying or confirming the identity of a plant drug. The transverse sections of leaves and stems will be of immense use in checking the identity of a medicinal plant. The powder study will help in finding out whether a powdered drug is genuine or adulterated. Locating a particular cell component, not reported from the source plant, in a powdered sample proves that the sample is adulterated. A little bit of plant debris settled at the bottom of a container having an extract will yield very valuable information on the source plant. The keys prepared for both leaves and stems of the medicinal plants of the Myrtales prove the utility of pharmacognostic markers beyond doubt.

Highlights

The highlights of the present investigation are the following.

1. The present study lead to a better understanding on the chemical inter-relationships among various taxa of Myrtales so that a logical grouping of these taxa could be arrived at. As a result a new grouping of the families is attempted. In this, the order **Myrtales** is divided to two suborders **Myrtineae** and **Lythrineae**. The former suborder contains Myrtaceae, Combretaceae, Melastomataceae, Onagraceae and Punicaceae and the latter contains a single family Lythraceae. The Rhizophoraceae and

Lecythidaceae are kept in separate, but closely placed orders **Rhizophorales** and **Lecythidales** respectively. Alangiaceae is kept away in the Order **Cornales.**

2. The chemistry of the **Rhizophoraceae** is closer to the Myrtales than to any other group. But it possesses sufficient characters to be an independent taxon. Therefore the formation of a separate order Rhizophorales is supported. But this order does not possess any of the typical chemical characters of as Rosales or Geraniales and therefore it is grouped alongwith the Myrtales.

3. The **Punicaceae** are distinct in having flavones in leaves and alkaloids of pyridine type in both stems and leaves and thus are distinct from the Lythraceae. These chemical characters do not support including Punicaceae in Lythraceae as a subfamily Punicoideae as practised by Thorne (2000).

4. The family **Lecythidaceae** is very similar to the rest of the Myrtales in containing gossypetin, its derivatives and other flavonols. But the differences in other characters such as lack of internal phloem and in having alternate leaves and bitegmic tenuinucellar ovules indicate that this family possesses distinct identity and therefore it may be treated as an order Lecythidales as practiced by Takhtajan (1980). The Lecythidales with their clear disc, calyptrate calyx, inferior ovary, peculiar fruits and arillate seeds are entirely different from the Theales or Malvales. The triterpenoids saponins of the Lecythidaceae also are not common in these orders. Therefore the shifting of Lecythidaceae (or Lecythidales) near to Theales and Malvales is not justified.

5. The **Alangiaceae** are very distinct in lacking gossypetin, the flavonol characteristic to the Myrtales and in containing isoquinoline alkaloids. These chemical differences support the shifting of this family to another order Cornales by all recent taxonomists.

6. The two subfamilies of Myrtaceae, **Myrtoideae and Leptospermoideae,** are two valid taxa in that gossypetin (and its methoxy derivatives) and a higher variety of phenolic acids are present in the former and their absence in the latter.

7. The present project resulted in finding out the **chemical characters and biomarkers** of all the parts of the plants selected. The biomarkers are extremely useful in identifying the genuineness of the drug and also to find out adulteration. The keys prepared using the chemical markers useful in identifying the various pants of the drugs provides ample proof for this claim. Here using the chemical markers two keys are prepared; 1. for the leaf drugs and 2. for the stem drugs.

8. The **pharmacognostic characters and biomarkers** also are found to be of great use in identifying or confirming the identity of a plant drug. The transverse sections of leaves and stems will be of immense use in checking the identity of a medicinal plant. The powder study will help in finding out whether a powdered drug is genuine or adulterated. Locating a particular cell component, not reported from the source plant, in a powdered sample proves that the sample is adulterated. A little bit of plant debris settled at the bottom of a container having an extract will yield very valuable information on the source plant. The keys prepared for both leaves and stems of the medicinal plants of the Myrtales prove the utility of pharmacognostic markers beyond doubt.

9. Almost all the medicinal plants and their parts are found to be rich in **bioflavonoids** and antioxidant phenolics. The recent data vindicating these compounds to have very many pharmacological properties, all the 15 medicinal plants of the Myrtales selected may exert these properties also.

10. Due to the **Bioprospecting** done in the present study, three new sources of combretastatins and 47 new sources of bioflavonoids were discovered.

11. The discovery of **gossypetin** as a characteristic compound of Myrtales is highly interesting. This flavonol, which is having a very restricted occurrence in the plant kingdom, is found only in a few members of the Malvales. The presence of this compound in both Malvales and Myrtales can be attributed to a common ancestry. Being

a polyhydroxy flavonol, gossypetin may effect a number of additional beneficial properties on the drug plants containing them.