A. Myrtales – Chemotaxonomy

Chapter 3.

Chemotaxonomical studies on some members of the Myrtaceae

Introduction

The family Myrtaceae consists of 140 genera and about 3000 species found in tropical and subtropical regions throughout the world and also well developed in temperate Australia. The plants included here are trees or shrubs with unicellular or sometimes bicellular or multicellular hairs bearing volatile oils (containing monoterpenes, sesquiterpenes or other terpenoids) in abundant scattered spherical or elongate schizogenous secretory cavities occurring in most of the unlignified tissues of the shoot. Leaves opposite or less often alternate, rarely whorled and always entire, often with an intramarginal vein. Stomata anomocytic or seldom paracytic. Stipules absent. Flowers in cymose or racemose inflorescences or single, commonly bibracteolate at the base, perfect or rarely unisexual (some flowers staminate in spp. of *Eucalyptus*), regular, epigynous [the hypanthium often prolonged beyond the ovary or seldom (in some Leptospermoideae)], only half epigynous or merely perigynous with well-developed hypanthium free from ovary. Sepals 4-5 imbricate or calyx sometimes undivided in bud and splitting irregularly or deciduous as a calyptra, or calyx much reduced. Petals 4-5, imbricate, sometimes (as in spp. of *Eucalyptus*) connivent to form a calyptra or sometimes wanting. Stamens numerous originating in centripetal sequence, borne at the rim or inner surface of hypanthium, anthers tetrasporangiate, the connective typically with an evident apical secretory cavity. Nectary disc borne on the summit of the ovary or lining the prolonged hypanthium. Gynoecium of 2-5 carpels, syncarpous, multilocular or seldom pseudomonomerous (in some Leptospermoideae). Style terminal, stigma capitate. Ovules 2-many in axile placentation, bitegmic. Fruit few seeded berry or a capsule or sometimes a drupe or nut. Seeds without endosperm.

Anatomical characters

This family is characterized by internal phloem occurring next to the pith. Secondary phloem of young stems commonly stratified tangentially into fibrous and soft layers, seldom wholly soft. Xylem vessels with simple or scalariform perforations and usually with some vestured pits. Imperforate tracheary elements commonly with evidently bordered pits, most of them in many species considered to be fibre tracheids, but those surrounding the vessels considered to be true tracheids. Wood rays heterocellular to sometimes homocellular, all uniseriate or a few 2-3 cells wide many of them containing amorphous deposits. Wood parenchyma largely paratracheal including some crystalliferous strands. Wood often with lysigenous canals.

Palynology

Pollen grains are binucleate, commonly tricolpate, seldom bicolpate or tricolpate, sometimes with a granular infratectal structure.

Previous Chemical Reports

Leaves of *Melaleuca leucadendron* yield volatile oil from which cajuputene is obtained. The chief constituent of cajuput oil is cineole (cajeputene hydrate, cajeputol $C_{10}H_{18}O$), terpineol ($C_{10}H_{18}O$), both free and as an ester of acetic acid, and small amounts of terpenes, such as laevo-pinene. The lowest fraction of the crude oil contained valeric aldehyde and benzoic aldehyde. Cineole, pinene, globulol, camphene, limonene, pinocarvone were the essential oils present in leaves of *Eucalyptus globulus.* Acetone extract of seeds of *Psidium guajava* gave five known flavonoids, two phenolic glycosides and two new phenylethanol glycosides i.e. *l*-O-3,4-dimethoxy-phenylethyl – 4- O-3,4- dimethoxy cinnamoyl – 6-O- cinnamoyl-β – D- glucopyranose and *l*-O-3,4-dimethoxy-phenylethyl – 6-O- cinnamoyl-β – D- glucopyranose (Joseline, 2004). From ethanolic extract of leaves, three pentacyclic triterpenoids including one new guajavonic acid and two known obtusinin and goreishic acid I have been isolated (Begum *et al.*, 2002). Chloroform fraction of alcoholic extract of aerial part of *Syzygium samarangense* (*Eugenia javanica*) gave triterpenoids and chalcone (Rachana, 1995). From leaves of *Eucalyptus cladocalyx*, a pentacyclic 28-nor-triterpene called

pentacyclic 28-nor-triterpene called cladocalol; ursulolactone acetate, ursolic acid; 3β-acetate-12, 20 (29)-lupadien-28-oic acids; β-sitosterol and a known flavonoid eucalyptine were isolated (Samira *et al.*,2005). Volatile constituents of Malay rose apple (*Syzygium malaccense*) were about 133 compounds of which 2-phenylethanol and its esters (2-phenylethyl acetate, 2-phenylethyl isopentanoate, 2-phenylethyl benzoate and 2-phenylethyl phenylacetate) were the major constituents which were responsible for its exotic aroma (Pino *et al.*, 2004).

Economic Importance

The family contains a good number of economically important plants. Commercial timber is provided by species of *Corymbia* and *Eucalyptus*. Edible fruits are produced by species of *Eugenia* (*E. uniflora*: Surinam cherry), *Psidium* (*P. guajava* – guava), *Myricaria*, *Myrteolea* and *Syzygium* (*S. jambos*: rose apple/ gulab Jamun; *S. cumini*: Jambolan or Java plum, *S. malaccensis*: mountain apple). Spices and volatile oils are obtained from *Eucalyptus* (medicinal oils from *E. leucoxylon*, *E. sideroxylon*, *E. elaeophora*, *E. globulus* – rich in cineole; industrial oils from *E. dives* and *E. amygdalina* rich in α- phellandrene and *l*-piperitone and perfumery oils from *E. macarthuri* and *E. citriodora* containing citronellol), *Pimenta* (*P. racemosa* yielding Bay oil and *P. dioica* – the popular All spice), *Leptospermum*, *Melaleuca* (*M. alternifolia* – tea tree oil), *Myrtus* and *Syzygium* (*S. aromaticum* – clove). The family provides many ornamentals such as *Callistemon and Calothamnus also.*

Taxonomy

Hooker (1862-1883) divided the family into three tribes Myrteae, Leptospermeae and Lecythideae. The last tribe Lecythideae is now considered as separate family Lecythidaceae.

The family is presently divided into two subfamilies

1) Leptospermoideae wherein the fruits are usually dehiscent, pericarp usually dry and leaves spirally arranged or opposite. Includes *Callistemon*, *Eucalyptus*, *Leptospermum*, *Melaleuca*, etc.
2) Myrtoideae wherein fruit usually is indehiscent, pericarp usually succulent and

leaves opposite. Contains *Eugenia, Myricaria, Psidium, Syzygium, Xanthomyrtus,* etc.

In the present work 17 members belonging to this family are screened for their secondary metabolites such as flavonoids, alkaloids, quinones, saponins, etc in both leaves and stems and the distribution of these compounds are used in taxonomic interpretations. The 17 members studied include two varieties of *Syzygium malaccense* (one with small fruit and the other with a big fruit) and two varieties of *Psidium guajava* (one with pink flesh and the other with white flesh).

Materials and Methods:

Most of the plants were collected from Baroda. *Syzygium* spp. have been collected from different localities of Kerala. Both the species of *Melaleuca* and *Leptospermum* have been collected from Victoria Gardens, Bombay and Ooty Botanical Garden respectively. The voucher specimens of these plants have been deposited in the Herbarium, Department of Botany, The Maharaja Sayajirao University of Baroda (BARO), Vadodara. Standard methods, presented in chapter 2 were followed for the extraction, isolation and identification of the phytochemicals.

Results

The results of screening of 17 members of this family for the flavonoids, alkaloids and quinones are presented in Table 1.

The family is found to be rich in flavonoids. The flavonoids located in this family include flavonols, flavones and anthocyanidins. Flavonols are the most common flavonoids in this family. The flavonols located are kaempferol, quercetin and its various methoxylated derivatives, myricetin and gossypetin and its derivatives. Kaempferol was rare and found in only two plants. Quercetin along with its derivatives such as 3'OMe quercetin, 3'4'diOMe quercetin and 7, 3', 4'triOMe quercetin was located in all the plants. Quercetin was located in 14 plants, 3'OMe quercetin in 15 plants and 3'4'diOMe quercetin in 12 plants. In only one member trimethyl quercetin was seen. Myricetin, the highly hydroxylated flavonol was located in 11 plants.

Table 1. (contd) The distribution of various chemical markers in some members of the Myrtaceae

Sr. No.	Name	Luteolin	Kaempferol	4' - OMe Kaempferol	Quercetin	3' - OMe Quercetin	3', 4' - diOMe Quercetin	7, 3', 4' - triOMe Quercetin	Myricetin	Gossypetin	3' - OMe Gossypetin	4' - OMe Gossypetin	3',4' -diOMe Gossypetin	Anthocyanins	Quinone	p -Hydroxy benzoic acid	Vanillic acid	Syringic acid	Gallic acid	Mellilotic acidacid	Gentisic acid	p -Coumaric acid	o -Coumaric acid	Ferulic acid	Tannins	Alkaloids
	Subfamily : Leptospermoideae																									
11	*Callistemon lanceolatus* DC (leaves)				+	+									+		+	+	+						+	
	———"——— (stem)				+	+									+		+	+	+						+	
12	*Eucalyptus globulus* Labill.(leaves)		+		+	+	+	+									+	+	+						+	
	———"——— (stem)				+	+	+		+								+	+	+						+	
13	*Pimenta dioica* (Linn.) Merrill(leaves)				+		+		+						+		+	+	+						+	
	———"——— (stem)														+		+	+	+						+	
14	*Melaleuca cajupute* Roxb.(leaves)					+	+										+	+						+		
	———"——— (stem)					traces											+	+								
15	*Melaleuca leucadendron* Linn.(leaves)					+	+										+	+								
	———"——— (stem)																+	+								
16	*Leptospermum scoparium* Forst(leaves)				+	+					+		+				+	+		+			+	+		
	———"——— (stem)				traces												+	+								
17	*Leptospermum flavescens* Sm.(leaves)				+	+			+								+	+								
	———"——— (stem)				traces												+	+								

Table 1.The distribution of various chemical markers in some members of the Myrtaceae

Sr. No.	Name	Luteolin	Kaempferol	4'- OMe Kaempferol	Quercetin	3' - OMe Quercetin	3', 4' - diOMe Quercetin	7, 3', 4' - triOMe Quercetin	Myricetin	Gossypetin	3' - OMe Gossypetin	4'- OMe Gossypetin	3',4' - diOMe Gossypetin	Anthocyanins	Quinone	p-Hydroxy benzoic acid	Vanillic acid	Syringic acid	Gallic acid	Melilotic acidacid	Gentisic acid	p-Coumaric acid	o-Coumaric acid	Ferulic acid	Tannins	Alkaloids
	Subfamily . Myrtoideae																									
1	*Syzygium malaccense* (Linn.) Merrill & Perry(leaves small fruits)				+	+			+	+							+	+	+	+					+	
	——"—— (stem small fruits)					+	+			+						+	+	+	+	+				+	+	
2	*Syzygium malaccense* (Linn.) Merrill & Perry(vc) (leaves big fruits)					+	+		+							+	+	+	+		+			+	+	
	——"—— (stem big fruits)									+	+					+	+	+	+					+	+	
3	*Eugenia uniflora* Linn. (leaves)				+	+	+								+		+	+	+				+		+	
	——"—— (stem)				+	+	+		+						+		+	+	+			+			+	
4	*Syzygium cuminii* (L.)Skeels (leaves)				+	+	+								+		+	+	+						+	
	——"—— (stem)				+				+					+	+		+	+	+						+	
5	*Syzygium jambos* (Linn.)Alston (leaves)				+	+									+		+	+	+						+	
	——"—— (stem)														+	+	+	+	+						+	
6	*Syzygium aromaticum* (Linn.)Merrill & Perry (leaves)		+	+	+												+	+	+						+	
	——"—— (stem)									+	+						+	+	+						+	
7	*Syzygium zeylanica* (Linn.) DC (leaves)				+	+	+								+		+	+	+						+	
	——"—— (stem)				+	+			+						+		+	+	+						+	
8	*Psidium guajava* (Linn.) (pink) (leaves)				+		+		+						+		+	+	+						+	
	——"—— (stem)									+	+	+			+		+	+	+						+	+
9	*Psidium guajava* (Linn.) (white) (leaves)				+	+	+		+						+		+	+	+	+				+	+	+
	——"—— (stem)					+				+			+		+		+	+	+	+					+	+
10	*Psidium cattleyanum* Sabine(leaves)				+	+			+	+					+		+	+	+			+				
	——"—— (stem)	+					+			+					+		+	+	+						+	

Gossypetin, a polyhydroxy flavonol which is otherwise rare in plant kingdom, was seen in 7 plants while 4 plants contained its methyl derivatives. Cyanidin, the anthocyanidin corresponding to quercetin was seen in stem of *Syzygium cumini*. Luteolin was the only flavone located from the stem of *Psidium cattleyanum*.

Proanthocyanins were not located in any plants. Quinones also were a common feature in this family having been located in ten plants. Only *Psidium guajava* tested positive for alkaloids.

The phenolic acids presents in this family were *p*-hydroxy benzoic, vanillic, syringic, gentisic, gallic, melilotic, *p*-coumaric, *o*-coumaric and ferulic acids. The first three acids were omnipresent in stems and leaves of all plants screened. Syringic acid in large amounts were present in stem of *Eugenia uniflora* and *Syzygium aromaticum*. *Eugenia* species was rich in gallic acid. Among the other phenolic acids which were comparatively rare, *p*-hydroxy benzoic acid was present in three plants, melilotic acid in three, *p*-coumaric in two, ferulic acid in five and *o*-coumaric in two.

Between the leaves and stems of the plants screened there were differences in chemical constitution. Flavonoids were more in leaves than stem. In plants like *Syzygium jambos* and *Pimenta dioica* stems were without flavonoids. The individual flavonoids also differed between leaves and stems. The stem of *Psidium cattleyanum* was entirely different from the leaves in containing a flavone, luteolin and a different flavonol, 3'4'- diOMe quercetin.

Between the two varieties of *Syzygium malaccense*, there exist differences in flavonoids and phenolic acids. Quercetin and melilotic acid were seen only in the variety with the small fruit whereas the other variety yielded 3'-OMe gossypetin and gentisic acid.

Psidium guajava with the pink fruit also was chemically different from the variety with the white fleshed fruit. 3'-OMe Gossypetin and 4' OMe Gossypetin was present only in former while 3'OMe quercetin, and ferulic acid in the latter.

Discussion

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*. All these taxa exhibit chemical identities and therefore are valid taxa. But more data are to be accrued to ascertain whether they have to be given a status of subspecies or not.

The family Myrtaceae is a chemically primitive family due to the abundance of flavonols, tannins and absence of flavones. The affinities with the Lecythidaceae will be discussed later with the latter family.