

## Chapter 4

### Chemotaxonomical studies on some members of the Combretaceae

#### Introduction

The family Combretaceae consists of 20 genera with 500 species of tropical trees or shrubs, often scandent. Leaves spirally arranged, opposite or whorled, simple, entire, leaf base often with two gland-containing flask shaped cavities at the base. Stipules minute or absent. Flowers in racemes, spikes or heads usually small, bisexual, regular and epigynous. Hypanthium often nectariferous within. Calyx 4-5 appearing as lobes of hypanthium, persistent, valvate or imbricate, sometimes. Corolla with 4-5 petals, imbricate or valvate often absent. Stamens twice the number of calyx and bicyclic, outer sometimes reduced or absent or rarely in pairs or triplets at times tricyclic. Anthers usually versatile, dehiscence longitudinal. Epigynous disc often present. Gynoecium of 2-5 carpels, unilocular with terminal style and 2-6 ovules, pendulous, anatropous, bitegmic with zig-zag micropyle. An elaborate obturator often produced on funicle. Fruit one seeded, indehiscent or drupaceous, generally ribbed. Seeds without endosperm. Embryo oily with 2 (or 3 in some *Terminalia* spp.) with folded or spirally twisted cotyledons.

#### Anatomical Characters.

The wood is mostly diffused porous, vessel segments with simple (oblique to transverse) perforations and vestured pits. Imperforated tracheary elements with small mostly simple pits, often some of the elements septate. Vascular tracheids and radial vessels sometimes present. Wood rays uniseriate or some of them 2 – 3 (6) seriate, homocellular or heterocellular, generally many of the cells containing amorphous gummy deposits. Wood parenchyma mainly paratracheal, commonly wing like and

including some vertical crystalliferous strands. Some apotracheal parenchyma sometimes also present. Internal phloem present next to the pith, intraxylary phloem also present as well.

### Previous Chemical Reports

The family is known to contain tannins, both hydrolysable and condensed. Flowers of *Calycopteris floribunda* contained gossypol, calycopterin and quercetin. Five biflavonoids such as 6''-demethoxyneocalycopterone, calyflorenone C, 6''-epicalyflorenone C and calyflorenone D were isolated from the green parts of this plant (Ralf, 2004). Leaves of *Quisqualis indica* contained rutin, trigonelline and quisqualic acid. Bark of *Terminalia arjuna* contained arjunine and pyrocatechol. Two pentacyclic triterpenoids glycosides from the bark were isolated (Ali *et al.*, 2003). Fruits of *T. bellerica* yielded tannins, gallic acid, ellagic acid and chebulagic acid and a new cardiac glycoside, bellericanin. Corilagin, ellagic, gallic and brevifolin carboxylic acids had been isolated from leaves and fruits of *T. catappa*. Its bark and wood contained ellagic and gallic acids, (+) - catechin, (+) - epicatechin and (+) - leucocyanidin. Leaves of *T. chebula* contained shikimic, dehydroshikimic and quinic acids (Anonymous, 1998). Acetone extract of leaves of *Anogeissus latifolia* yielded of corilagin, gallic acid, chebulagic acid, myrtigallic acid, shikimic acid, quinic acid and ellagic acid.

Combretastains are group of stilbene dimers isolated from *Combretum caffrum*, a native of Africa. These compounds consist of biaryls connected by an unsaturated ethylene bridge. They showed cytotoxic activity against cancer cells. The activity of these compounds is due to their restriction of blood flow to the tumour and thus starving the cells of oxygen. They inhibit microtubule formation also (Pettit, 1987).

### Useful Plants

A number of plants in this family are of economic importance. *Terminalia catappa* yields a nut which is edible known as Indian Almond. *Quisqualis indica* (Rangoon Creeper) is an ornamental plant grown for its beautiful flowers. *Anogeissus latifolia* provides gum ghatti, used as a substitute of gum Arabic. Both *Terminalia chebula* and *T. bellerica* yield tannins, so the former is useful in the production of sole-leather due to

high ellagitannic acid. The former plant is an important medicinal plant, the fruit rind of which is a component of "Triphala", a well known rasayana drug. *Terminalia arjuna* bark is a well known cardiogenic, also used as a diuretic, febrifuge, tonic, antidiarrheal and cures wounds and urinary diseases. Its ashes are prescribed in scorpion sting. The leaves are used externally as a cover for sores and ulcers. It is also used as anti-HIV. Juice of fresh leaves is used for earache. Unripe fruits of *T. chebula* are more purgative and ripe ones are more astringent. It is highly useful in chronic diarrhoea and dysentery, flatulence, vomiting, hiccup, colic and enlarged spleen and liver. *Combretum sondaicum* is a Chinese herb, the roasted leaves of which are used as an opium antidote. *Combretum caffrum* is a source of combretastatins used in cancer therapy. Leaf juice of *Anogeissus latifolia* is good for otopyorrhoea. Bark of this plant is used for ulcers, inflammations, diabetes, diarrhoea, haemorrhages, haemoptysis, dysentery, skin diseases, leprosy, ophthalmia and general debility.

In the present work leaves and stems of 13 plants of the Combretaceae have been screened for their phytochemicals and the distribution of the same have been used for taxonomic considerations. In addition, stem of four members *Combretum coccineum* Lam., *C. extensum* Roxb., *Quisqualis indica* Linn. and *Terminalia arjuna* W. & A. (bark) have been bioprospected for combretastatins.

### Materials and Methods

All the plants were collected from Baroda and environments. 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. The methodology followed for screening the plants for combretastatins is as follows:

Forty grams of coarse stem powder was subjected to cold extraction for 48 hours using methanol as a solvent. This extract was filtered and the filtrate was concentrated by distillation of methanol. The concentrated extract was partitioned between n-Hexane and H<sub>2</sub>O: MeOH (2:3) to remove chlorophyll. The aqueous methanolic extract thus

obtained was further extracted with  $\text{CH}_2\text{Cl}_2$  to give a  $\text{CH}_2\text{Cl}_2$  extract. The remaining methanolic fraction was again extracted with ethyl acetate. Both these extracts were analysed for combretastatins.

The  $\text{CH}_2\text{Cl}_2$  fraction was loaded on Silica gel G TLC plates. The plates were developed using the solvent system ethyl acetate: methanol: water (40: 5.4: 4). The separated compounds were visualized by spraying freshly prepared vanillin spray reagent (0.1 gm vanillin + 28 ml MeOH + 1 ml  $\text{H}_2\text{SO}_4$ ). The plates were heated on a hot plate at around 100-105° C until the optimum color was developed. Some of the plates were partly sprayed and heated. The colored bands thus obtained were compared with the unsprayed part under UV light and the fluorescent bands were marked out. These bands were scraped out and eluted using spectroscopic grade methanol and the UV absorption spectrum was recorded in the range of 240–400 nm.

## Results

The distribution of various flavonoids, phenolic acids and quinones are presented in Table 2. Tannins are found to be present in all the plants. Flavonols, flavones, biflavones and anthocyanidins were the flavonoids located in the family Combretaceae. Flavonols were the dominant compounds found in great variety in both leaves & stems of all plants screened. Kaempferol and quercetin were more frequent. These compounds along with methoxylated derivatives were present in all the plants screened. Myricetin was comparatively rare, found in leaves of only two plants. Gossypetin along with their derivatives were observed in 6 plants. Flavones located in this family were apigenin, acacetin and luteolin. Apigenin was seen in both the species of *Quisqualis* while *Quisqualis malabarica* contained luteolin also. Luteolin was located in leaves in *Terminalia catappa* also. Acacetin was seen in *Calycopteris*. Cyanidin was identified in the bark of *Terminalia arjuna* leaves and stem of *Calycopteris*. Amentoflavone, a biflavone, was located in both the stems and leaves of *Calycopteris floribunda*.

Quinones are located from three species of *Terminalia* i.e. *T. arjuna* and *T. crenulata* *T. Paniculata*, *Combretum ovalifolium* and *Calycopteris*.

Altogether 9 phenolic acids were identified from the members screened. Vanillic and syringic acids were located in all the plants and all organs. Gallic acid was found

from all plants except *Combretum coccineum* and *Anogeissus latifolia*. Melilotic, *p*-coumaric and ferulic acids were present in less than half the number of plants. Protocatechuic acid and *o*-coumaric acids were seen in only one plant each. Alkaloids and saponins were found to be absent.

It is found that stems of three plants, *Combretum coccineum*, *C. extensum* and *Terminalia arjuna* (bark) contained compounds having the typical fluorescence and spectral maxima as of combretastatins. The stem of *Combretum coccineum* contained four compounds which gave the test for combretastatins. *C. extensum* stem yielded three compounds which appeared to be combretastatins. The bark of *Terminalia arjuna* possessed five compounds which fluoresced in UV and having peaks between 275-280 nm. *Quisqualis indica* did not contain any compound having the specific fluorescence and spectra.

### Discussion

The near omnipresence of flavonols such as kaempferol, quercetin and gossypetin and their derivatives in all plants screened indicate the homogeneity of the family. The near absence of glycoflavones and fewer incidences of flavones are the other features binding all the members together. The flavone containing members like *Quisqualis* and *Calycopteris*, which incidentally are lianas, may be considered advanced members of the family.

The presence of gossypetin, a rare flavonol found common in Myrtaceae indicates the close-relationships of the Combretaceae to that family. Incidentally this flavonol is found absent in the Lythraceae with which Thorne grouped Combretaceae in the suborder Lythrineae. Thus the present investigation indicate that both the Myrtaceae and Combretaceae are closer than to the Lythraceae and thus Thorne's treatment does not get any support from chemical evidences.

The presence of compounds having the fluorescence and spectra of combretastatins in two species of *Combretum* i.e. *C. coccineum* and *C. extensum* and *Terminalia arjuna* is highly promising. Detailed studies on these plants isolating the compounds in large amounts and subjecting them to NMR, Mass spectra etc. will lead to identification of new combretastatins from these plants.