Chemotaxonomic Investigation on the Family Dipterocarpaceae Using Flavonoid Analysis

Kunjani Joshi
M.Sc. (Tribhuvan)

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Faculty of Science
University of Colombo

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ABSTRACT

Flavonoids are a group of phenolic compounds having the basic configuration C_6- C_3- C_6 and ubiquitous occurrence. Flavonoids have been used to solve the problems at different taxonomic levels and the three classes of flavonoids frequently used for these studies are anthocyanidins, flavones and flavonols. Moreover, the flavonoids are also used to solve the problems of plant identification where flowering and fruit development does not occur frequently.

Dipterocarpaceae is an important woody plant family mostly dominating the wet zone forests in Sri Lanka. There are two views put forward by Ashton (1980) and Kostermans (1992) regarding the classification of the family Dipterocarpaceae of Sri Lanka showing significant differences in generic, subgeneric and species boundaries. Flowering or fruiting of most of the Dipterocarpaceae members are very periodical and there is need for a method, which does not use reproductive parts to solve such problems. In this investigation leaves of 45 species with subspecies reported by Ashton (1980), *Stemonoporus moonii* reported by Kostermans (1992) and the only species of Dipterocarpaceae present in Nepal, *Shorea robusta* were used either in the form of fresh or dried leaves.

The aglycones (flavone, flavonol, and anthocyanidin from the break down of proanthocyanidins) and glycosides (with sugars) were isolated using standard techniques of paper chromatography, thin layer chromatography, spectrophotometry and other specific techniques. All compounds have been confirmed either using commercially purchased markers or markers obtained from known sources.

All species show a remarkable variation in flavonoid profiles. Among the detected flavonoid aglycones, the significant constituents found were of flavonols quercetin, kaempferol, and flavones luteolin and apigenin . However, flavonol myricetin was notably detected in only three species of *Shorea* : *S. affinis*, *S. trapezifolia*, and *S. gardneri*, the first record of this compound in Dipterocarpaceae. Another distinctive occurrence is the presence of proanthocyanidins in the leaves of thirteen species of the genus *Shorea* and two species of the genus *Dipterocarpus*. These two genera can be regarded as the most primitive in flavonoid patterns among the total species surveyed and other five genera : *Cotylelobium*, *Hopea*, *Stemonoporus*, *Vateria,* and *Vatica* would have the more advanced flavonoids among which *Stemonoporus* is the most advanced.

In this investigation, six flavonoid glycosides : quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 3-xylosylglucoside, kaempferol 3,5-glucoside, luteolin 7- glucoside and apigenin 5- glucoside have been isolated and both aglycones and sugars have been characterized for the first time in Dipterocarpaceae. Among six isolated glycoside compounds, quercetin 3-glucoside and quercetin 3-rutinoside are most common where as kaempferol 3,5-glucoside was only detected in five genera: *Cotylelobium, Hopea*, *Shorea, Stemonoporus*, and *Vatica*. Apigenin 5-glucoside was found to be common in five genera : *Cotylelobium, Dipterocarpus, Hopea, Stemonoporus* and *Vatica*. Quercetin 3-xylosylglucoside was detected only in two species of the genus *Hopea,* while Luteolin 7- glucoside was found only in the species of the genus *Shorea,* which can be regarded as very significant character of these two genera.

Cluster analysis of the aglycone results do not completely agree with either classifications. However, it clearly favors Kostermans view of upgrading the subspecies of *Hopea* as two species, *Hopea jucunda* and *H. modesta,* which has been further strengthened by the SEM study of the leaf surface. Analysis also supports Kostermans view in separating *Cotylelobium lewisianum* from *C. scabriusculum*. However, the data of the

present study do not agree with the sectional arrangement of *Shorea* as proposed by Ashton or the separation of the genera *Shorea* and *Doona* by Kostermans. The species of *Stemonoporus* show very complicated interlinkages with other species of Dipterocarpaceae.The flavonoid pattern of *Vatica chinensis* is different from other two species of *Vatica.* Aglycone analysis also identifies two groups within the genus *Dipterocarpus* which has not been identified by both classifications. Although the cluster analysis carried out using 20 biometric characters of the leaves indicated that there are grouping tendencies with considerable overlapping giving chain species, it also supports the grouping of the genus *Dipterocarpus* as in the chemical analysis.

The flavonoid distributions in the species of Dipterocarpaceae do not completely agree with either of the existing classification, but it has given important markers especially for species delimitation.