CHEMOTAXONOMIC INVESTIGATION OF *COTYLELOBIUM* SPECIES (DIPTEROCARPACEAE) USING FLAVONOID ANALYSIS

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Abstract: During the chemotaxonomic investigation of Dipterocarpaceae, three flavonoid aglycones : flavonol quercetin, flavonol kaempferol and flavone apigenin and four glycosides : Quercetin 3-glucoside, apigenin 5- glucoside, kaempferol 3,5-glucoside and quercetin 3-rutinoside were isolated from the leaves of two species of *Cotylelobium* (*C. lewisianum*, and *C. scabriusculum*). The isolated flavonoids can be used as chemotaxonomic markers. Myricetin, luteolin and proanthocyanidins were not decteted in this investigation. Both the species of *Cotylelobium* can be regarded as advanced in flavonoid patterns because of the absence of myricetin and loss of proanthocyanidins. The data of the flavonoid patterns and the outcome of cluster analysis are taxonomically useful to resolve the controversies over the systematic arrangement of the species and also suggest the need for a revision of classification of the genus *Cotylelobium.*

Key words: Dipterocarpaceae; Flavones. Flavonols; Chemotaxonomy; Cluster analysis.

INTRODUCTION

The genus *Cotylelobium* Pierre (Dipterocarpaceae) is rich in species diversity with 6 species distributed in Sri Lanka, Thailand, Malaysia, Indonesia, Brunei and Philippines (Dayanandan *et al* 1999; Joshi, 2001). The taxonomy of this taxa has always been a point of discussion. Recently it was realised that the controversy relating to taxonomy of the species can be solved by studying chemical constituents and their chemical characters i.e. the presence or absence of different phenolic compounds like flavonoids and tannins in the plants for species delimitation, systematic arrangement and tracing phylogenetic relationship of species. Among the chemical constituents, flavonoids are already proved as potentially important markers for taxonomic studies due to its characterisitics such as structural variability, chemical stability, ubiquitous occurrence and easy and rapid identification (Harborne, 1984; Heywood, 1984; Markham, 1982; Joshi, 2001, 2002, 2005; Willams *et al.* 1991). Moreover, the flavonoids are also used to solve the problems of plant identification where flowering and fruit development does not occur frequently (Joshi, 2003b).

The information on the chemical constituents of the species of *Cotylelobium* is very limited. Previous sporadic works were mainly concentrated on the isolation and identification of some triterpenoids, steroids and phenolic compounds including some flavonoids (Gunawardana *et al.* 1980; Joshi, 2001). During the survey of leaf flavonoid patterns of Dipterocarpaceae, some flavonoids have been isolated and identified in the species of *Cotylelobium.* In the present paper, an attempt has been made to present the leaf flavonoids isolated from the leaves of two species of *Cotylelobium* (C. *lewisianum* (Trimen ex Hook. f.) Ashton and *C. scabriusculum* (Thw.) Brandis).

MATERIALS AND METHODS

The following materials and methods were used in the investigation of flavonoids.

Plant materials

The herbarium specimens of *Cotylelobium* (C. *lewisianum* (Trimen ex Hook. f.) Ashton and *C. scabriusculum* (Thw.) Brandis) from the National Herbarium, Sri Lanka were used for isolation and identification of flavonoids in this investigation.

Extraction and Identification of flavonoids

The flavonoid constituents were extracted from leaf materials using 70% hot ethanol and run two dimensionally on Whatman No. 1 chromatography paper in BAW (n-butanol, acetic acid and water, 4:1:5, top layer) and 15% HOAc (acetic acid) using rutin as an authentic marker compound to obtain a profile for each taxon. Acid hydrolysis of the extracts was carried out in 2N HCl at 100^0 C for 30 to 45 min. These were extracted into ethyl acetate and run one-dimensionally on Whatman No 1 and TLC (thin layer chromatography) plates against the authentic flavone and flavonol markers in BAW (*n*-butanol-acetic acid-water, 4:1:5), 50% HOAc, Forestal (acetic acid, conc. HCl and water, 30:3:10) and PhOH (phenol saturated with water). Aglycones were identified by their chromatographic properties in these solvent systems, their colour in UV (360nm) with and without NH_3 and their UV visible spectra and comparison with authentic marker compounds (Harborne, 1973, Joshi, 2003a; Joshi *et al*. 2004).

Glycosides were separated and purified from direct 70 % EtOH extracts by paper chromatography on Whatman No. 3 (Harborne, 1984; Markham, 1982). They were based on UV

spectral, shift measurements, Rf comparisions, and hydrolysis to yield aglycone and sugars. They were further determined by co-chromatography in four solvents with authentic markers to confirm identification (Joshi, 2001; 2003a).

Cluster Analysis

In order to investigate the co-relation between the species of the same taxa and other species, cluster analysis was performed using morphological and chemical data.

RESULTS

The results of flavonoid survey are presented in Table 1. The detectable amount of flavonoid, especially flavonol quercetin and flavone apigenin, were present in the leaves of both species, whereas flavonol kaempferol was only detected in *C. scabriusculum*. Proanthocyanidin was not decteted in this investigation. The interesting finding of this investigation was the presence of high amount of flavone apigenin and absence of proanthcyanidin.

Both species showed the presence of flavonol and flavone glycosides in their leaves. In this taxa, flavonoid glycosylation in 3- position, 3, 5- position and 5- position are common. Quercetin 3-glucoside and apigenin 5- glucoside were found common to both species of *Cotylelobium* surveyed, whereas kaempferol 3,5-glucoside and quercetin 3-rutinoside were detected in *C. scabriusculum* and *C. lewisianum* respectively.

The dendrogram, an outcome of the cluster analysis of chemometric data, shows a great tendency to form a complex grouping of the species. (Fig.1). Each group is heterogenous and clustering of various species and their retionships among themselves and with other groups are complex and difficult to acertain. The species of *Cotylobium* indicate hetrogenous nature showing linkages with the species of *Hopea*, *Stemonoporus, Vateria* and *Vatica*.

DISCUSSION AND CONCLUSION

One of the most significant present findings in the present investigation is the detection of flavonoid aglycones : flavonol quecetin, flavonol kaempferol, flavone luteolin, and glycosides: quercetin 3-glucoside, quecetin 3-rutinoside, kaempferol 3,5 - glucoside, and apigenin 5- glucoside in the species of *Cotylelodon*. These flavonoids can be regarded as taxonomic markers.

Another notable result of the present work is the absence of myricetin and proanthocyanidin in the leaves of the studied species. From the taxonomic viewpoint, presence and absence of myricetin and proanthocyanidin character is very significant. Their presence is considered as a primitive character in dicots, particularly in woody plants (Bate-Smith & Whitemore, 1959; Bate Smith, 1962; Harborne, 1966). Thus both the species of *Cotylelobium* can be regarded as advanced in flavonoid patterns because of the absence of myricetin and loss of proanthocyanidins.

Since more than two decades, the taxonomy of the genus *Cotylelobium* has been a point for discussion. Time to time, various taxonomists have tried to classify the species of this genus on the basis of morphological and anatomical characters. Some species are excluded in the respective groups, some are placed in different groups, or some species are integrated in new genus by the workers. Ashton (1980) has reported two species of *Cotylelobium* : *C. lewisianum* and *C. scabriusculum* in his classification of Dipterocarpaceae, whereas Kostermans (1992) has made a revision of the classification of Dipterocarpaceae and included *Cotylelobium* in *scabriusculum* under *Sunaptea* as *S. scabriuscula*, and *Cotylelobium lewisianum* assigned as *Vatica lewisiana* . In the present investigation, it was found that both species of *Cotylelobium* fall close with each other showing their morphological similarity, which give support to Ashton's view. However, flavonoid pattern data and dendrogram indicate that there is no linkages between the species of *Cotylelobium.* Thus, the present investigation supports the view of Kostermans (1992) and recommended for the revision of the classification.

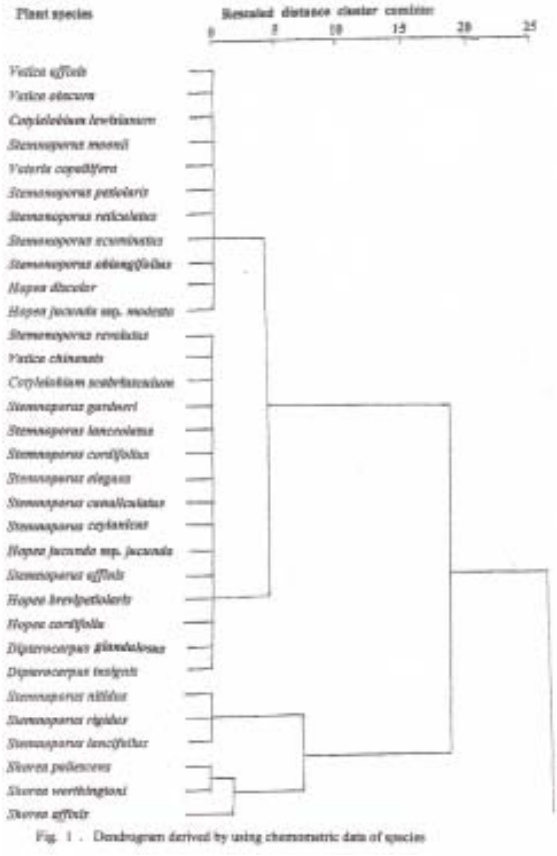

Fig. 1 . Dendrogram derived by using chemometric data of species

In conclusion, the species of *Cotylelobium* could be categorized on the basis of flavonoid pattern. Both species of the *Cotylelobium* have advanced flavonoid patterns due to absence of myricetin and loss of proanthocyanidins. The present findings are useful to resolve the controversies relating to the taxonomy of *Cotylelobium*. But more comprehensive investigation on other areas, such as molecular, cytological, ecological, palenobotanical as well as biogeographical aspects are also needed to draw the phylogenetic relationships of the species.

Table 1: Flavonoid patterns in the species of *Cotylelobium*

Scientific name	Flavonoid aglycones							Flavonoid glycosides			
	Flavonol			Flavone		Proanthocyanidin					
	M	Q	K	L	A	D	C	1	2	3	4
Cotylelobium lewisianum	–	+	–	–	+	–	–	+	+	–	+
Cotylelobium scabriusculum	–	+	+	–	+	–	–	+	–	+	+

Key: M = myricetin, Q = quercetin, K = kaempferol, L = luteolin, A = apigenin, D = delphinidin, C = cyanidin,
1 = quercetin 3- glucoside, 2 = quercetin 3- rutinoside, 3 = kaempferol 3,5 - glucoside, 4 = apigenin, 5 = glucoside,
+ = detected, – = not detected,

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