Vatica species (Dipterocarpaceae) of Sri Lanka: Species Diversity and Chemotaxonomy Using Flavonoid Analysis

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Vatica species (Dipterocarpaceae) of Sri Lanka: Species Diversity and Chemotaxonomy
Using Flavonoid Analysis

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Abstract

Taxonomy of Sri Lankan *Vatica* is very controversial and has always been a point of discussion. During the chemotaxonomic study, the species diversity of *Vatica* was investigated and three flavonoid aglycones: flavonol quercetin, flavonol kaempferol and flavone apigenin and four glycosides: quercetin 3-glucoside, quercetin 3-rutinoside, kaempferol 3, 5-glucoside and apigenin 5- glucoside were isolated from the leaves of three species of *Vatica* (*V. affinis, V. chinensis* and *V. obscura)*. The isolated flavonoids can be used as chemotaxonomic markers. Myricetin, luteolin and proanthocyanidins were not decteted in this investigation. All the species of *Vatica* can be regarded as advanced in flavonoid pattern 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 suggest the need for a revision of classification of the genus. .

Key words: Chemotaxonomy Dipterocarpaceae, Flavones, Flavonols, cluster analysis

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Introduction

The genus *Vatica* (Dipterocarpaceae) is rich in species diversity with 65 species distributed in Sri Lanka, Thailand, Malesia, Burma, Indo-china, South and East India, South China and Bangladesh (Dayanandan *et al* 1999; Joshi, 2001). The taxonomy of these taxa has been the subjects of several recent monographs and showes controversy in generic and species boundaries. Recently it was realised that the controversy relating to taxonomy of the species can be solved by studying molecular patterns and chemical constituents and their chemical characters 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 (Markham, 1982; Hegnauer, 1982; Harborne, 1984; Heywood, 1984; Willams *et al*. 1991; Joshi, 2001, 2002, 2005). Moreover, the flavonoids are also used to solve the problems of plant identification where flowering and fruit development does not occur frequently (Joshi, 2003b).

Taxonomy of Sri Lankan *Vatica* is very controversial and has always been a point of discussion (Ashton 1980; Kostermans, 1992). The information on the chemical constituents of the species is very limited. Previous sporadic works were mainly concentrated on the isolation and identification of some triterpenoids, steroids and phenolic compounds (Gunawardana *et al.* 1979, 1980; Joshi, 2001). In the present paper, an attempt has been made to present the species diversity and leaf flavonoids isolated and identified from the leaves of *Vatica*, which might help to assist to fill up

the gaps in our knowledge and resolve the controversies on systematics of the species.

Materials and Methods

Plant materials

The fresh materials of V. chinensis was collected from Royal Botanical garden, Perediniya and the herbarium specimens of V. affinis and V. obscura from the National Herbarium, Sri Lanka were used for isolation and identification of flavonoids in this investigation. Voucher specimens were lodged in the Department of Botany, University of Colombo, Sri Lanka.

Extraction and Identification of flavonoids

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 1000 C for 30 to 45 min. They were extracted into ethyl acetate and run one dimensionally on Whatman no 1 and TLC (thin layer chromatography) plates for descending 1-D chromatography against the authentic flavonol markers myricetin, quercetin, and kaempferol and the flavone marker apigenin in four solvents: HOAc (50% acetic acid); BAW (*n*-butanol-acetic acid-water, 4:1:5) top layer; Forestal (acetic acid-conc.HCl-water, 30:3:10) and PhOH (phenol saturated with water). The presence of proanthocyanidins was observed by further extraction with amyl alcohol and was run on solvent BAW, Forestal and Formic acid. Aglycones were identified by their chromatographic properties in these solvent systems, their colour in UV (360nm) with and without NH3 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 extract by paper chromatography on Whatman 3 paper (Markham, 1982; Harborne, 1984). They were based on UV spectral, shift measurements, Rf comparisions, and hydrolysis to yield aglycone and sugars. Sugars were identified by co-chromatography against markers in three solvents system including toluene pyridine acetic acid water (4:1:1:3) which gives a clear separation of glucose and galactose. Flavonoid quercetin 3-xylosylglucoside was further characterized by partial acid hydrolysis in order to confirm xylosylglucoside. Co-chromatography with authentic markers was carried out to confirm identification.

Cluster Analysis

In order to study the co-relation between the species, cluster analysis was also performed using chemical data based only on leaves.

Results

Three species of Vatica (V. affinis V. chinensis and V. obscura) show great variation in morphological characters. The results of flavonoid patterns are presented in Table 1. The detectable amount of flavonoid, especially flavonol quercetin and flavone apigenin, were present in the leaves of all three species, whereas flavonol kaempferol was only detected in V. chinensis. 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.

The glycoside patterns of the taxa showed the great variation at species level. Quercetin 3-glucoside was found common to all species of *Vatica* surveyed, whereas quercetin 3-rutinoside was detected in *V. affinis* and *V. obscura* and kaempferol 3, 5-glucoside, and apigenin 5- glucoside only in *V. chinensis.*

Table 1. Flavonoid patterns in the species of *Vatica*

S. No.	Scientific Name	M	Q	K	L	A	D	C	1	2	***3***	4	5	6
1	Vatica affinis	-	+	-	-	+	-	-	+	+	-	-	-	-
2	*Vatica chinensis*	-	+	+	-	+	-	-	+	-	-	+	-	+
3	*Vatica obscura*	-	+	-	-	+	-	-	+	+	-	-	-	-

Key: M = myricetin, Q = quercetin, K = kaempferol, L = luteolin, A = apigenin, D = delphinidin, C = cyanidin, 1 = quercetin 3- glucoside, 2 = quercetin 3- rutinoside, 3 = quercetin 3-xylosylglucoside, 4 = kaempferol 3,5 - glucoside, 5 = luteolin 7- glucoside, 6 = apigenin 5-glucoside,

+ = detected, - = not detected,

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 *Vatica* indicate hetrogenous nature showing linkages with the species of *Cotylelobium*, *Hopea,* and *Stemnoporus.*

[IMAGE]

Fig.1. Dendrogram derived by using chemometric data obtained from flavonoids of species of the family Dipterocarpaceae

Discussion

One of the most significant present findings in the present investigation is the detection of flavonoids i.e. aglycones: flavonol quecetin, flavone apigenin, and glycosides: quercetin 3-glucoside, quecetin 3-rutinoside, kaempferol 3,5 - glucoside, and apigenin 5- glucoside in the species of Vatica. These flavonoids can be regarded as taxonomic markers for the systematic arrangement of the species. Among the flavonoid patterns of the studied species, V. chinensis showed the remarkably different aglycones and glycosides from other two endemic species (V. affinis and V. obscura) having flavonol kaempferol and kaempferol 3, 5 - glucoside, and apigenin 5-glucoside.

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 (Bate-Smith and Whitemore, 1959; Harborne, 1966). Their presence is considered as a primitive character in dicots, particularly in woody plants (Bate-Smith & Whitemore, 1959; Bate Smith, 1962; Harborne, 1966). Thus all the species of Vatica can be regarded as advanced in flavonoid patterns because of the absence of myricetin and loss of proanthocyanidins.

Since more than two decades, the species diversity and taxonomy of Vatica have 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 and some are placed in different groups and some species are assigned as synonyms by the workers. Ashton (1980) reported three species of Vatica: V. affinis, V. chinensis and V. obscura in his classification of Dipterocarpaceae, whereas Kostermans (1992) has made a revision and included four species V. affinis, V. paludosa (syn. V. chinensis), V. obscura and V. lewisiana. In his revised classification, Kosterman (1992) has included V. chinensis as synonyms of V. paludosa which is endemic to Sri Lanka. Similarly, he has removed Cotylobium lewisiana from the classification of Ashton (1980) and is assigned as Vatica lewisiana. Joshi (2008) has also supported the removal of Cotylobium lewisiana from the classification of Cotylobium complex from the flavonoid pattern and cluter analysis.

In the present investigation, both flavonoid pattern data and dendrogram outcomes indicate that there is no linkages between the V. chinensis and other species of Vatica. In cotext to the systematic arrangement of the taxa, there is still need for further comprehensive studies in various relevent areas.

In conclusion, the species of Vatica could be categorized on the basis of flavonoid pattern. All three species of the Vatica 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 Vatica. But more comprehensive investigation on other areas, such as molecular, cytological, ecology and biogeographical aspects are also needed to draw the phylogenetic linkages with species.

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