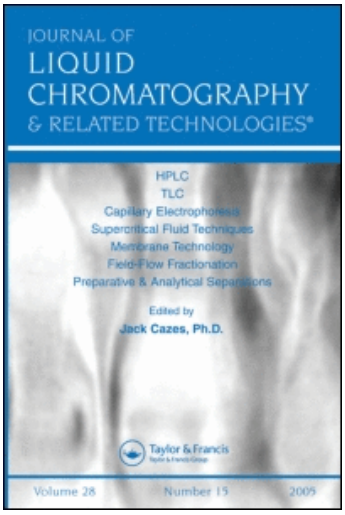

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Identification of the Flavonoids in Papilionaceae Flowers Using Paper Chromatography

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IDENTIFICATION OF THE FLAVONOIDS IN PAPILIONACEAE FLOWERS USING PAPER CHROMATOGRAPHY

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ABSTRACT

The flavonoid of the flowers of sixteen members of the Papilionaceae were examined using paper chromatography. Thirty flavonoids were identified on the basis of colour reactions and where necessary co-chromatography with authentic markers. A flavonoid profile of each plant has been constructed. This is the first report of such a comprehensive biochemical study and this work contributes to the understanding of the chemistry of this important flora and their application in local medicine.

Two new solvent systems were developed in the course of this work which will aid flavonoid research immensely.

INTRODUCTION

The Leguminoseae (Fabaceae) forms a large family of the flowering plants. Recent estimates put them at 590-690 genera and 12-17,000 species (1,2,3). They are important, economically as food, fodder, timber and in Nigeria as part of concoctions in local medicine (4,5,6). Little is known about their flavonoids apart from scanty reports by Pomilio et at. (7) and Purshothaman et al. (8). Interest in this work was rekindled by the discovery

of Parvez and Ogbeide (9). This work is an attempt towards the study of the flavonoids in the plants and hence determine their role in local medicine.

EXPERIMENTAL

Materials:

Fresh flowers samples from sixteen members of the Papilionaceae were obtained from Centrosema pubescenes, Crotalaria falcata, C. retusa, C. verrucosa, Desmodium velutinum, D. gangeticum, D. triflorum, D. adscendens, Erythrina vogellii, E. senegalensis, E. sigmoideae, E. excelsa, E. glauca, E. addsoniae, E. mildbraedii and Millettia zechina when they are in flower, in Benin (lat 6.5°N, Long 6.0°W). Most of the plants commonly feature as parts of concoctions in local medicine. A specimen of each plant after identification has been preserved in the Herbarium of Bendel State University, Ekpoma.

Extraction

Flower specimens were extracted several times with 80% methanol containing 1.5M hydrochloric acid (HCL) to improve the extractions of glucosides (5:1) and the combined dilute extract was evaporated in vacuo in an all glass evaporator at 30°C. The concentrated extract was used for chromatography.

Hydrolysis of the extract:

Methanol extract was routinely hydrolysed with 2M HCL at 100°C for 30 min.

Chromatography:

Aglycones were separated by two-dimensional chromatography on Whatman No. 1 paper. Butanol-acetic acid - water (B.A.W. 5: 1: 4) and acetic acid 2% were used as solvents. The aglycones were detected under UV light, eluted, purified

TABLE 1. GENERAL DISTRIBUTION OF FLAVONOIDS IN THE FLOWERS OF PAPILIONACEAE SPECIES

NOS. OF FLAVONOIDS		CROTALARIA PUBESCENES	CROTALARIA FALCATA	CROTALARIA RETUSA	CROTALARIA VERRUCOSA	DESMODIUM VELUTINUM	DESMODIUM GANGETICUM	DESMODIUM TRIFLORUM	DESMODIUM ADSCENDENS	ERYTHRINA VOGELII	ERYTHRINA SENEGALENSIS	ERYTHRINA SIGMOIDEAE	ERYTHRINA EXCELSA	ERYTHRINA GLAUCA	ERYTHRINA ADDISONIAE	ERYTHRINA MILDBRAEDII	MILLETTIA ZECHIANA	TOTAL OF EACH CLASS
1	ANTHOCYANINS	1	1	1	0	2	3	3	2	2	2	2	2	2	2	1	3	23
2	FLAVONES	2	0	0	1	1	1	1	1	0	0	0	1	2	1	1	0	12
3	FLAVONOLS	1	2	2	2	0	0	0	1	2	2	2	0	1	2	2	4	23
4	ISOFLAVONES	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	13
5	FLAVANONES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	FLAVANONOLS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	TOTAL	4	4	4	4	4	5	5	5	5	5	5	4	5	6	5	7	76

TABLE II: DISTRIBUTION OF FLAVONOIDS IDENTIFIED IN THE FLOWERS OF PAPILLIONACEAE SPECIES

ANTHOCYANINS	Pel 3-O-gluco	3-O-rham	5-O-gluco	3,5-O-diglUco	Peo 3-O-gluco	3-O-arab	Cya 3-O-gluco	3-O-soph	3-O-rham	3-O-rutI	3,5-O-dIglUco	Del 3-O-rham	Mal 3,5-O-dIglUco	FLAVONES	Ape 7-O-gluco	7-O-rutI	7-O-glucu	Lute 7-O-gluco	Kaem 3-O-gluco	3-O-rutI	3-O-soph	3-O-rham	Quer 3-O-gluco	3-O-soph	8-hydrox 7-glucos	3-metHyLether	Myr FlAv 4 metHyLether	ISOFLAVONES	genIstein	IrIgenIn	IrIdIn	tectorIgenIn
Centrosema pubescenes																	+	+								+						
Crotalaria falcata												+							+				+									
Crotalaria retusa		+																	+					+								
Crotalaria verrucosa																		+		+										+		
Desmodium velutinum				+			+											+					+									
Desmodium gangeticum	+						+									+														+		
Desmodium triflorum						+	+									+														+		
Desmodium adscendens		+						+							+			+													+	
Erythrina vogellii									+																				+			
Erythrina senegalensis		+			+					+								+													+	
Erythrina sigmoideae					+																								+			
Erythrina excelsa				+				+																								+
Erythrina glauca			+				+										+									+						
Erythrina addsoniae		+									+						+	+	+					+			+					
Erythrina mildbraedii			+									+						+				+	+							+		
Milettia zechina		+									+		+									+			+	+	+					

and identified by hRf values and by standard procedure (10, 11). Anthocyanins were chromatographed two dimensionally on 20 x 20 sheets of Whatman No. 3 MM paper in TLC developing tank (No. z12, 619-5 Aldrich, Chemical Co. Ltd., Gillingham) using the solvent system.

(i) BAW, n-butanol-acetic glacial - water top layer within 1 - 2 hours of mixing (4: 1:5). Separation was also effected by descending chromatography on Whatman No. 1MM paper with two standard (ii), (iii) and two new solvent systems (iv), (v).

(ii) BAW, (iii) H_2O - HCL, (97:3) (iv) BAFW:n-butanol-acetic acid glacial-formic acid-water (3:1:1:5) (v) BFTW n-butanol-formic acid-toluene-water (5:1:1:3) gave very high resolutions.

RESULTS AND DISCUSSION

The flavonoid composition of the flowers of sixteen taxa of medicinal plants consisting five genera of the papilionaceae were investigated by extraction and chromatography (12). They afforded thirty flavonoids in four groups namely anthocyanidins, flavones, flavonols and isoflavones (Table 1). Anthocyanins were found exclusively in the form of 3 - O,

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Abbreviations in table 11: pel, pelargonidin; peo, peonidin; cya, cyanidin; del, delphinidin; mal, malvidin; ape, apegenin; lute, luteolin; kaem, kaempferol; quer, quecertin; myr, myristin; gluco, glucoside; rham, rhamnoside; arab, arabinoside; soph, sophoroside; ruti, rutinoside; glucu, glucuronide.

5 - O - monoglucosides and 3 - O, 5 - O-diglucosides in Papilionaceae species.

Table(11) shows the different flavonoids identified in the family. The anthocyanidin pelagronidin occurred as 3-O - glucoside in D. gangeticum, as 3-O-rhamnoside in C. retusa, D. adscendens, E. senegalensis, and M. zechina. Pelargonidin 3-O-glucoside was found in Centrosema pubescens, E.glauca, E. mildbraedii. It occurred as 3-O,5-O-diglucoside in D velutinum and E. exelsa. Peonidin occurred in E. senegalensis and E. sigmoideae as the 3-O-glucoside in E. vogellii as 3-O-arabinoside. Cyanidin was found as its 3-O glucoside in D. velutinum,D. gangeticum, D. triflorum, and E. glauca, whereas it occurred as its 3-O-sophoroside in D. adscendens, E. excelsa. The 3-O rhamnoside of cyanidin was found in only E. vogellii, and its 3-O-rutinoside in only E.senegalensis, cyanidin 3-O, 5-O- diglucoside was in E.addisoniae and M. zechina. Delphinidin as 3-O rhamnoside was in C. falcata and D. gangeticum only, malvidin 3-O, 5-O-diglucose in only M. zechina.

Of the flavones, apigenin was identified as its 7-O-glucoside in D.adscendens alone, apigenin 7-O-rutinoside in D. gangeticum, D. triflorum, and E. execelsa. Apigenin-O-glucuronide was in Centrosema pubescens, E. glauca, E. addisoniae. Luteolin 7-O-glucoside was isolated from Centrosema pubescens, C. verrycosa D. velutinum and E. addisoniae.

The flavonol Kaempferol was found as 3-O-glucoside in C. falcata, C. retusa, D. adscendens, E. senegalensis, E. sigmoideae and E. addisoniae.

Kaempferol 3-O-rutinoside occurred in only C. Verrucosa while its 3-O-sophoroside was also in only E. vogellii. The

3-O-rhamnoside of kaempferol was isolated in E. mildbraedii and M. zechina. Quercetin 3 - methyl ether was found in C. pubescens and E. glauca. As its 3-O-glucoside it was found in C. falcata, C. verrucosa, E. senegalensis, E. sigmoideae, E. mildbraedii, M. zechina: and quercentin 3-O-sophoroside was in C. retusa, and E. addisoniae.

Quercetin 8-hydroxy 7-glucoside occurred in M. zechina only along with its flavonol 3-methlether or isorhamnetin occuring in C. pubescens and E. glauca. A new flavonol myricetin 4-methlether was discovered from the islote of M. zechina (9).

Isoflavones which are established characteristic constituents of the family papilionaceae were found mostly in the aglycone form in the species of the Papilionaceae examined.

The aglycone genistein was identified in C. falcata, retusa, D. velutinum and E. addisoniae. Irigenin in E. vogellii only; iridin in C. verrucosa, D. gangeticum, D. triflorum, E. sigmoideae. Tectorigenin occurred in D. adscendens, E. senegalensis, E. excelsa and E. mildbraedii.

During the course of this work, serious difficulties were often encountered in the accurate measurement of the hRf of the flavonoids due mainly to poor separation in standard/ existing solvent systems. Two new solvent systems BFTW; n-butanol formic acid-toluene-water (5:1:1:3) and BAFW n-butanol-acetic acid glacial - formic acid-water (3:1:1:5) were developed. With these systems it was possible to obtain new reliable and reproduceable hRfs where values obtained from old solvents became doubtful.

Despite the size and widespread distribution of the family Papilionaceae in the tropics of both hemispheres, some genera have received very little attention for a systematic study of their flavonoids. Report of these flavonoids in some species of the genera Centrosema, Crotalaria, Desmodium Erythrina and Millettia are scanty more so for the other important medicinal taxa. Pomilio et at. (7) identified perlagonidin 3-O-glucoside, 3-O-sophoroside and cynidin 3-O-glucoside in the flowers of

Erythrina falcata. Also Purushothaman et al (8) about the same time reported the isolation of the isoflavonoid of gangetin from the leaves of D. gangeticum. This work has revealed that the plants investigated have simple flavonoid profiles. Further investigation is currently in progress in this laboratory as to how best to apply these information especially in chemotaxonomy and phylogeny of the plants.

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