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Flavonoid Distribution in Asplenioid Ferns

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ABSTRAK

Tinjauan ke atas taburan flavonoid di dalam 15 spesies paku-pakis Asplenioid menunjukkan kehadiran kaemferol di dalam 80%, kuersetin di dalam 53% dan proantosianidin di dalam 13% spesies yang dikaji. Kaemferol 3 O-*metil eter hanya dijumpai di dalam* A. marinum. *Akasetin dan genkwanin dahulunya telah dijumpai di dalam paku-pakis,* Notholaena bryopoda *(Polypodiaceae). Kini, ianya dikesan pula di dalam pinna* A. normale. *Ini merupakan laporan kali kedua mengenai kehadiran flavon jenis ini di dalam paku-pakis. Hispidulin dan pektolinarigenin telah dijumpai di dalam pinna* A. glaucophyllum *dalam bentuk sureh kerana kepekatan yang rendah dan sampel pinna yang sedikit. Skutellarein dijumpai di dalam* A. belangeri. *Dalam pengetahuan kami, penemuan hispidulin, pektolinarigenin dan skutellarein dari kajian ini merupakan laporan yang pertama kali hadir di dalam pinna-pinna paku-pakis.*

ABSTRACT

In a survey of 15 representatives of the Asplenioid ferns for the pinnae flavonoids, kaempferol was found in 80%, quercetin 53% and proanthocyanidin 13% of the species studied. Kaempferol 3 O-*methyl ether was found in* A. marinum *only. Acacetin and genkwanin have been previously found in the fern,* Notholaena bryopoda *(Polypodiaceae), but for the first time in* A. normale. *This is the second report of the occurrence of this type of flavone in ferns. Hispidulin and pectolinarigenin were detected in* A. glaucophyllum *in trace amounts due to the small sample and at a relatively low concentration. Scutellarein was detected in* A. belangeri. *To our knowledge, this is the first report of the existence of hispidulin, pectolinarigenin and scutellrein in ferns.*

INTRODUCTION

The presence of flavonoids in the Asplenioid ferns, genus *Asplenium* was previously reported by Voirin (1967). He found kaempferol 4'-*O*-methyl ether in *A. diplaziosorum.* Later, Voirin (1970) reported the distribution of flavonoid aglycones in 19 species of *Asplenium.* He studied mainly European species and a few species from Central Africa. He found kaempferol in 18 species, quercetin in 6 species and proanthocyanidins in 12 species. Harborne *et al.* (1973) studied the flavonoid characters of plants and hybrids of Appalachian species of *Asplenium* and found a series of kaempferol derivatives in the fronds of three parental species of the Appalachian *Asplenium* complex. *A. platyneuron* is characterized by the presence of the 7-*O*-glucoside of kaempferol, 3,4'-*O*-dimethyl ether and kaempferol 3,7-*O*-diglucoside with an aliphatic acyl attachment. By contrast, *A. rhizophyllum* contains a caffeoyl complex of kaempferol glycosides. *A. montanum,* in addition to having the glycosyl xanthones (Smith and Harborne 1971) has another two kaempferol glycosides (Harborne *et al.* 1973). Further flavonoid studies of Italian *Asplenium* were carried out by Imperato (1985 and references therein) who found several kaempferol glycosides in six *Asplenium.* As part of an ongoing project on the chemistry of *Asplenium,* the present study aims to add to the number of reports on the flavonoid profiles of the genus. For this purpose, we extracted the pinnae of the *Asplenium* species collected in local habitats from Malaysia and three species from England.

MATERIALS AND METHODS

Plant Sources

Fronds of twelve Malaysian species of Aspleniaceae were collected from various localities and habitats in West Malaysia. Fronds of *A. scolopendrium*, *A. marinum* and *A. adiantum-nigrum* were collected in Berkshire, England. The identities of the plants were verified by J.M. Camus and H.J.M. Bowen, and voucher specimens are deposited in the herbarium at the Department of Botany, University of Reading.

Flavonoid Analysis

A method similar to the one described by Harborne (1984) was used for acid hydrolysis. Dried pinnae were heated with 2 M hydrochloric acid (2 M HCI) at 100°C for about 30 min in a boiling water bath. The resulting extracts were cooled, filtered and then extracted, first with ethyl acetate and then with *iso*-amyl alcohol. The ethyl acetate extracts were evaporated to dryness and the concentrated extracts developed on Whatman no. 1 paper against standard markers in the following solvents: *n*-butanol-acetic acid-water (BAW, 4:1:5, top layer), Forestal (acetic acid-conc. hydrochloric acid-water, 30:3:10), 50% acetic acid, phenol (125 ml water in 500 g of phenol) and chloroform-water-acetic acid (CAW, 30:15:2). Interfering phenolic acids were removed by first over-running all chromatograms (Whatman no. 1 paper) in water and drying before further chromatography in those five solvent systems. The anthocyanidins delphinidin and cyanidin, formed by the breakdown of proanthocyanidin polymers were identified in the *iso*-amyl alcohol extracts by one-dimensional thin layer chromatography on microcystalline cellulose (Merck) in Forestal against two marker substances, cyanidin and delphinidin. The identity of flavonoid aglycones were established by direct comparison of their chromatographic behaviour and spectral data with authentic markers. Flavone *C*-glycosides were confirmed by their resistance to two hours of acid hydrolysis with 2M HCI at 100°C. They were extracted with *iso*-amyl alcohol and identified by one-dimensional chromatography, against vitexin and orientin as marker.

RESULTS AND DISCUSSION

As expected, proanthocyanidins were detected as shown by the formation of cyanidin and delphinidin on acid treament of the pinna (Table 1 and *Figure 1*). The flavonols quercetin and kaempferol (*Figure 2*) were also found in the acid hydrolysates of the fronds of many species examined. Twelve of the fifteen species examined contain kaempferol and all but four of these also possess quercetin. Only in the samples of *A. scolopendrium* and *A. marinum* could these flavonols not be detected, but kaempferol 3-*O*-methyl ether was found in *A. marinum*. Previous surveys of the Aspleniaceae (Berti and Bottari 1968; Voirin 1970; Harborne *et al.* 1973) for flavonoid aglycones have indicated that kaempferol is more common in this group than quercetin. The present finding in 80% of the Malaysian *Asplenium* studied confirms this pattern.

Proanthocyanidins based on delphinidin and cyanidin were present in *A. amboinense* and *A. adiantum-nigrum*. Voirin (1970) studied nineteen species of *Asplenium*, mainly European species and a few species from Central Africa. He found that proanthocyanidins based on delphinidin and cyanidin were widely distributed, being present in twelve of nineteen species investigated. On the other hand, in the present investigation of *Asplenium*, proanthocyanidins were present in only one of the eleven Malaysian species studied viz: *A. amboinense*. Therefore, it seems that Malaysian species of *Asplenium* in general do not accumulate proanthocyanidins, in contrast to the European species. Perhaps there are phytogeographical differences in the chemistry between European and Malaysian *Asplenium* in this respect.

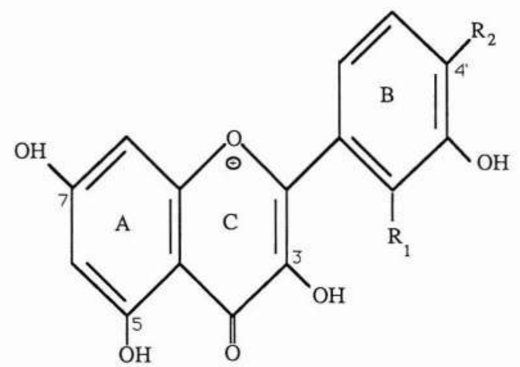
In the present study, the methylated flavone appeared to have a restricted distribution. Genkwanin and acacetin (*Figure 2*) were detected only in *A. normale*. They were first reported in the fern, *Notholaena bryopoda* (Polypodiaceae) (Wollenweber, 1982). This is therefore the second report of the occurrence of this type of flavone in ferns. Hispidulin and pectolinarigenin (*Figure 2*) were detected in *A. glaucophyllum* in trace amounts due to its relatively low concentration and the small amount of sample available. Scutellarein was found previously in leaves of the plant *Scutellaria galericulata* and in roots of *S. rivularis* (Barberan 1986). In the present survey, it was detected in *A. belangeri*. To our knowledge, this is the first report of the existence of hispidulin, pectolinarigenin and scutellarein in ferns.

Flavone *C*-glycosides, namely vitexin and orientin, were only detected in one species studied, *A. amboinense*. The occurrence of vitexin in ferns has previously been reported in *Cyathea faurier* and *Sphenomeris chusana* by Ueno *et al.* (1962). Orientin is a common constituent in five *Cyathea* species (Hiraoka and Hasegawa 1975). Both vitexin and orientin were also reported to occur in seven *Deparia* species (Hiraoka 1978). Thus, the presence of these flavone *C*-glycosides in the species studied increases the number of fern taxa with these types of flavones.

TABLE 1
Flavonoid survey of 15 Asplenioid fern species

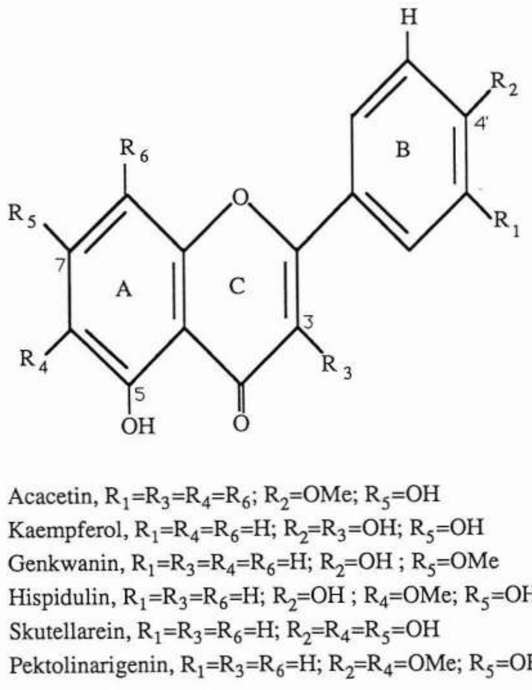
Taxon, collector and collection number	Proanthocyanidins		Flavonols			Flavones				*Flavone C-glycosides*	
	PC	PD	Kml	Km2	Qu	Genk	Aca	Hisp	Pect	Vitexin	Orientin
Asplenium L.											
A. adiantum-nigrum L.; UmiKalsom **235**, Cornwall, England	+	+	+	–	–	–	–	–	–	–	–
A. amboinense Willd.; UmiKalsom **230**, Pahang, West Malaysia	+	+	+	–	+	–	–	–	–	+	+
A. belangeri (Bory) Kze; UmiKalsom **219**, Pahang, West Malaysia	–	–	–	–	–	–	–	–	–	–	–
A. caudatum Forst; UmiKalsom **205**, Pahang, West Malaysia	–	–	+	–	+	–	–	–	–	–	–
A. glaucophyllum Aldrew; UmiKalsom **220**, Pahang, West Malaysia	–	–	+	–	+	–	–	+	+	–	–
A. longissimum Bl.; UmiKalsom **180**, Wilayah Persekutuan, West Malaysia	–	–	+	–	–	–	–	–	–	–	–
A. marinum L.; UmiKalsom **236**, Wilayah Persekutuan, West Malaysia.	–	–	–	+	–	–	–	–	–	–	–
A. nidus L.; UmiKalsom **179**, Negeri Sembilan, West Malaysia	–	–	+	–	+	–	–	–	–	–	–
A. normale Don. UmiKalsom **231**, Pahang, West Malaysia	–	–	+	–	+	+	+	–	–	–	–
A. pellucidum Lam; UmiKalsom **193**, Selangor, West Malaysia	–	–	+	–	–	–	–	–	–	–	–
A. phyllitidis Don; UmiKalson **212**, Perak, West Malaysia	–	–	+	–	+	–	–	–	–	–	–
A. scolopendrium L.; UmiKalsom **234**, Berkshire, England	–	–	–	–	–	–	–	–	–	–	–
A. scortechinii L.; WODJ **583**, Perak, West Malaysia	–	–	+	–	–	–	–	–	–	–	–
A. tenerum Forst.; UmiKalsom **221**, Pahang, West Malaysia	–	–	+	–	+	–	–	–	–	–	–
A. unilaterale Lam.; UmiKalsom **228**, Pahang, West Malaysia	–	–	+	–	+	–	–	–	–	–	–

Key: PC = procyanidin, PD = prodelhinidin, Kml = kaempferol, Km2 = kaempferol 3 *O*-methyl ether, Qu = quercetin, Genk=genkwanin, Aca = acacetin, Hisp-hispidulin, Pect = pectolinarigenin.



Cyanidin, R_1=OH; R_2=H
Delphinidin, R_1=R_2=OH

Fig. 1: Structure of proanthocyanidins



Acacetin, R_1=R_3=R_4=R_6; R_2=OMe; R_5=OH
Kaempferol, R_1=R_4=R_6=H; R_2=R_3=OH; R_5=OH
Genkwanin, R_1=R_3=R_4=R_6=H; R_2=OH ; R_5=OMe
Hispidulin, R_1=R_3=R_6=H; R_2=OH ; R_4=OMe; R_5=OH
Skutellarein, R_1=R_3=R_6=H; R_2=R_4=R_5=OH
Pektolinarigenin, R_1=R_3=R_6=H; R_2=R_4=OMe; R_5=OH

Fig. 2: Sructures of flavones and flavonols

CONCLUSION

Flavonoid aglycones based on kaempferol and quercetin are common to Angiosperms; here they were found in 80% and 53% of the species studied. Proanthocyanidin, flavone *C*-glycosides, methylated and hydroxylated flavones seem to have a restricted distribution in Malaysian, *Asplenium* ,being found in 13%, 7%, 13% and 7% respectively.

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