

## Phytochemical Screening of Flavonoids in Three Hybrids of *Nepenthes* (Nepenthaceae) and their Putative Parental Species from Sarawak and Sabah

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**Abstract:** Screening of flavonoids of leaf materials of nine dry herbarium specimens of three natural hybrids and six of their putative parental species collected from Sarawak and Sabah have been carried out. Eight spots containing phenolic acids, flavonols, flavones, leucoanthocyanins and unknown 1 and 3 were identified from the chromatographic profiles. Phenolic acid and ellagic acid, quercetin, kaempferol, unknown 1 and unknown 3 were prevalence: quercetin and kaempferol were present in all taxon except *Nepenthes tentaculata* and *Nepenthes gracilis* respectively; phenolic acid was undetected in *Nepenthes rajah* and *Nepenthes muluensis*, similarly ellagic acid was not recorded in *Nepenthes rajah* and *Nepenthes gracilis*. Flavonoids compound termed here as unknown 2 and 3 were equally prevalence in all the taxon screened but they were absent from *Nepenthes muluensis* and *Nepenthes gracilis*, *Nepenthes burbidgeae*, *Nepenthes tentaculata* and *Nepenthes gracilis* respectively. Luteolin was found in *Nepenthes rajah* and *Nepenthes muluensis* and hybrids *Nepenthes x Alisaputriana* and *Nepenthes x Sarawakiensis* only whereas cyanidin was detected in *Nepenthes rajah*, *Nepenthes mirabilis* and *Nepenthes gracilis* and hybrids *Nepenthes x Alisaputriana* and *Nepenthes x Ghazallyiana*. Myricetin and apigenin were absent in all these taxon studied. Chromatographic patterns of the three hybrids studied showed complementation of their putative parental species.

**Key words:** *Nepenthes*, Sabah, Sarawak, hybrid, flavonoids

### Introduction

The application of chemical data in taxonomy dates back as early as 1699, when J. Petiver wrote information on the relationship between chemical properties and certain morphological groupings of plants and he used herbal Umbelliferae together with the Labiatae and Cruciferae to explain his hypothesis that the morphologically similar plants produce chemical constituents with similar therapeutical effects (Fairbrothers et al., 1975). Fairbrothers et al. (1975) suggested that photochemistry offered the tool to check proposed classification based on morphological characters alone.

The role of biochemical systematics in the study of hybridizing populations and the analysis of past hybridization and introgression was demonstrated by Alston and Turner (1963), Smith and Levin (1963), Torres and Levin (1964), Garber and Strommaes (1965). The chemical work on hybridization was well reviewed by Harborne and Turner (1984). Heywood (1976) indicated that the chromatographic pattern of flavonoids has proved extremely valuable in the analysis of hybridization for example in *Baptisia* and *Asplenium* (fern). Chemical studies have been used in taxonomy to solve the problems caused by the specific and intraspecific taxa alike (Heywood, 1976; Harborne, 1973). Heywood stressed that chemical studies may be of particular value to solve population problems below the species level, especially in situations where hybridization or introgression is occurring or suspected to occur. The application of flavonoids in taxonomic study has been reported in *Pinus* (Erdtman, 1963), *Centrospermae* (Mabry, 1966), *Malus* and *Pyrus* (Williams, 1966) and *Leguminosae* (Alston and Turner, 1963). Harborne (1973) indicated that flavonoids can be used as taxonomic markers because they possess structural variability, chemical stability, widespread distribution in the plant kingdom and easy and rapid identification.

Very little work on flavonoids has so far been done on the Photochemistry of *Nepenthaceae*. Previous work on the phytochemistry of *Nepenthaceae* was carried out by Adam and Wilcock (1992 a,b, 1995), Som (1988) and Jay and Lebreton

(1972). Som (1988) worked on Malay Peninsula species and using HPLC and TLC recognized 16 different types of phenolic compounds including phenolic acids (protocatechuic acid and ellagic acid), 5 types of hydroxycinnamic acids, 4 types of coumarins, the flavonols quercetin and kaempferol, the flavones apigenin, the flavonones narigenin and catechin. According to Som (1988) the phenolic compound profile of Malay Peninsula species is taxonomically useful at both the specific and generic level. Jay and Lebreton (1972) carried out photochemical screening of *N. destillatoria*, *N. rafflesiana*, *N. morganiana x Veitch* and *N. chelsonii x Veitchii*. They found leucocyanidin, quercetin and traces of kaempferol.

The present study was carried out with the following objectives: firstly, to determine the different types of flavonoids found in the leaf materials of three hybrids of *Nepenthes* and their respective parental species; secondly, to determine the taxonomic significance of these flavonoids types in delimiting these hybrids and their parental species.

### Materials and Methods

Leaf materials of three natural hybrids *Nepenthes* and their six respective parental species for photochemical screening were obtained from nine dry herbarium specimens. All of the materials were rapidly dried and not given any chemical treatment.

**Hydrolysis treatment:** The screening of the leaf flavonoids of three *Nepenthes* hybrids and six of their putative parental species from Sarawak and Sabah studied was undertaken using hydrolyzed extracts follow the treatment of Harborne (1973).

About 1-2 g of dried leaves were cut into small pieces and extracted in 20 ml of 2M HCl, then boiled in the waterbath at 100 °C for 40 min. The hydrolyzed extract was taken left to cool and filtered through filter paper to remove debris from the extract.

The filtrate was treated twice with ethyl acetate; the upper layer containing flavones and flavonol was separated from lower aqueous layer by a separating funnel. Amyl alcohol was added to the latter layer to extract anthocyanidins. These extracts were left

to evaporate to dryness overnight in a dark fume chamber, 5 drops of ethanol (95 %) and methanol (100 %) were added to dissolve flavones, flavonol and leucoanthocyanins respectively ready for spotting into the plates.

**Thin layer chromatography (TLC) and spots identification:** Chromatographies of the hydrolyzed extracts were run one-dimensionally in solvent 1, at room temperature of 20-25 °C. The concentrated extracts were spotted on the lower left of the TLC plate by 5 µl micropipette. Fifteen loads of the extracts were applied and allowed to dry using a hair dryer before each subsequent load. The diameter of the spot in each chromatogram was normally about 5 mm. Authentic markers of flavones (luteolin and apigenin) and flavonols (myricetin, quercetin and kaempferol) obtained commercially were co-chromatographed.

Identification of the hydrolyzed compounds of these extracts was made by examination of the spots under UV light and by changes in colour under light after application of ammonia. R<sub>f</sub> values of these spots in comparison with the R<sub>f</sub> values of authentic markers, coupled with those values given for each known compound in Harborne (1973), were of great help in identification of these spots. The general flavonoid and anthocyanins profile patterns of each species were obtained through careful examination of repeated plates. Examinations of anthocyanins were carried out in daylight and under UV.

**Results and Discussion**

Eight chromatographic spots were identified in this study. They are phenolic acids (spots 1-2), flavones (spots 6-7), flavonols

(spots 6-7), leucoanthocyanins (spot 11) and two unknown pots (Table 1).

Two types of phenolic acids that is ellagic acid (spot 2) and unknown phenolic acid (spot 1) were recognized (Table 2). Ellagic acid and phenolic acid were detected in all taxon except *Nepenthes rajah* and *Nepenthes gracilis*, *Nepenthes rajah* and *Nepenthes muluensis* respectively. According to Bate-Smith and Swain (1965), ellagic acid is one of the commonest phenolic compounds in the leaves of the angiosperms. Som (1988) found ellagic acid in eight of the Malay Peninsula species. It was not detected in *Nepenthes destillatoria*, *Nepenthes rafflesiana*, *Nepenthes x Morganiana* and *Nepenthes x Chelsonii* (Jay and Lebreton, 1972).

Two common flavonols (spots 4-5) were detected in the taxon studied. They were quercetin and kaempferol. The presence of quercetin (spot 4) was recorded in all the hybrids and their six putative parental species studied. Whereas kaempferol (spot 5) was undetected in *Nepenthes gracilis* only. Flavones apigenin (spot 7) was absent in all taxon studied whereas luteolin (spot 6) was not recorded in *Nepenthes rajah*, *Nepenthes muluensis*, *Nepenthes x Alisaputriana* and *Nepenthes x Sarawakiensis*.

One type of leucoanthocyanins (Spot 11) viz. cyanidin was detected (Tables 1, 2). Cyanidin was found in *Nepenthes x Alisaputriana*, *Nepenthes rajah*, *Nepenthes mirabilis*, *Nepenthes gracilis* and *Nepenthes x Ghazallyiana*.

Presence of flavonols quercetin and kaempferol and the absence of myricetin in three *Nepenthes* hybrids and six of their putative parental species agree with the finding of previous authors (Som, 1988; Jay and Lebreton, 1972). The absence of a widely distributed compound like myricetin among these *Nepenthes* suggests that it may provide additional diagnostic information for these six species.

Table 1: Properties of phenolic compounds and leucoanthocyanins of the hydrolyzed leaf extracts of three hybrids of *Nepenthes* and their six putative parental species from Sarawak and Sabah

Spot No.	Mean Rf (x 100) in Forestal	Mean Rf (x 100) marker	Mean Rf (x 100) Harborne (1973)	Flavonoids	CR in day light	CR under UV	CR UV-ammonia
1	22	-	-	Phenolic acid	NV	Blue	Blue
2	29	-	-	Ellagic acid	NV	Purple	Yellow
4	38	44	41	Quercetin	NV	Yellow	BRY
5	55	60	55	Kaempferol	NV	Yellow	Yellow
6	66	60	66	Luteolin	NV	Ochre	BRY
7	-	79	83	Apigenin	NV	Ochre	DY
8	68	-	-	Unknown 1	NV	Purple	Purple
10	92	-	-	Unknown 3	Yellow	BRY	BRY
11	43	-	49	Cyanidin	Pink	Magenta	Magenta

Absent or not available, +: present, Spot 1-2: Phenolic acids, Spot 4-5: Flavonols, Spot 6-7: Flavones, Spot 11: Leucoanthocyanins, CR: Colour reaction, BGR: Blue green, BRY Bright yellow, DY: Dull yellow, NV: Not visible, UV: Ultraviolet

Table 2: Distribution of phenolic compound and leucoanthocyanins in three *Nepenthes* hybrids and their putative parental from Borneo

Taxa (section)	1	2	4	5	6	8	10	11	Specimen
<i>Nepenthes burbidgeae</i> (Regiae)	3+	++	3+	3+	-	+	-	-	J2484
<i>N. x alisaputriana</i>	+	++	3+	3+	+	++	3+	+	J2442
<i>In vitro</i>	+	++	3+	3+	+	++	+	+	
<i>Nepenthes rajah</i> (Regiae)	-	-	+	(+)	++	++	3+	+	J2443
<i>Nepenthes muluensis</i> (Vulgatae)	-	+	++	+	+	-	3+	-	J2400
<i>N. x sarawakensis</i>	+	+	+	+	+	+	3+	-	
<i>In vitro</i>	+	+	+	+	+	+	++	-	
<i>Nepenthes tentaculata</i> (Vulgatae)	+	+	-	(+)	-	+	-	-	J2392
<i>Nepenthes mirabilis</i> (Vulgatae)	+	++	3+	3+	-	++	++	++	J2141
<i>N. x ghazallyiana</i>	++	++	3+	3+	-	+	+	3+	J2476
<i>In vitro</i>	++	++	3+	3+	-	+	+	++	
<i>Nepenthes gracilis</i> (Vulgatae)	++	-	+	-	-	-	-	(+)	J853

Key: (+) +: (very) weak spot, ++: Strong spot, 3+: Very strong spot, -: Absent, J = Jumaat; Spot number refer to Table 1

Leaves of the three hybrids and their six putative parental species contained the flavones luteolin but not apigenin. Jay and Lebreton (1972) failed to detect either types of flavones but Som (1988) showed that luteolin was absent from Malay Peninsula species. She detected apigenin and was confined to highland species in the Malay Peninsula. This study however failed to find even a trace of apigenin in leaves of all the taxon studied. On the other hand, the presence of luteolin was detected. It was confined to highland taxon thus shows similar result of Som (1988) for apigenin.

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#### References

- Adam, J.H. and C.C. Wilcock, 1995. A new natural hybrid of pitcher plant from Sabah, Malaysian Borneo, *Borneo Science*, 1: 1-7.
- Adam, J.H. and C.C. Wilcock, 1992a. A new natural hybrid of *Nepenthes* from Mt. Kinabalu (Sabah). *Reinwardtia*, 11: 35-40.
- Adam J.H. and C.C. Wilcock, 1992b. One new natural hybrid of *Nepenthes* from Borneo. *The Sarawak Museum*, J. Vol. XLII NO. 64 (new series): 291-293.
- Alston, R.E. and B.L. Turner, 1963. *Biochemical Systematics*. Prentice-Hall, Englewood Cliffs., New Jersey.
- Bate Smith, E.C. and T. Swain, 1965. Recent developments in the chemotaxonomy of flavonoid compounds. *Lloydia*, 28: 313-331.
- Erdtman, G., 1963. Some aspects of chemotaxonomy. In T. Swain (ed.). *Chemical Plant Taxonomy*, pp: 89-125. Academic Press London, New York.
- Fairbrothers, D.E., 1968. Chemosystematics with Emphasis on Systematic Serology. pp: 141-174. In V.H. Heywood (ed.). *Modern Methods in Plant Taxonomy*. Academic Press London.
- Fairbrothers, D.E., J.J. Mabry, R.L. Scogin and B.L. Turner, 1975. *Ann. Missouri Botanical Garden*, 62: 765-800.
- Garber, E.D. and O. Strommaes, 1965. A paper chromatographic study of flower extracts from fourteen species and four interspecific hybrids. *Botanical Gazette*, 125: 96-106.
- Harborne, J.B., 1973. *Phytochemical Methods*. Chapman & Hall London.
- Harborne, J.B. and B.L. Turner, 1984 *Plant Chemosystematics*. Academic Press, London and New York.
- Heywood, V.H., 1976. *Plant Taxonomy*. The Institute of Biology's Studies in Biology No. 5. 2<sup>nd</sup> edition. Reprint 1979. Edward Arnold.
- Jay, M. and P. Lebreton, 1972. Chemotaxonomic research on vascular plants. The flavonoids of Sarraceniaceae, Nepenthaceae, Droseraceae and Cephalotaceae, a critical study of the order Sarraceniales. *Naturaliste Canadienne*, 99: 607-613.
- Mabry, T.J., 1966. The Betacyanins and Betaxanthins. In: T. Swain (ed.). *Comparative Phytochemistry*. pp: 231-244. Academic Press, London, New York.
- Smith, D.M. and D.A. Levin, 1963. A chromatographic study of reticulate evolution in the Appalachian *Asplenium* complex. *Am. J. Bot.*, 50: 952-958.
- Som, R.M., 1988. Systematic studies on *Nepenthes* species and hybrids in the Malay Peninsula. Ph.D. Thesis. Fakulti Sains Hayat, Universiti Kebangsaan Malaysia, UKM Bangi, Selangor Darul Ehsan.
- Torres, A.M. and D.A. Levin, 1964. A chromatographic study of *Cespitose zinnias*. *Am. J. Bot.*, 51: 639-643.
- Williams, A.H., 1966. Dihydrochalcones. In T. Swain (ed.). *Comparative Phytochemistry*. pp: 297-307. Academic Press London, New York.