Chemotaxonomy of South Indian *Piper*[1]

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ABSTRACT

A chemotaxonomical study was carried out on South Indian taxa of *Piper* to understand their interrelationships. Fourteen taxa were analysed for their flavonoids and based on presence or absence of these compounds, percentage similarity indices were calculated. The results in general supported the species delimitation and taxonomical relationships arrived at by conventional taxonomy using morphological characters. A chemical dichotomy was evident between the two sub genera - *Pipali* (having erect spikes) and *Maricha* (having pendant spikes) thereby supporting the validity of the sectional classification.

Key words: chemotaxonomy, flavonoids, *Piper* spp.

The genus *Piper* (Piperaceae) is distributed mainly in Central America, Northern and Southern America and Southern Asia. In Southern Asia, the genus is mainly distributed in the Indo-Malayan region, South India and Sri Lanka. The humid evergreen forests of Western Ghats of South India are considered to be the centre of origin and diversity for *P. nigrum* L., the dried mature fruits of which constitute black pepper, which is the most important spice of the world. About 15 species of *Piper* are reported from South India. No study has been conducted on the taxonomy and inter relationships of South Indian *Piper* except for species enumeration and floristics (Gamble 1925; Hooker 1886) and a preliminary study on the species occurring in Karnataka (Rahiman & Nair 1987).

A large collection of *Piper* germplasm is available at the National Research Centre for Spices (NRCS), Calicut. These collections were used in a biosystematic study for understanding the taxonomy and interrelationships of the species occurring in the region. The present paper deals with a chemotaxonomic study on South Indian taxa of *Piper* based on flavonoid analysis.

Materials and methods

The following species of *Piper* were studied: *P. argyrophllum* Miq., *P. attenuatum* Ham ex. Miq., *P. galeatum* Miq. C. Dc., *P. hymenophyllum* Miq., *P.*

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longum Linn., *P. mullesua* Ham ex. D. Don., *P. schmidtii* Hook. F., *P. silentvalleyensis* Ravindran and Asokan, *P. trichostachyon* Miq. C. Dc., *P. wightii* Miq., *P. sugandhi* Ravindran, Babu & Naik, *P. sugandhi* var. *brevipilis* Ravindran, Babu & Naik, *P. nigrum* Linn. (7 distinct collections) and *P. nigrum* var. *hirtellosum* Asokan & Ravindran. Among the species reported from the region, the two endangered ones, *P. barberi* Gamble and *P. hapnium* Gamble could not be included in the study due to paucity of materials. Voucher specimens of the species are deposited at the NRCS herbarium and live specimens are available at the germplasm conservatory at NRCS.

For chemical analysis, composite leaf samples from various collections of each species were dried in shade, powdered and 10 g of the powder extracted with methanol for 48 h at room temperature and then with 80:20 methanol-water for another 24 h. After 24 h the flasks were refluxed for 1 h, cooled and filtered. The two extracts were combined and then extracted with hot benzene to remove chlorophyll and fatty impurities. The final extract was used for analysis of flavonoids.

The methanol extract was analysed for flavonoids by standard techniques (Mabry, Markham & Thomas 1976; Markham 1982) with Whatman 3mm paper (56 cm x 48 cm) and using the solvent system t-butanol-acetic acid-water (6:1:3). The running time was 18 h at an ambient temperature of 30±3°C. Only unidirectional separation was used because the spot numbers were less and good separation was obtained in the solvent system. The chromatograms were examined under long UV (356 nm) after development, before and after exposure to ammonia vapours. One set of chromatograms were also sprayed with 1% Aluminium chloride in methanol and examined under UV. All chromatograms were prepared in duplicates and the Rf values given are means of two observations. The spot patterns were used for computing paired affinity indices (PAI), which is defined as:

$$PAI = \frac{\text{Number of spots similar to A \& B}}{\text{Total number of spots in A \& B}} \times 100$$

PAI is a measure of chemical affinity between any two taxa.

Results and discussion

The number of flavonoid spots varied from 10-16 in the 14 taxa studied, the lowest being in *P. silentvalleyensis* and the highest in *P. sugandhi.*

The flavonoid spot pattern is represented in Table 1 and the spot characters and the probable flavonoid types are given in Table 2. Based on the data in Table 1, PAI were computed for the taxa (Table 3). The PAI provides a measure of chemical affinity among the taxa studied. The following conclusions can be drawn from the results.

P. argyrophyllum showed high chemical similarity with *P. attenuatum* (78.5%) and *P. hymenophyllum* (78%) followed by *P. schmidtii* and *P. trichostachyon* (71%). The affinity was lowest with *P. longum* and *P. sugandhi* var. *brevipilis* (47% and 38%, respectively). *P. attenuatum* had high similarity with *P. schmidtii* (78%) followed by *P. wightii* (71%). *P. longum* did not show much chemical affinity with any other taxa, the highest being with *P. mullesua* (69%) which in turn did not show high affinity to any other taxa. *P. silentvalleyensis* did not show much

Table 1. Flavonoid spot patterns of *Piper* spp.

Species	Rf values																			
	.25	.32	.40	.45	.50	.53	.56	.60	.63	.65	.70	.72	.75	.80	.84	.86	.88	.90	.93	.95
P. argyrophyllum	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	-	+
P. attenuatum	+	+	-	+	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-	+
P. galeatum	+	-	+	+	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+
P. hymenophyllum	-	+	-	+	+	-	+	+	+	+	+	-	+	-	-	+	+	+	-	+
P. longum	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	-	+	+	-	+
P. mullesua	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	+
P. nigrum	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	+
P. nigrum var. *hirtellosum*	+	+	+	+	-	-	+	+	+	-	+	-	+	+	+	+	-	+	-	+
P. schmidtii	-	+	+	+	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-	+
P. silentvalleyensis	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+
P. trichostachyon	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	+	-	+	-	+
P. wightii	+	+	+	-	+	+	-	+	-	+	+	-	+	-	+	-	-	+	+	+
P. sugandhi var. *brevipilis*	+	+	+	+	-	+	-	+	+	-	+	+	-	+	+	-	-	+	+	+
P. sugandhi	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+

chemical affinity with other species, the highest being with *P. schmidtii* (69%). *P. trichostachyon* displayed close chemical affinity with *P. galeatum* (89%). The cultivated *P. nigrum* showed high affinity with *P. nigrum* var. *hirtellosum* (87%) which in turn showed 70% and 71% affinity with *P. galeatum* and

Table 2. Spot characters and probable flavonoid types in *Piper* spp.

Rf	Colour under UV	Colour under UV+NH_3	Probable flavonoid type
0.25	Light blue	No colour change	Isoflavones lacking a free -OH group
0.32	-do-	-do-	-do-
0.40	-do-	-do-	-do-
0.45	-do-	-do-	-do-
0.50	Purple	Yellowish green to yellow	5-OH flavones or flavonols
0.53	Dull yellow	No colour change	Flavones with a free 3-OH
0.56	Bluish green	-do-	Flavonols with a free 3-OH and with or without a free 5-OH
0.60	Purple	-do-	Flavones or 3-OH substituted flavonols with 5-OH (but lacking a free 4-OH)
0.63	-do-	-do-	-do-
0.65	Yellowish brown	-do-	Flavones with a free 3-OH
0.70	Bluish green	-do-	5-hydroxyflavonol (+ve reaction with $AlCl_3$ spray)
0.72	Greenish blue	-do-	Flavonols with a free 3-OH and with or without a free 5-OH
0.75	-do-	-do-	-do-
0.80	Pale blue	Fluorescent bluish green	Flavonols with a free 3-OH but lacking a free 5-OH or flavones lacking free 5-OH
0.84	Yellowish brown to dull yellow	No colour change	Flavonols with a free 3-OH and with or without a free 5-OH
0.86	-do-	-do-	-do-
0.88	-do-	-do-	-do-
0.90	-do-	-do-	-do-
0.93	-do-	-do-	-do-
0.95	Orangish red	Bluish green	Anthocyanin 3, 5-OH diglycoside

P. trichostachyon, respectively. *P. sugandhi* and *P. sugandhi* var. *brevipilis* had 88% affinity between them and they displayed moderate affinity with *P. nigrum* and *P. galeatum* (70 and 71%, respectively).

From the chemical tests, the flavonoid types occurring in *Piper* spp. were found to be mainly flavonols, flavones, isoflavones and 5-hydroxy flavononones. Rare flavanols were absent. Vanillin-HCl spray did not give a positive test reaction, indicating the absence of catechins, proanthocyanidins, flavanones and dihydroxyflavanole (i.e. absence of flavonoids possessing A-ring oxidation pattern in combination with a saturated C-ring). Ferric chloride spray did not give a positive reaction thereby indicating the presence of O-methylation of the flavonoid nuclei. Extraction of different flavonoids and their chemical characterisation was not attempted since such a study was beyond the scope of the present investigation.

Chemical evidences are being increasingly used in taxonomy and results from various sources indicate that it is a powerful tool in elucidating taxonomic and phylogenetic relationships. Gottlieb (1972) showed that in Lauraceae, secondary metabolites are taxonomically important; he has also indicated the probable evolutionary trends in the family based on chemical constituents. Excellent studies in chemotaxonomy have been carried out in Rosaceae (Challice 1973), Ulmaceae (Giannasi 1978) and in many other families and genera (Harborne & Turner 1984). Unfortunately, in Piperaceae no such studies have been carried out. Though many species of *Piper* were studied chemically, there was no attempt to correlate the chemical information with taxonomy except for a preliminary study by Rahiman & Subbaiah (1984). They found close chemical similarities between certain species known to be morphologically related. According to them chemical evidence supported the conclusions of the conventional taxonomists.

The present study is the most elaborate on South Indian *Piper*. Reasonable chemical affinities based on flavonoids were noted between the following taxa :

P. galeatum - *P. trichostachyon* 87%

P. attenuatum - *P. argyrophyllum* 79%

P. argyrophyllum - *P. hymenophyllum* 78%

P. galeatum - *P. sugandhi* 82%

P. sugandhi - *P. sugandhi* var. *brevipilis* 88%

P. nigrum - *P. nigrum* var. *hirtellosum* 87%

P. galeatum - *P. sugandhi* var. *brevipilis* 70%

These chemical relationships strongly support the morphological and taxonomical relationships arrived at by conventional tools. These results are also supported by similar results from a cluster analysis study (Ravindran, Babu & Balakrishnan 1992).

P. longum, *P. mullesua* and *P. silentvalleyensis* were the three species among which chemical relationships were comparatively low.

P. longum - *P. mullesua* 69%

P. longum - *P. silentvalleyensis* 35%

P. mullesua - *P. silentvalleyensis* 57%

Table 3. Paired affinity indices (PAI) between *Piper* taxa

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P. argyrophyllum	100	79	59	78	47	62	50	62	71	64	71	53	38	55
P. attenuatum		100	64	63	47	68	59	68	78	60	67	71	53	52
P. galeatum			100	55	50	53	70	70	68	66	87	53	70	82
P. hymenophyllum				100	59	64	50	59	56	44	53	50	35	45
P. longum					100	69	37	50	56	35	50	50	58	61
P. mullesua						100	47	47	63	57	42	53	55	58
P. nigrum							100	87	53	50	65	61	71	72
P. nigrum var. *hirtellosum*								100	63	56	69	50	65	67
P. schmidtii									100	69	56	67	53	55
P. silentvalleyensis										100	53	53	39	47
P. trichostachyon											100	63	65	70
P. wightii												100	59	67
P. sugandhi var. *brevipilis*													100	88
P. sugandhi														100

These three species belong to the sub-genus *Pipali* (Ravindran 1990) having erect spikes. *P. mullesua* and *P. silentvalleyensis* resemble very closely in vegetative characters and they are difficult to distinguish unless they bear spikes. These three taxa also showed low affinity to other species. Morphologically also these three species are quite distinct from all other species which come under the sub-genus *Maricha* (Ravindran 1990). Thus, in general, this result also substantiates the earlier conclusions based on morphological characters.

The members of the two sub-genera are chemically very distinct thereby lending support to the validity of the subgeneric classification. This conclusion was further supported by a cluster analysis study in which *P. mullesua* and *P. silentvalleyensis* were in a closely related cluster while *P. longum* was distinct from all other species (Ravindran *et al.* 1992); Hooker (1886) included *P. mullesua* (syn. *P. brachystachyum*) under the section Chavica, along with the species *P. longum*. Gamble (1925) also treated them as closely related. *P. mullesua* is very distinct and is the only South Indian species with erect globose spikes. *P. silentvalleyensis* is a unique species with erect, flexuous, filiform spikes and is the only wild bisexual species in South India. *P. longum* on the other hand has a trailing habit and has cylindrical female spikes with laterally-fused flowers. This species is also highly apomictic. Anatomically also *P. longum* is very distinct from all other species, an observation supported by workers like Murty (1959) and Dutta & Dasgupta (1977).

In *Piper*, alkaloids form an important group of compounds. One such alkaloid is the isoquinoline group of alkaloids present in many families having a Magnolian - Ranalian ancestry (Gottlieb *et al.* 1989). An investigation into the alkaloid pattern may be useful in understanding the phylogenetic sequences and relationships in Piperaceae. Gottilieb *et al.* (1989) while discussing the chemical dichotomies of the Magnolian complex suggested that neolignans and benzylisoquinoline type of alkaloids are important in the taxonomic phylogentic consideration of Piperaceae. They suggested that pyrones and amides form a link between Piperaceae and Lauraceae while cinnomoylamides could be related to Chloranthaceae. Further chemosyste - matic investigations could be useful in elucidating the phylogenetic lines leading to Piperaceae in general and *Piper* in particular.

References

Challice J S 1973 Phenolic compounds of the subfamily Pomiodeae : a chemotaxonomic survey. Phytochemistry 12 : 1045-1101.

Dutta P C & Dasgupta A 1977 Comparison of the vegetative anatomy of Piperales. 1. Juvenile xylem of twigs. Acta biol. Acad. Sci. Hung. 28 : 81-96.

Gamble J S 1925 Flora of Presidency of Madras Vol.2. Reprint. Botanical Society of India, Calcutta.

Giannasi D E 1978 Generic relationship in the Ulmaceae based on the flavonoid chemistry. Taxon 27 : 331-334.

Gottlieb O R 1972 Chemosytematics of the Lauraceae. Phytochemistry 11 : 1537-1570.

Gottlieb O R, Kaplan MAC, Kubitzski K & Toledo Barros J R R 1989 Chemical dichotomies of the Magnolian complex. Nord. J. Bot. 8 : 437-444.

Harborne J B & Turner B L 1984 Plant Chemosystematics. Academic Press, London.

Hooker J D 1986 The Flora of the British India Vol.V. Reprint. L. Reeve and Co., London.

Mabry T J, Markham K R & Thomas M B 1976 The Systematic Identification of Flavonoids. Springer - Verlag, Berlin.

Markham K R 1982 Techniques of Flavonoid Identification. Academic Press, New York.

Murthy Y S 1959 Studies in the order Piperales. Phytomorphology 10 : 50-59.

Rahiman B A & Nair M K 1987 The genus *Piper* Linn. in Karnataka, India. J. Bombay Nat. Hist. Soc. 84 : 66-83.

Rahiman B A & Subbaiah C C 1984 Flavonoid analysis in eight species of *Piper* from the Western Ghats. Pl. Physiol. & Biochem. 11 : 26-32.

Ravindran P N 1990 Studies on black pepper and some of its wild relatives. PhD Thesis. Submitted to University of Calicut.

Ravindran P N, Nirmal Babu K & Balakrishnan R 1992 Numerical taxonomy of *Piper* spp.1. A cluster analysis study. Rheedea 2 : 55-61.