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CHEMOTAXONOMY OF *CINNAMOMUM* SCHAEF. OCCURRING IN WESTERN GHATS

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A chemotaxonomical investigation was carried out in *Cinnamomum* as part of a biosystematic study on this genus occurring in the Kerala state. Eight species occurring in the Western Ghats were analysed for the terpenoids, steroids and flavonoids. *C. malabatrum*, the most common and complex species was studied in more detail. The results indicated much chemical variability among the species. Chemically *C. verum*, *C. camphora* and *C. perrottetii* were the most complex, while some collections of *C. malabatrum* were the least complex. Chemically *C. amphora* was the most distinct species. *C. verum*, *C. camphora*, *C. cassia* and *C. riparium* are very distinct chemically. Much infraspecific variability was noticed in *C. malabatrum*. The complexity in flavonoid pattern in this genus, resulted from O-methylation which is considered as an advanced character, Flavonols were found to be replaced by flavones. Both these are advanced characters in the evolutionary history of flavonoids.

Key words: Chemotaxonomy, *Cinnamomum*, Western Ghat.

Cinnamomum Schaeffer (Lauraceae) is an important genus consisting of about 450 reported species (Kostermans, 1964). This genus is of great economic importance. *C. verum* Bercht & Presl. gives the famous spice cinnamon; *C. cassia* Bercht & Presl gives the Chinese cassia; *C. burmannii* C.G. and Th. Nees is the Indonesian cassia; *C. loureirii* Nees gives the Saigon cassia and *C. camphora* (Linn.) Bercht. & Presl is the camphor tree. The members belonging to this genus are mostly small trees inhabiting mainly the plains and lower elevations of the evergreen forests of Western Ghats. Some species are restricted to the higher elevation, such as *C. macrocarpum* and *C. perrottetii*. *C. malabatrum* is the most widely distributed of all the species occurring in South India.

The Malabar Coast of India was once famous for the cinnamon export. In spite of its economic importance from ancient times practically very little is known about the *Cinnamomum* spp. occurring in the Western coastal region of India. Efforts are in progress in recent years to collect, conserve and study this genus especially with reference to the South Indian species. The present study forms part of a larger biosystematic study on the species occurring in the Kerala region of South India.

MATERIALS AND METHODS

Eight species occurring in the Western Ghats and adjoining regions of the Kerala State were studied. They are *C. verum Bercht & Presl.*, *C. cassia* Bercht & Presl., *C. camphora* (L) Bercht & Presl., *C. riparium* Gamble, *C. perrottetii* Meissner, *C. nicolsonianum* Manilal and Shylaja, *C. macocarpum* Hooker and *C. malabatrum* (Burman f.) Bercht & Presl. *C. malabatrum*, being the most heterogenous and most widely distributed, was studied in a more detailed manner by using nine collections from varying agroecological conditions. Voucher specimens were deposited at the Calicut University Herbarium (CALI)

The chemotaxonomic studies were carried out based on triterpenoids, steroids and flavonoids. Leaf materials collected from the various taxa were dried in the shade, powdered and 10 g of the powder was extracted in a soxhlet extraction unit. The materials were extracted with petroleum ether (60-80) for 12h followed by extraction with methanol for another 12h. The petroleum ether extract was concentrated under vacuum and used for the analysis of petroleum ether soluble triterpenoids and steroids. The methanol extract was concentrated and used for analysis of flavonoids.

The petroleum soluble compounds were analysed by thin layer chromatography using silica gel following standard procedure. (Harborne, 1973). The solvent used was benzene - ethyl acetate (9 : 1). The separation was carried out in standard sized plates

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(20x20cm) and the plates after run were drived and sprayed with concentrated H_2SO_4 and heated to 100°C for 30 minutes. The spots after heating were studied. The other detection reagents used were 5% 2,4-DNPH in 2N. HCl; 1% $KMnO_4$ in water and 1% $FeCl_3$ in methanol. Three replications were used and the Rf values given are averages of three readings.

The methanol extracts were analysed by paper chromatography following the technique of Mabry, Markham and Thomas (1970) and Markham (1982) using Whatman No. 1 paper and the solvent system t-butanol-acetic acid-water (3:1:1). The Chromatograms were dried and observed under long u.v. light (360 nm) both before and after exposure to ammonia vapour and $AlCl_3$ (1% in methanol) spray. The Rf values given are mean of three replications.

The chromatographic pattern of the various accessions were compared and the paired similarity indices (PSI) or paired affinity indices (PAI) were computed. The PSI between A and B is calculated by

$$PSI = \frac{\text{Number of spots common to A and B}}{\text{Total number of spots in A and B}} \times 100$$

where A and B stands for any two species. The PSI is a measure of chemical similarity between any two species or collections. The chromatographic data were used for a mumerical analysis also. For this the presence or absence of a compound is represented by binary values 1 and 0 and the data analysed by using the centroid clustering technique following the method of Engelman (1981). The analysis was done at the Computer Centre of the Carneigie-Mellon University, Pittsburgh, USA, using the BMDP-81 programme package developed by the University of California.

OBSERVATIONS AND RESULTS

A total of nine spots were observed in TLC of petroleum ether extract (Table 1). The methanol extract gave a total of eleven spots (Table 2). The results indicated that the Indian and Sri Lankan samples of *C. verum* are chemically identical showing absolute chemical similarity. This species was one of the most complex, a status it shared with *C. camphora* and *C. perrottetii*. *C. verum* showed resemblances with *C. capmphora* in petroleum soluble compounds but differed significantly in flavonoid patterns.

C. cassia, again a cultivated species, was found to have high similarity with *C. camphora* but here also flavonoid pattern showed differences between the two. The least similarity was found between *C. cassia* and *C. macrocarpum*.

Two collections of *C. riparium* were found to show significant differences in the total chemical characteristics, but they showed only marginal differences in the flavonoid pattern. *C. perrottetii* was distinctly different from all the other species but was showing similarity to one collection of *C. malabatrum*, but the flavonoid patterns of these were very different. *C. nicolsonianum* and *C. malabatrum* appear to be chemically related.

In terms of overall chemical complexity, *C. verum*, *C. camphora* and *C. perrottetii* were the most complex, each having eleven spots, followed by *C. cassia*, one collection each of *C. riparium* and *C. malabatrum*. Three collections of *C. malabatrum* and *C. nicolsonianum* were the least complex, while the other species occupied middle ranks in the order of complexity. The chemical relationships among the taxa are depicted in the form of a dendrogram (Fig. 1).

The chromatographic data were subjected to a numerical analysis in an attempt to group the taxa by taking the binary values 1 and 0 to represent the presence (=) or absence (-) of a compound. The results arrived at from this analysis are :-

1. Indian and Sri Lankan collections of *C. verum* are identical chemically, thereby supporting the results obtained from morphometric studies.
2. *C. camphora* is the most distant species, thereby lending support to the conclusions arrived at by morphological analysis.
3. *C. verum*, *C. camphora*, *C. cassia* and *C. riparium* appear to be very distinct chemically, both among themselves and from other species.
4. Much variability was noticed within the species *C. malabatrum*. OTUS 10 and 11 stand out distinctly from others.
5. Chemical relationships were evident between *C. perrottetii* and *C. macrocarpum* and between *C. malabatrum* and *C. nicolsonianum*.

DISCUSSION

Though Gottlieb and coworkers (Gottlieb, 1972) carried out extensive studies on most of the genera in Lauraceae, no published works are available on the chemotaxonomical aspects on *Cinnamomum*. The

Table 1: Spot pattern of triterpenoids and steroids (Petroleum ether soluble compounds).

No.	Species	Spot Numbers									
		1	2	3	4	5	6	7	8	9	Total
1.	*C. verum*	+	+	+	+	-	-	+	-	-	5
2.	*C. verum* (Ceylon)	+	+	+	+	-	-	+	-	-	5
3.	*C. cassia*	+	+	+	-	+	-	+	-	*	6
4.	*C. camphora*	+	+	+	+	-	-	+	-	*	6
5.	*C. riparium*	+	+	-	+	+	+	+	+	-	7
6.	*C. riparium*	+	+	-	-	+	-	-	+	-	4
7.	*C. nicolsonianum*	+	+	+	-	+	-	-	+	-	5
8.	*C. perrottetii*	+	+	-	+	+	-	+	+	-	6
9.	*C. macrocarpum*	+	+	-	+	-	-	+	-	-	4
10.	*C. malabatrum*	+	+	+	+	+	-	+	-	-	6
11.	-do-	+	+	+	+	+	-	-	-	+	5
12.	-do-	+	+	+	-	+	-	+	+	-	6
13.	-do-	+	+	-	-	+	-	+	-	-	4
14.	-do-	+	+	-	-	+	-	+	+	-	5
15.	-do-	+	+	-	-	+	-	-	+	-	4
16.	-do-	+	+	+	-	+	-	-	+	-	5
17.	-do-	+	+	-	-	+	-	-	+	-	4

Table 2: Flavonoid patterns in *Cinnamomum* spp.

No.	Species	Spot Numbers											
		1	2	3	4	5	6	7	8	9	10	11	Total
1.	*C. verum*	+	-	+	-	-	+	-	+	+	+	-	6
2.	*C. verum* (Ceylon)	+	-	+	-	-	+	-	+	+	+	-	6
3.	*C. cassia*	+	-	-	-	+	-	-	-	+	+	-	4
4.	*C. camphora*	+	+	-	-	+	+	-	-	+	-	-	5
5.	*C. riparium*	+	-	-	+	-	-	-	-	+	-	-	3
6.	*C . riparium*	+	-	-	+	-	+	-	+	-	-	-	4
7.	*C. nicolsonianum*	+	-	-	-	+	-	-	-	-	-	-	2
8.	*C. perrottetii*	+	-	-	+	+	-	+	-	+	-	-	5
9.	*C. macrocarpum*	+	-	-	-	+	-	+	-	+	-	-	4
10.	*C. malabatrum*	+	-	-	+	-	-	-	-	+	-	*	4
11.	-do-	+	-	-	-	*	-	-	+	-	-	-	3
12.	-do-	+	-	+	-	-	+	-	-	-	-	-	3
13.	-do-	+	-	-	-		+	-	+	-	-	-	3
14.	-do-	+	-	-	+	+	-	+	-	-	-	-	4
15.	-do-	+	-	-	-	+	-	-	-	+	-	-	3
16.	-do-	+	-	+	-	-	+	-	-	-	-	-	3
17.	-do-	+	-	-	+	-	-	-	+	-	-	-	3

existing information on *Cinnamomum* pertain mainly to the volatile oil composition of some of the economically important species such as *C. verum* and *C. camphora*. Bate-Smith (1962) noted the presence of flavonoids in *Cinnamomum* in the course of his study on the presence of these compounds in plant kingdom.

The present study shows the presence of flavonoids in all the species of *Cinnamomum* investigated. The number of spots differed from two in *C. nicolsonianum* to six in *C. verum*. The chemical variability is further evident in the petroleum ether soluble compounds. The total number of spots obtained varied from seven in *C. nicolsonianum* and some of the *C. malabatrum* to eleven in *C. verum*, *C. camphora* and *C. perrottettii*.

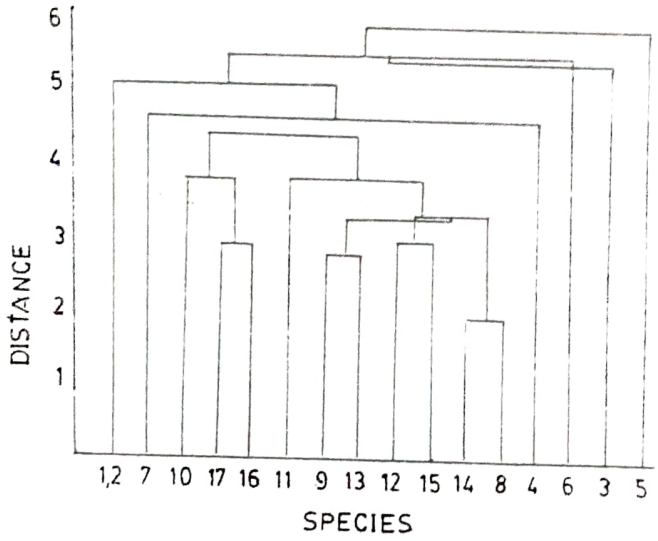
Within species variability in the terpenoid compounds were reported by earlier workers in *C. camphora* (Tetenyi, 1970). Many chemical varieties each differing in its constitutent volatile oil compounds (chemovars) had been recognised in *C. camphora*. (Fujita, 1967). Similarly infraspecific flavonoid dif-

Table 3: Percentage similarity indices (PSI) between the various taxa of *Cinnamomum*.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	100	100	60.9	57.1	63.6	38.1	31.6	70.0	44.4	33.3	31.6	52.6	44.4	52.6	44.4	36.4	21.1
2.		100	60.9	57.1	63.6	38.1	31.6	70.0	44.4	33.3	31.6	52.6	44.4	52.6	44.4	36.4	21.1
3.			100	60.0	47.6	40.0	22.2	52.6	47.1	35.3	44.4	44.4	47.1	33.3	47.1	38.1	22.2
4.				100	76.2	40.0	33.3	63.2	70.6	58.8	55.5	44.4	70.6	44.4	47.1	38.1	22.2
5.					100	28.6	42.1	60.0	55.5	44.4	42.1	42.1	55.5	42.1	33.3	47.6	33.3
6.						100	33.3	42.1	47.1	47.1	55.5	55.5	58.8	44.4	58.8	50.0	22.2
7.							100	23.5	40.0	40.0	37.5	37.5	46.7	37.5	46.7	31.6	37.5
8.								100	75.0	50.0	58.8	70.6	62.5	82.4	62.5	40.0	23.5
9.									100	71.4	80.0	66.6	85.7	66.6	71.4	44.4	26.7
10.										100	80.0	53.3	71.4	53.3	57.1	44.4	40.0
11.											100	75.0	80.0	62.5	66.7	63.2	37.5
12.												100	66.4	75.0	93.3	42.1	25.0
13.													100	66.7	71.4	85.6	26.7
14.														100	66.6	42.1	25.0
15.															100	22.2	23.5
16.																100	42.1
17.																	100

1 & 2: *C. verum*, 4. *C. cassia*, 5. *C. camphora*, 6. *C. riparium*, 9. *C. nicolsonianum*, 16. *C. perrottetii* 17. *C. macrocarpum* Others *C. malabatrum*.

$$*PSI = \frac{\text{Number of similar spots}}{\text{No. of similar spots + No. dissimilar spots}} \times 100$$



ferences are known in many cases. Bohm (1987) cites many examples of infraspecific flavonoid variation in his recent review.

Chemically the most distant species are *C. verum* and *C. macrocarpum* and also *c. macrocarpum* and some of the *C. malabatrum* collections. *C. cassia* and *C. camphora* exhibited an overall chemical similarity of 76.2%, but the picture is quite different when flavonoid pattern alone is considered. *C. cassia* and *C. camphora* share only three spots, thereby indicating that flavonoids are better taxonomic markers.

Two collections of *C. riparium* from different ecological conditions differed each other when total chemical characters were considered but their flavonoid patterns were very similar. Such differences in terpenoid compounds were also recorded in *C. camphora* (Fujita, 1987). Based on the volatile components it can be said that the two collections of *C. riparium* represent two distinct chemical races, but at the same time flavonoid chemistry shows that they belong to the same taxa, thereby supporting the taxonomic categorisation based on morphological differences.

C. perrottetii is a very distinct species having restricted distribution (above 1000m). Chemically also it is very distinct from all other species, except from a collection of *C. malabatrum*. It is difficult to provide an explanation for such parallel chemical affinities between distinct species, but as Crawford and Levy (1978) had pointed out identical chemical profiles need not necessarily indicate genetic identity. Moreover a comparison with flavonoids indicates distinct differences between these two.

C. macrocarpum, which also has a very restricted distribution occurring above 1500 m only does not show any chemical similarity with other species. *C. nicolsonianum* has shown close chemical similarity with *C. malabatrum*, though the distinction between the two can be justified based on distinct morphological characters.

Centroid clustering analysis of the chromatographic data sorted out *C. verum*, *C. riparium*, *C. cassia* and *C. camphora* as independent entities. Close clustering resulted between *C. perrottetii* and *C. macrocarpum* as well as between *C. malabatrum* and *C. nicolsonianum*. In terms of flavonoid pattern, the most complex species was found to be *C. verum* followed by *C. camphora*, the least complex being *C. nicolsonianum*. Chemical complexity has generally been associated with advancement but this conclusion

has been contested by some other workers. There are instances to show that more advanced taxa have fewer and structurally simpler compounds relative to those found in more primitive members of the main genus. Such trends have been observed in *Parthenium, Vernonia, Hymenoxys,* and *Ambrosia* (Mabry, 1973). Loss mutations thus seem to occur more frequently than do gain mutations during the course of speciation within a genus.

The complexity in flavonoid pattern in the genus results from O-methylation (as most of the spots not answering $FeCl_3$ test).O-methylation is considered as an advanced character in the evolution of flavonoids (Harborne, 1967; Gottlieb, 1972; Gornall and Bohm, 1978). Gottlieb (1972) reported that flavonoids in Lauraceae were represented by simple derivatives like flavonols. Flavonols and leucoanthocyanidins are considered as primitive characters (Harborne, 1967). The reaction with sodium amalgam and magnesium and acid when tested on the materials used for study show the presence of flavones rather than flavonols. Replacement of flavonols by flavones is also an advanced character in the evolutionary history of flavonoids (Crawford, 1978). Such advanced flavonoids are also reported from some other members of Lauraceae such as *Lindera* (Gottlieb, 1972).

A qualitative study of flavonoids itself thus seems to be of much use in the taxonomic study of the genus *Cinnamomum.* The simple techinque of paper chromatography is useful in the study of *Cinnamomum* populations in the field and it is also helpful to identify types that are close to the cultivated types for breeding purposes.

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REFERENCES

Asen S 1984 High pressure liquid chromatographic analysis of flavonoid chemical markers in petals from *Gerebera* flowers as an adjunct for cultivar and germplasm identification. *Phytochemistry* **23** 2523-2526.

Bohm B A 1987 Ifraspecific flavonoid variation. The *Bot Rev* **53** 197-279.

Bohm B A & Collins, F W 1979 Flavonoids of some species of *Chrysoplenium Bioch. Syst. Ecol.* **7** 195-201.

Engelman L 1981 Cluster analysis of cases pp. 456-463 In BMDP '81 Manual; W J Dixon (ed.) Uni. California Press California.

Crawford D J 1978 Flavonoid chemistry and angiosperm evolution *Bot. Rev.* **44** 431-456.

Crawford D J & Levy M 1978 Flavonoid profile affinities and genetic diversity *Syst. Bot.* 3 369-373.

Crawford D J & Mabry T J 1978 Flavonoid chemistry of *Chenopodium fremontii* : infraspecific variation and systematic implication at the interspecific level. *Bioche. Syst. Ecol.* **6** 189-192.

Fujita Y 1967 Classification and phylogeny of the genus *Cinnamomum* in relation to the constituent essential oils. *Bot. Mag.* Tokyo **80** 261-271.

Gornall R J & Bohm B A 1978 Angiosperm flavonoid evolution-a reappraisal. *Syst. Bot.* **3** 353-368.

Gottlieb O R 1972 Chemosystematics of the Lauraceae. *Phytochemistry* **11** 1537-1570.

Harbone J B 1967 Comparative biochemistry of the flavonoids Academic press London.

Harbone J B 1973 Phytochemical methods Chapman and Hall, London.

King B L 1977 Flavonoid analysis of hybridization in *Rhododendron* section *Pentanthera.* (Ericaceae) Syst Bot **2** 14-27.

King B L 1980 The systematic implication of flavonoids in *Rhododendron* subgen. *Pentanthera.* Pages 163-185 in J L Luteyn and M E O Brien (eds.) Contributions towards classification of *Rhododendrons.* New York Bot. Gar., New York.

Kostermans A J G H 1964 Bibliographia Lauracearum Ministry of National Res. Djakarta.

Kabry T J 1973 The chemistry of disjunct taxa In Chemistry in Bot. Classification (Bends G & Santesson J eds.) Nobel Foundation Stockholm.

Mabry T J Markham K R & Thomas M B 1970 The systematic identification of flavonoids Springer-Verlag Berlin.

Markham K R 1982 Techniques of flavonoid identification Academic Press London.

Tetenyi P 1970 Infraspecific chemical taxa of medicinal plants. Chemical Pub. Co. New York.