Silica gel chromatographic study of phenolic compounds in some cultivated cucurbits

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Phenolic compounds in the leaves of cultivated cucurbits viz. *Trichosanthes dioica* Roxb., *Lagenaria siceraria* (Molina) Standl., *Luffa cylindrica* (L.) Roem., and *Luffa acutangula* (L.) Roxb. were carried out through silica gel chromatographic separation to ascertain their relative phylogenetic position. On phytochemical analysis, paired affinity, group affinity and isolation value supported the inclusion of these species in the same tribe Cucurbiteae on the basis of earlier cytotaxonomic studies. The two species of *Luffa* showed the closest phytochemical affinity and occupied as intermediate position between *Lagenaria* and *Trichosanthes*. *Luffa* was distantly related to other two genera having paired affinity values of less than 50%.

Key words: Silica gel chromatography, separation of phenolic compounds, cultivated cucurbits

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Introduction

The secondary metabolites such as alkaloids, terpenes and phenolics including flavonoids can be employed to study phylogenetic affinitiy in many plant genera. The thin layer chromatography was employed successfully for the separation of phenolic compounds in a number of genera like Secale (Frost 1966, Dedio et al. 1969), Aegilops (Kaltsikes and Dedio 1970), Hordeum and Triticum (Frost and Holm 1973), Cucumerineae (Das et al. 1974) etc. for substantiating earlier conclusions drawn on the status of their taxa on the basis of cytogenetic evidence. Among the earlier reports of chemosystematics in the Cucurbitaceae, Enslin and Rehm (1958) used the distribution of cucurbitacins as an index in the taxonomy of the Cucurbitaceae. On the basis of distribution of phenolics, Das et al. (1974) concluded that Citrullus vulgarishad closer relationship with Lagenaria than Citrullus vulgaris var fistulosus and suggested the possible evolution of Citrullus vulgaris from Lagenaria or vice-versa. They also showed the close relationship between Lagenaria and Luffa.

The present investigation on the distribution of phenolics was carried out in four morphologically related species of cucurbits to examine their relative phyletic distance as evidenced from their biochemical picture.

Materials and methods

Four species of cucurbits viz. *Trichosanthes dioica* Roxb., *Lagenaria siceraria* (Molina) Standl., *Luffa cylindrica* (L.) Roxb. and *Luffa acutangula* (L.) Roxb. were studied in the present investigation. The leaves from the apical portion of all the species of same age were collected for biochemical assay. The leaves were first washed thoroughly in running tap water and dried at 40°C in an oven for 24 hrs. The leaves were crushed and kept in a 50% solution of pertoleum ether (BP 40-60°C) and aqueous methanol for 24 hrs in order to get phenolic extracts. Each extract, on evaporation under vacuum pump, yields a sticky residue.

A chromatographic plate was prepared with silica gel. 0.1 ml aqueous methanolic extract was applied at the starting point of

the plate. It was then dipped in the solvent TCA (toluenechloroform-acetone) and allowed to develop chromatogram. The chromatogram was first treated with ammonia vapour, then with iodine vapour and finally with 1% lead acetate as recommended by Block et al. (1953) to distinguish the spots. Ammonia vapour gave distinct colour under visible and UV light in case of some phenolic spots. The spots of other phenolic compounds became apparent after treatment with iodine vapour and lead acetate. The visible spots were traced on a transparent paper. The RF (relative distance) of each spot was used as a basis for comparison and specification of various phenolic compounds obtained. On the basis of colour and position, spots assumed to be indentical in two or more species were assigned the same number. The chromatographic results were subjected to numerical taxonomic treatment as an aid to establish phenolic relationship in the different species of the family Cucurbitaceae.

Analysis of phytochemical data

The method adopted by Ellison et al. (1962) was followed to make the suitable comparisons in the form of qualitative relationships. Species were compared on the basis of their biochemical affinityies.

Values of paired affinity (PA), group affinity (GA) and isolation value (IV) were calculated as follows:

$$PA = \frac{Spots \ common \ in \ species \ A \ and \ B}{Total \ spots \ in \ A \ and \ B} \quad x \ 100$$

$$GA = Total PA value + 100$$

$$IV = \frac{\text{Number of unique spots in a species}}{\text{Total number of spots in all species}} \times 100$$

Results

The total number of spots obtained in all the species was 20, out of which eight were found in *T. dioica*, nine in *L. siceraria*, eight in *L.* →

RESEARCH PAPERS

cylindrica and nine in *L. acutangula* (Figure 1). From the observation of the composite chromatogram it was distinct that *L. cylindrica* and *L. acutangula* had six spots in common. A relative distribution of all the spots has been shown in Table 1.

The PA value calculated on the basis of presence and absence of the phenolics is shown in **Table 2**. The highest PA value 70.68% was observed between *L. cylindrica* and *L. acutangula*. The lowest PA value (25%) was found between *L. cylindrica* and *L. siceraria*. The PA value between *T. dioica* and *L. siceraria* was 35.29%. These values showed that two species of *Luffa* were closely related but showed a distant relationship with both *T. dioica* and *L. siceraria*. Above observations showed the intermediate position of *Luffa* species between *L. siceraria* and *T. dioica*.

Group affinity values also showed the close relationship between *L. cylindrica* (230.57) and *L. acutangula* (230.31). The *Luffa* species were also related to *L. siceraria* (215.02) on one hand and *T. dioica* (195.58) on the other (**Table 3**).

The isolation value was found to be 20% each in *T. dioica* and *L. siceraria* while for *L. cylindrica* and *L. acutangula* it was just half i.e. 10% **(Table 3)**.

Discussion

Although phenolics are considered to be metabolically inert, they are present in the cell wall of plants in considerable amounts and are stable and characteristic end products (Bate-Smith 1958). In the present investigation a number of phenolic compounds were spotted but they were not identified qualitatively. Chromatographic spots are regarded as excellent markers and are much more important than the chromosome numbers in taxonomy of plants (Grant 1968).

In the present study spot no 4 was present in all the species and appeared to be the characteristic spot for all the 4 species. Spot no 20 was found in three species viz. *L. siceraria, L. cylindrica* and

L. acutangula. Its absence in *T. dioica* indicated that in comparison to *T. dioica, L. siceraria* was closer to the *Luffa* spp.

Higher PA value was considered as an indication of close affinity between different species. PA value of 50% and above was considered as a marker of close relationship. In this regard, the two species of *Luffa* (with PA value of 70.58%) were most closely related and appeared distantly related with *Lagenaria* and *Trichosanthes* conforming the conclusions drawn from cytotaxonomy.

The PA value was supported by the GA value, on the basis of which it could be said that *T. dioica* was distantly related to the other species; *L. cylandrica* and *L. acutangula* were very close and showed some closeness to *Lagenaria siceraria*.

According to Ayyangar (1967), on the basis of a number of criteria like chromosome number, chromosome morphology, meiotic behaviour, secondary association, satellites, nucleoli, chiasma statistics, developmental morphology, amino acid assay, geographical distribution pattern

Colour of the spots: Bl: Blue, Or: Orange, Gr: Green, Vi: Violet, Ye: Yellow Reagents used: a: ammonia, b: iodine, c: 1% lead acetate Concentration of the spots: +++ more intense, ++ less intense, + trace, - absent

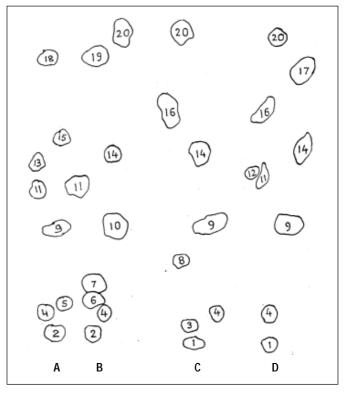


FIGURE 1. A composite chromatogram showing distribution of phenolic compound in

A - Trichosanthes dioica C - Luffa cylindrica B - *Lagenaria siceraria* D - *Luffa acutangula*

TABLE 1. Thin layer chromatographic separation of phenolics in four cucurbits revealing colour of spots, their RF values and concentration

SN	Colour	RF values T/C/A	Tricosanthes dioica	Lagenaria ciceraria	Luffa cylindrica	Luffa acutangula
1	Bl (c)	0.032	-	-	++	++
2	Ye (c)	0.054	+	+	-	-
3	Ye (c)	0.075	-	-	+	-
4	Bl (c)	0.108	+++	+	++	++
5	Vi (b)	0.118	++	-	-	-
6	Gr (c)	0.140	-	+	-	-
7	Or (a)	0.182	-	+	-	-
8	Bl (c)	0.254	-	-	+	-
9	Vi (b)	0.351	+	-	+	+
10	Or (a)	0.356	-	+	-	-
11	Ye (c)	0.464	++	+	-	+
12	Ye (c)	0.491	-	-	-	+
13	Bl (c)	0.497	+	-	-	-
14	Ye (c)	0.545	-	++	+++	++
15	Or (c)	0.556	+	-	-	-
16	Or (a)	0.659	-	-	+	+
17	Vi (b)	0.767	-	-	-	+
18	Ye (c)	0.778	+	-	-	-
19	Or (a)	0.806	-	+	-	-
20	Vi (b)	0.875	-	+	++	++

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in conjuction with conventional morphological characters a system is proposed in which *Trichosanthes*, *Luffa* and *Lagenaria* were placed in the same tribe Cucurbiteae and were closely related, with *Luffa* occupying intermediate position between *Trichosanthes* on one hand and *Lagenaria* on the other. The distribution of phenolic compounds as reveled in the present study also supports the classification and phylogeny suggested by Ayyangar (1967).

It has been mentioned by Griesbach (1972) that the presence and concentration of given substance depend on the physiological growth condition of a plant and on its stage of the development. It was found that the same chromatographic patterns of the flavonoids from the leaves of one and the same plant varied with age and environment (Harborne1967, Armstrong 1968, Parks et al. 1972). Therefore, the most suitable leaves for the study of phenolic compounds were considered the apical leaves obtained from the plants of same age.

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TABLE 2. Paired affinity value (PA) of different species

	Tricosanthes dioica	Lagenaria siceraria	Luffa cylindrica
Lagenaria siceraria	35.29%		
Luffa cylindrica	25%	35.29%	
Luffa acutangula	35.29%	44.44%	70. 58%

TABLE 3. Group affinity, number of unique spots and isolation value of phenolic compounds in cucurbits

Species	GA	No of unique spots	Isolation value (%)
Tricosanthes dioica	195.58	4	20
Lagenaria siceraria	215.02	4	20
Luffa cylindrica	230.57	2	10
Luffa acutangula	250.31	2	10

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