

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 Cucurbitaceae on the basis of earlier cytotoxic studies. The two species of *Luffa* showed the closest phytochemical affinity and occupied an 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 affinity 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), Cucurbitaceae (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 vulgaris* had 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 petroleum 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 (toluene-chloroform-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 identical 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 affinities.

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

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

$$GA = \text{Total PA value} + 100$$

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

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.* ➔

in conjunction with conventional morphological characters a system is proposed in which *Trichosanthes*, *Luffa* and *Lagenaria* were placed in the same tribe Cucurbitaceae 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 revealed 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 (Harborne 1967, 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

| | | | |
|----------------------------|----------------------------|----------------------------|-------------------------|
| | <i>Tricosanthes dioica</i> | <i>Lagenaria siceraria</i> | <i>Luffa cylindrica</i> |
| <i>Lagenaria siceraria</i> | 35.29% | | |
| <i>Luffa cylindrica</i> | 25% | 35.29% | |
| <i>Luffa acutangula</i> | 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 (%) |
|----------------------------|--------|--------------------|---------------------|
| <i>Tricosanthes dioica</i> | 195.58 | 4 | 20 |
| <i>Lagenaria siceraria</i> | 215.02 | 4 | 20 |
| <i>Luffa cylindrica</i> | 230.57 | 2 | 10 |
| <i>Luffa acutangula</i> | 250.31 | 2 | 10 |

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