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Studies in the Identification of *Compositae* Taxa by Paper Chromatography

By

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With 1 Figure

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Introduction

Paper chromatographic techniques have recently been used for identification of various plant taxa (RILEY & BRYANT 1961; ELLISON et al. 1962; TORRES & LEVIN 1964; TURNER & MABRY 1964). However, for *Compositae*, one of the largest dicotyledonous angiosperm taxon, such data is not available. Further, *Compositae* are a well defined and a closely inter-related family in which "the taxa tend to be founded on slighter differences than in other families" (SMALL 1917). Prompted by these, the present chemotaxonomic investigation of *Compositae* of Delhi State (with special reference to the tribe *Heliantheae*) was undertaken to elicit information from biochemical compounds for identification purposes.

Organic compounds like, etheral oil, sesqueterpeulactone, diterpene, sterine, paraffine, saponine, carotinoids, wadelolactone, patulitrin, acetylene-polyene, alkaloids, cyanogen derivatives, aurones, chalcones, flavones, phenolic derivatives, sugars, organic acids, etc., have been reported to occur in *Compositae* taxa (PURI & SESHADRI 1954; BANNERJEE & SESHADRI 1956; GOVINDACHARI et al. 1956; SESHADRI & THAKUR 1960; HEGNAUER 1964). However, none of these compounds have been studied from the taxonomic viewpoint. BATE-SMITH 1962 demonstrated the taxonomic significance of phenolic constituents of plants, and indicated that *Compositae* like *Leguminosae* and unlike other *Campanulatae* have an exceptional number and diversity of phenolic compounds.

In the present investigation, therefore, the usefulness of the phenolics and their varied fluorescence types have been taken as the criterion for identification of 15 *Compositae* taxa.

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Materials and Methods

Aerial vegetative parts of 15 *Compositae* taxa (of tribe *Heliantheae*) occurring in and around Delhi, were collected during June to November 1968 and air-dried. The extraction of the phenolics from the dried plant tissue was done by acidic methanol. Whatman No. 1 chromatographic paper was used for separation and the chromatograms were developed by the descending method with BAW (*n*-Butanol: Glacial Acetic Acid: Water — 3:1:1). The developed chromatograms were scanned under UV with and without ammonia. Spots so developed (in UV, with and without NH_3) were noted and numbered on their fluorescence types (Fig. 1), and their R_f's were calculated. The following abbreviations were used for denoting the colour and intensity of the spots: Br = brown; Bl = blue; Gr = green; V = violet; W = white; Yl = yellow; Fl fluorescent; P = pale; In = intense; Or = orange; R = red.

The arrangement of taxa in the figures and table follows that of "Flora of British India" (HOOKER 1882).

Results

Twenty-seven major spot types could be distinguished in 15 species studied. All taxa investigated showed spot No. 1 which fluoresced as Br under UV and did not change on fuming with NH_3. The R_f of this spot varied from 0,914 to 0,946 in different taxa (Table 1). The profile patterns of the taxa investigated are illustrated in Fig. 1. Based on the profile patterns of individual taxa, the species studied can be grouped in four categories:

Category A (6 to 12 spots in the profile; 2 or more spots showed changes in fluorescence with NH_3): *Helianthus tuberosus*, *Glossocardia linearifolia*, *Bidens bipinnata*, *Bidens* sp., *Hymenatherum tenuifolium*.

Category B (only 3 spots in the profile; no change with NH_3): *Parthenium hysterophorus*, *Xanthium strumarium*, *Verbesina encelioides*, *Bidens biternata*, *Tridax procumbens*.

Category C (4 to 6 spots in the profile; 1 or 2 spots show change in fluorescence with NH_3): *Sclerocarpus africanus*, *Blainvillea rhomboidea*, *Cosmos sulphureus*, *Gaillardia pulchella*.

Category D (a characteristic aggregation of spot numbers 11, 12, 13 and 14): *Eclipta erecta*.

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Category A

Helianthus tuberosus (9 spots), *Glossocardia linearifolia* (9 spots), *Bidens bipinnata* (9 spots), *Bidens* sp. (12 spots), and *Hymenatherum tenuifolium* (6 spots) showed similarity in the distributional pattern of the spots (Fig. 1). Each taxon, however, could be identified by changes observed after exposing the chromatograms to fumes of NH_3. In *Helianthus tuberosus*

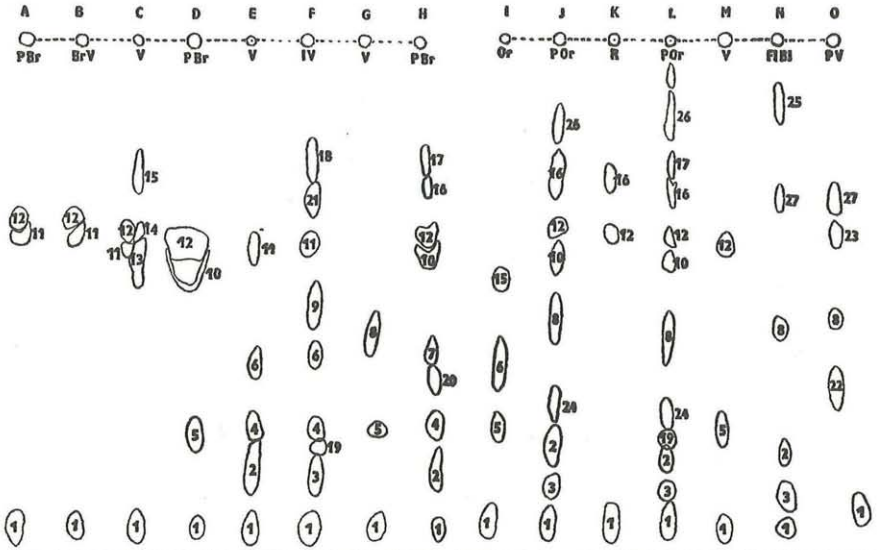

Fig. 1. Uni-directional chromatographic patterns (with BAW) of aerial vegetative part extracts from 15 *Compositae* taxa. A = *Parthenium hysterophorus*, B = *Xanthium strumarium*, C = *Eclipta erecta*, D = *Sclerocarpus africanus*. E = *Blainvillea rhomboidea*, F = *Helianthus tuberosus*, G = *Verbesina encelioides*, H = *Glossocardia linearifolia*, I = *Cosmos sulphureus*, J = *Bidens bipinnata*, K = *Bidens biternata*, L = *Bidens* sp., M = *Tridax procumbens*, N = *Hymenatherum tenuifolium*, O = *Gaillardia pulchella*. (Bl = blue; Br = brown; Fl = fluorescent; I = intense; Or = orange; P = pale; R = red; V = violet).

and *Glossocardia linearifolia*, spot No. 4 was common and fluoresced as FlBl under UV and became InFlBl with NH_3 fumes. In addition to spot No. 4 the profile of *H. tuberosus* had spot Nos. 19 (R_f 0.788) and 21 (R_f 0.320) which developed Yl fluorescence on exposure to NH_3 fumes. *Glossocardia linearifolia* did not show spot Nos. 19 and 21 and was thus separated from *H. tuberosus*. Spot No. 4 did not occur in any other taxon of this category. The two species of *Bidens* (*B. bipinnata* and *Bidens* sp.) and *Hymenatherum tenuifolium* possessed spot Nos. 2 and 3. However, spot No. 2 fluorescing as

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Table 1

R_f values and colours of spots developed in the chromatographic profile pattern UV: Bl = blue, Br = brown, Gr = green, V = violet, W = white, Yl = yellow, NH_3 are indicated in brackets.

Spot Nos.	Spot Types Fluorescence under UV	Taxa A	B	C	D	E	F
1	Br	0.945	0.934	0.941	0.941	0.945	0.945
2	FlGrBl					0.829 (InFlGrBl)	
3	FlBl						0.853
4	FlBl					0.748 (InFlBl)	0.755 (InFlBl)
5	PV				0.764		
6	FlPBlYl					0.622	0.611
7	FlGrYl						
8	PV						
9	PV						0.513
10	FlW				0.480 (InFlW)		
11	VYl	0.382	0.377	0.408		0.403	0.393
12	InV	0.349	0.342	0.373	0.397		
13	InV			0.443 (InFlBl)			
14	FlBlV			0.375			
15	FlV			0.257			
16	Yl						
17	PBr						
18	BrYl						0.233
19	—						0.788 (Yl)
20	—						
21	—						0.320 (Yl)
22	—						
23	—						
24	PBl						
25	InBr						
26	PYl						
27	PBrV						

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Table 1

of 15 Compositae taxa A—O (see explanation to Fig. 1). Fluorescence under Fl = fluorescent, P = pale, In = intense. — Colours of spots developed with

Taxa								
G	H	I	J	K	L	M	N	O
0.941	0.938	0.931	0.934	0.936	0.930	0.946	0.946	0.914
	0.829		0.787		0.810		0.80	
			(GrYl)		(GrYl)		(InFlGrBl)	
			0.861		0.872		0.893	
			(Yl)		(GrBl)		(GrBl)	
	0.740							
	(InFlBl)							
0.753		0.746				0.776		
		0.627						
		(InFlBlYl)						
	0.593							
0.567			0.554		0.578		0.563	0.543
	0.425		0.425		0.430			
	(InFlW)							
	0.373		0.363	0.376	0.383	0.404		
		0.253						
	0.283		0.261	0.266	0.30			
			(YlV)					
	0.229				0.263			
					0.774			
					(Yl)			
	0.655							
	(Yl)							
								0.670
								(BlGr)
								0.383
								(GrYl)
			0.702		0.723			
					(GrBl)			
							0.127	
			0.165		0.149			
							0.308	0.308

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FlGrBl became GrBl with NH_3 in the two species of *Bidens*, while InFlGrBl in *Hymenatherum tenuifolium*. The two species of *Bidens* (*B. bipinnata* and *Bidens* sp.) were separated from each other by spot No. 3 which being common to both showed variations in fluorescence with NH_3; in *B. bipinnata*, spot No. 3 changed from FlBl to Yl, whereas in *Bidens* sp., spot No. 3 changed from FlBl to GrBl. In addition, *Bidens* sp. showed spot No. 19 (R_f 0.774) which developed Yl fluorescence with NH_3, and spot No. 24 (R_f 0.723) which changed from PBl to GrBl with NH_3.

Category B

Parthenium hysterophorus, *Xanthium strumarium*, *Verbesina encelioides*, *Bidens biternata*, and *Tridax procumbens* possessed 3 spots each. None of the spots showed any change on fuming with NH_3. Individual taxa in this category could be delineated on their spot types, R_f values of the spots, and on the fluorescences of the starting point on the chromatogram. Thus, *Parthenium hysterophorus* and *Xanthium strumarium* which showed identical spot types (spot Nos. 1, 11, and 12) could be separated from each other on the R_f values (0.945, 0.934; 0.382, 0.377; 0.349, 0.342 respectively) of the spots, and on the fluorescence of the starting point on the chromatogram. In *Parthenium hysterophorus*, the starting point fluoresced as PBr, while in *Xanthium strumarium* it was BrV. The above mentioned characteristic spots (Nos. 1, 11, and 12) of *Parthenium* and *Xanthium* were not found in the remaining taxa of this category. The other taxa could be identified by their spot types alone: *Verbesina encelioides*: Spot Nos. 1, 5, and 8; *Bidens biternata*: Spot Nos. 1, 12, and 16; *Tridax procumbens*: Spot Nos. 1, 5, and 12.

Category C

Sclerocarpus africanus (4 spots), *Blainvillea rhomboidea* (6 spots), *Cosmos sulphureus* (4 spots), and *Gaillardia pulchella* (5 spots) showed similar distributional pattern of spots. Of these, *Sclerocarpus africanus* possessed spot No. 10 (R_f 0.480) which fluoresced as W under UV and as InW with NH_3. This spot was absent in the other three taxa of this category. Spot No. 15 fluorescing as FlV was present in *Cosmos sulphureus* and *Blainvillea rhomboidea* but, whereas the former had 4 spots the latter had 6, and thus could be distinguished from one another. *Gaillardia pulchella* could be identified from other taxa of this category by spot Nos. 22 (R 0.670), 23 (R_f 0-383), and 27 (R_f 0.308). These spots were absent in other taxa.

Category D

Eclipta erecta was identified by its profile which contained an aggregation of four spots, Nos. 11, 12, 13, and 14 (besides spot Nos. 1 and 15), which was not found in any other taxon investigated.

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Conclusions

Spot types and profile patterns in 15 species investigated showed marked variations. Also, each taxon had a characteristic profile pattern suggesting the reliability of the utility of biochemical compounds for taxonomic purposes. This supports the views of FAIRBROTHERS 1968, HARBOURNE 1967, and MIROV 1967 about the possibility of employing chemical constituents in helping to characterize, describe, and classify taxa. Further, the importance of present investigation lies in the fact that identification of the taxa could be made by studying the biochemical compounds present in the vegetative parts rather than the reproductive parts of the plants (PURI & SESHADRI 1954; BANNERJEE & SESHADRI 1956; GOVINDACHARI et al. 1956; SESHADRI & THAKUR 1960). It was possible to identify not only different genera, but also different species of the same genus. Thus by this method three species of *Bidens* investigated could be distinguished easily by their profile pattern. The data presented above suggest the need for further biochemical investigations of *Compositae* taxa.

Summary

Fifteen *Compositae* taxa (belonging to tribe *Heliantheae*) were identified through paper chromatography of biochemical compounds extracted from vegetative parts. On the basis of profile pattern developed on the chromatograms, the taxa studied were grouped in four categories. Further identification of taxa within each category was possible through R_f values and fluorescence of spots.

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