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Numerical chemotaxonomy of *Bauhinia*

G NAGESHWAR, M RADHAKRISHNAIAH and L L NARAYANA*

Department of Botany, Nizam College, Osmania University, Hyderabad 500 001, India
* Department of Botany, Kakatiya University, Vidyaranyapuri, Warangal 506 009, India

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Abstract. The chemotaxonomy of twelve species of *Bauhinia* is presented. From the quantified data on the distribution pattern of different chemical constituents, including the free aminoacids, it is inferred that the taxa are closely related and do not warrant splitting of the genus. However, the division of the genus based on certain selected phenolic constituents does not conform to the one on morphological grounds.

Keywords. *Bauhinia*; secondary metabolites; free amino acids; affinity; splitting.

1. Introduction

The chemotaxonomy of *Bauhinia* received very little attention in the multi-voluminous work on the chemotaxonomy of flowering plants in general (Gibbs 1974) and of the leguminous taxa (Harborne *et al* 1971) in particular. The present account deals with the distribution pattern of different secondary metabolites and free amino acids and estimates the extent of kinship among them and the desirability of splitting the genus.

2. Material and methods

The twelve species of *Bauhinia* presented in table 1 are either procured from the Indian Botanic Garden, Howrah or from various localities in Hyderabad. To detect different secondary metabolites, fresh leaves and stems and 80 % ethanolic extracts of the shade-dried material were used. Following standard procedures/specific tests (Gibbs 1974), the material was screened for alkaloids, anthraquinones, aucubin compounds (Ehrlich test), aurones (Aurone test A), catechol-tannins (HCl/methanol or Isenberg Buchnann's test), cyanogenic glycosides (HCN test A), ellagic acid, flavonoid pigments (Shinoda test), indoles, Juglone (Juglone test A), leucoanthocyanins (leucoanthocyanin test A), lignans, methylene dioxy compounds (Labat test), phenols, saponins, syringin (Syringin test), syringyl radicals (Maule test), free tannins (Tannin test A) and triterpenoids/steroids (Liebermann-Burchard and Salkowski tests). The activity of the enzyme polyphenolase is measured in terms of its intensity by cigarette and hot water tests (Gibbs 1974).

To detect free amino acids, an equally concentrated alcoholic extract of the mature leaves was treated with chloroform (1 : 3 ratio) in a separating funnel and left overnight. The supernatant liquid was used for spotting the chromatograms by unidirectional ascending paper chromatography on Whatman No. 1 paper in butanol: acetic acid: water (4 : 1 : 1 v/v) solvent system. The dried chromatograms were sprayed with 0·2 % ninhydrin in acetone and the amino acids were identified by comparing the Rf values and spot colours, in visible light with those of the standards, chromato-

Table 1. List of taxa studied.

	Name of the taxon	Place of collection	Native distribution
1	*B. scandens* L var *horsfieldii* (Miq.) Ohashi (= *B. anguina* Roxb)	Indian Botanic Garden, Howrah	Asia
2	*B. corymbosa* Roxb ex DC	-do-	China
3	*B. diphylla* Buch Ham	-do-	India
4	*B. galpinii* N E Brown	-do-	S. Africa
5	*B. hookeri* F Muell	-do-	Australia
6	*B. petersiana* Bolle	-do-	Africa
7	*B. ferruginae* Roxb	-do-	Asia
8	*B. retusa* Buch Ham ex Roxb (= *B. roxburghiana* Voigt)	-do-	India
9	*B. rufescens* Lam	-do-	W. Africa
10	*B. tomentosa* L	Chikkadpally, Hyderabad	Africo-Asia
11	*B. vahlii* Wight & Arn	Indian Botanic Garden, Howrah	India
12	*B. variegata* L var *alboflava* De Wit	Public Garden, Hyderabad	Asia
13	*B. variegata* L var *variegata* De Wit	Osmania Univ. Campus, Hyderabad	Asia

graphed under identical laboratory conditions. The unidentified aminoacids were designated by their hRf (100 × Rf) values.

3. Observations

3.1 *Secondary metabolites*

The distribution of various secondary metabolites presented in table 2 shows uniform absence of alkaloids, aucubin compounds, cyanogenic glycosides, lignans, syringin and tannins and uniform occurrence of similar broad flavonoid patterns, phenols, syringyl radicals and triterpenoids. However, there is restricted distribution of certain other chemical compounds in the taxa studied. Thus the anthraquinones are present in *B. galpinii*, *B. hookeri* and *B. scandens* var *horsfieldii*; aurones in *B. tomentosa*; catechol-tannins in *B. diphylla*, *B. galpinii*, *B. hookeri*, *B. scandens* var *horsfieldii*, *B. tomentosa*, *B. vahlii* and *B. variegata* var *variegata* as in *B. divaricata* (Gibbs 1974); ellagic acid in *B. corymbosa*, *B. diphylla*, *B. galpinii*, *B. hookeri*, *B. retusa*, *B. scandens* var *horsfieldii*, *B. tomentosa*, *B. vahlii* and *B. variegata* var *alboflava*; indoles in stems of both the varieties of *B. variegata* and leaves of *B. variegata* var *alboflava*; juglone in *B. ferruginae*, *B. petersiana* and *B. rufescens* (Gibbs 1974 also reported the absence of juglone in *B. galpinii*), leucoanthocyanins in all the taxa except *B. tomentosa*; methylene dioxy compounds in *B. ferruginae*, *B. galpinii*, *B. petersiana* and *B. tomentosa*; saponins in *B. corymbosa* and steroids in all the taxa except *B. diphylla*, *B. petersiana* and *B. tomentosa*. The enzyme polyphenolase is negative in *B. galpinii*, *B. hookeri* and *B. vahlii* and in other members it is either immediately or slowly positive.

Table 2. Distribution of secondary metabolites.

Chemical constituent	Name of the taxon*												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Alkaloids	–	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	+	–	–	+	+	–	–	–	–	–	–	–	–
Aucubin compounds	–	–	–	–	–	–	–	–	–	–	–	–	–
Aurones	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA
Catechol-tannins	+	–	+	+	+	–	–	–	–	+	+	–	+
Cyanogenic glycosides	–	–	–	–	–	–	–	–	–	–	–	–	–
Ellagic acid	+	+	+	+	+	–	–	+	–	+	+	+	–
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+
Indoles	–	–	–	–	–	–	–	–	–	–	–	+	+
Juglone	–	–	–	–	–	+	+	–	+	–	–	–	–
Leucoanthocyanins	+	+	+	+	+	+	+	+	+	–	+	+	+
Lignans	–	–	–	–	–	–	–	–	–	–	–	–	–
Methylene dioxy compounds	–	–	–	+	–	+	+	–	–	+	–	–	–
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	–	+	–	–	–	–	–	–	–	–	–	–	–
Steroids	+	+	–	+	+	–	+	+	+	–	+	+	+
Syringin	–	–	–	–	–	–	–	–	–	–	–	–	–
Syringyl radicals	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	–	–	–	–	–	–	–	–	–	–	–	–	–
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+
Activity of Polyphenolase	+	+	+	–	–	+	+	+	+	+	–	+	+

* same as in table 1; + = positive; – = negative; NA = not applicable

3.2 *Free aminoacids*

The distribution pattern of the free amino acids (protein and nonprotein) is presented in table 3. Out of a total of 23 amino acids in the free pool 18 could be identified as protein amino acids and the remaining 5, presumably the nonprotein ones, are designated by their hRf values. Of the several identified protein amino acids in the free pool, threonine seems to be more common. Its presence is noticed in all the taxa except *B. vahlii*. Tyrosin occupies the second position in distribution. It is present in all the taxa except *B. corymbosa*, *B. diphylla*, *B. ferruginae* and *B. galpinii*. Methionine and glutamine are spotted in 8 out of the 13 taxa studied. The rest of the amino acids have restricted distribution in the free pool. Thus, alanine could be seen in *B. diphylla*, *B. ferruginae*, *B. hookeri*, *B. petersiana*, *B. retusa* and *B. scandens* var *horsfieldii*; aspartic acid in both the varieties of *B. variegata*; glutamic acid in *B. ferruginae*, *B. retusa*, *B. rufescens* and *B. variegata* var *variegata*; glycine in *B. rufescens* and *B. scandens* var *horsfieldii*; histidine in *B. galpinii* and *B. variegata* var *alboflava*; leucine in *B. galpinii*, *B. petersiana*, *B. rufescens*, *B. tomentosa* and *B. variegata* var *variegata*; lysine in *B. diphylla*, *B. galpinii* and *B. retusa*; phenyl alanine in *B. diphylla*, *B. retusa*, *B. variegata* var *variegata*; serine in *B. corymbosa*, *B. tomentosa* and *B. vahlii*; tryptophan in *B. galpinii* and *B. hookeri* and valine in *B. diphylla*, *B. hookeri* and *B. scandens* var *horsfieldii*. Isoleucine and norleucine are present in *B. ferruginae* and *B. galpinii* respectively.

From among the nonprotein aminoacids γ-amino butyric acid is present in all the taxa except *B. hookeri* and *B. scandens* var *horsfieldii*; hRf 20 is observed in all the

Table 3. Distribution of amino acids.

	Name of the taxon*												
Aminoacid	1	2	3	4	5	6	7	8	9	10	11	12	13
Alanine	+		+		+	+	+	+					
γ-amino butyric acid		+	+	+		+	+	+	+	+	+	+	+
Aspartic acid												+	+
Glutamic acid							+	+	+				+
Glutamine	+	+	+		+	+	+				+		+
Glycine	+								+				
Histidine				+								+	
Leucine				+		+			+	+			+
Isoleucine							+						
Norleucine				+									
Lysine			+	+				+					
Methionine	+	+	+	+	+				+			+	+
Phenylalanine			+					+					+
Serine		+								+	+		
Threonine	+	+	+	+	+	+	+	+	+	+		+	+
Tryptophan				+	+								
Tyrosine	+				+	+		+	+	+	+	+	+
Valine	+		+		+							+	
hRf 20				+	+		+	+	+		+	+	
hRf 26						+					+		
hRf 28									+			+	+
hRf 30	+	+	+					+		+			
hRf 50					+		+						

* same as in table 1.

members except *B. corymbosa*, *B. diphylla*, *B. petersiana*, *B. scandens* var *horsfieldii*, *B. tomentosa* and *B. variegata* var *variegata*. The other unidentified amino acids such as hRf 26 (in *B. petersiana* and *B. vahlii*) hRf 28 (in *B. rufescens* and both the varieties of *B. variegata*) hRf 30 (in *B. corymbosa*, *B. diphylla*, *B. retusa*, *B. scandens* var *horsfieldii* and *B. tomentosa*) and hRf 50 (in *B. ferruginae* and *B. hookeri*) are limited in distribution.

4. Discussion

The chemical constituents studied (except the free-protein amino acids) are the terminal products of biosynthetic pathways. They accumulate in the plant tissues and thus the secondary plant product profile remains relatively stable under various environmental conditions (Flück 1963).

A perusal of the distribution of different chemical constituents indicates that they are in concurrence with those of the earlier workers (Harborne *et al* 1971; Gibbs 1974) on a few species of *Bauhinia*. The distribution of the chemical constituents is suggestive of a fair degree of kinship among the taxa studied and the same may be expressed in the form of synthetic numerical indices (Ellison *et al* 1962; Alston and Turner 1963). There is uniformity in the occurrence of similar broad flavonoid pigments, phenols, syringyl

radicals and triterpenoids and the absence of a few other chemical constituents as stated in the preceding paragraphs. The uniform presence or absence of a particular compound, of course, has little taxonomic significance. Further the similarities in the negative characters are to be ignored (Runemark 1968). Thus the paired affinity indices are calculated on the basis of distribution of the remaining chemical compounds, closely following Ellison *et al* (1962) and employing the positive matches and the differences (table 4). They are further depicted in the polygonal graphs (Hutchinson 1936) in figures 1–13 to indicate the degree of affinity at a glance. The summation of paired affinity indices known as group affinity index (GAI) (Ellison *et al* 1962) expresses the affinity of one taxon with all others. Thus GAI should be 1300 in the present context if there is 100 % affinity and 100 if it is least. The GAI (table 4) which range from 671–948 also speak of close chemical ties among the taxa studied.

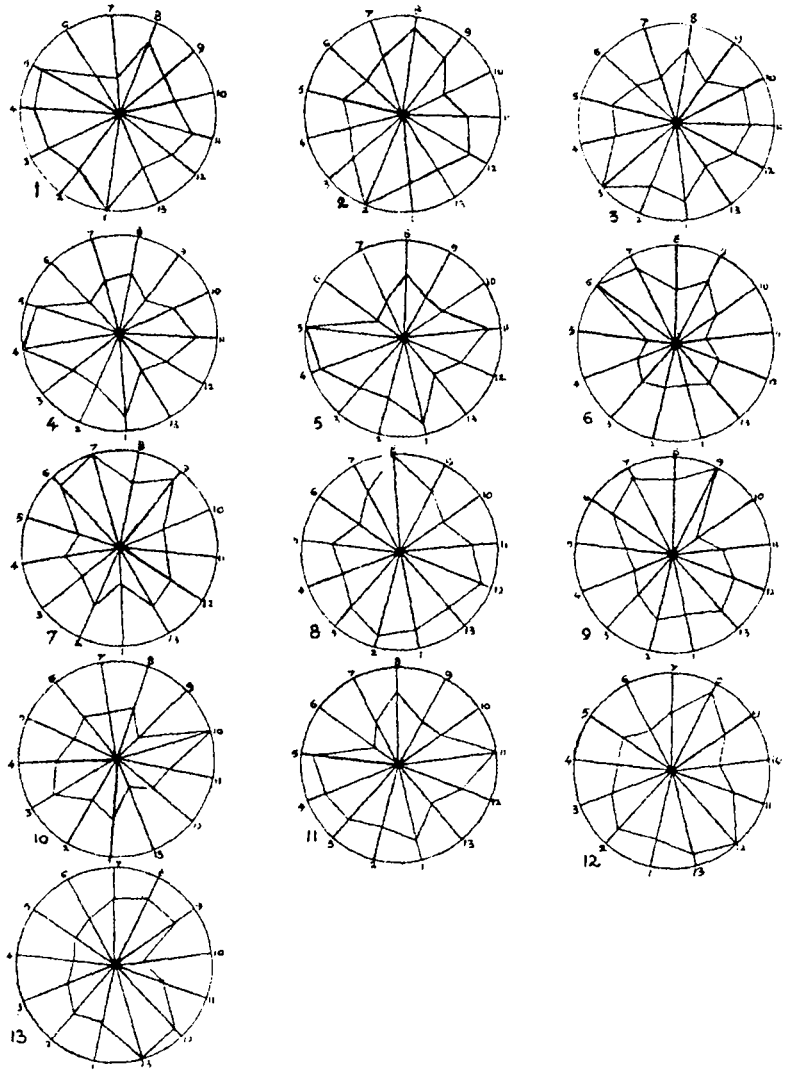
Bate-Smith (1962) distinguished the species and the families of dicotyledons into four classes on the basis of presence or absence of leucoanthocyanins (a, a_0) and trihydroxy constituent, the ellagic acid (b, b_0) and found the trend of evolution to be in the direction of $ab \rightarrow a_0b_0$ with transitional forms a_0b and ab_0. Viewed from this angle a majority of the species of *Bauhinia* (present study) are to be regarded as primitive (ab class), *B. yunnanensis* (Bate-Smith 1962) as highly advanced (a_0b_0 class) and *B. tomentosa* (a_0b class), *B. ferruginae*, *B. petersiana*, *B. rufescens* and *B. variegata* var *variegata* (ab_0 class) (present study) forming the evolutionary bridge.

The protein amino acids are of ubiquitous occurrence, but they exhibit considerable quantitative variation depending upon the metabolic threshold of the tissue and environmental conditions. Hence their distribution pattern in the free pool, has little taxonomic significance. The nonprotein amino acids, on the other hand, are not universally present and their incidence has relevance in taxonomic considerations at all levels of hierarchy up to the family (Gershenzon and Mabry 1983). The species of *Bauhinia* have been assigned to four different genera *viz Lasiobema* (Korth) Miq, *Phanera* Lour, *Piliostigma* Hochst and *Bauhinia* L (*s. s.*). The commonness to a large

Table 4. Paired and group affinity values.

Name of the taxon*	Name of the taxon*													Group affinity
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1	100	72	80	83	90	40	36	80	60	60	80	72	60	913
2		100	66	54	60	44	60	88	66	44	66	80	66	866
3			100	60	66	50	44	75	50	75	75	66	50	857
4				100	90	40	54	60	40	60	80	54	40	815
5					100	22	40	66	44	44	88	60	44	814
6						100	88	50	75	50	25	44	50	678
7							100	66	88	44	44	60	66	790
8								100	75	50	75	88	75	948
9									100	25	50	66	75	814
10										100	50	44	25	671
11											100	66	50	853
12												100	88	888
13													100	789

* same as in table 1



Figures 1–13. Polygonal representation of the paired affinity values of the 13 taxa (as mentioned in table 4) to all others. Affinity values are expressed along the radii from 0–100 % beginning at the centre.

extent in the distribution of nonprotein amino acids, besides several similarities in chemical characters does not seem to warrant the splitting of the genus as has been done on morphological grounds. However, the division of *Bauhinia* based on the distribution of leucoanthocyanins and trihydroxyacid, as stated in the preceding paragraph does not conform to the one on traditional grounds. Hence it is suggested that *Bauhinia* be treated in a comprehensive sense. Further a perusal of the hRf values indicates that they are on an increase in the native species of those from Africa to Asia and then to Australia, with exception of the Malayan taxon *B. ferruginae*, which strikes a mild discordant note among the Asian species.

The species now studied could be identified on the basis of some of the chemical characters.

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References

Alston R E and Turner B L 1963 *Biochemical systematics* (New Jersey: Prentice Hall)

Bate-Smith E C 1962 The phenolic constituents of plants and their taxonomic significance; *J. Linn. Soc. (Bot)* **58** 95–174

Ellison W L, Alston R E and Turner B L 1962 Methods of presentation of crude biochemical data for systematic purposes with special reference to the genus *Bahia* (Compositae); *Am. J. Bot.* **49** 599–604

Flück H 1963 Intrinsic and extrinsic factors affecting the production of secondary plant products in: *Chemical plant taxonomy*, (ed.) T Swain (London: Academic Press), 167–186

Gershenzon J and Mabry T J 1983 Secondary metabolites and the higher classification of angiosperms; *Nordic. J. Bot.* **3** 5–34

Gibbs R D 1974 *Chemotaxonomy of flowering plants* III (Montreal and London: McGill Queen's Univ. Press)

Harborne J B, Boulter D and Turner B L (eds) 1971 *Chemotaxonomy of Leguminosae* (New York and London: Academic Press)

Hutchinson A H 1936 Polygonal representation of polyphase phenomena; *Trans. R. Soc. Can.* **3** 19–26

Runemark H 1968 Critical comments on the use of statistical methods in chemotaxonomy; *Bot. Not.* **121** 29–43