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A STUDY OF FOLIAR FLAVONOIDS OF SIX SPECIES OF *BAUHINIA*

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The chromatograms of foliar flavonoids of the six species of *Bauhinia* investigated here revealed 45 spots. Of these 22 spots could be identified which represent 7 flavonoids. Two of the 7 identified flavonoids viz. Quercetin, Kaempferol were common to all the six species. While Apigenin was present in three species viz. *B. blakeana, B. purpurea* and *B. variegata,* Myrecitin was present only in *B. racemosa* where as Isorhamnetin was present in two species viz. *B. blakeana* and *B. purpurea*. Luteolin present only in *B. tomentosa Bauhinia* species can be distinguished by the presence of derivatives of Quercitin viz. Qurecitin-3-glucoside (Isoquercitrin) was present in *B. tomentosa*, Qurecitin-3-rutinoside (Rutin) was present in three species viz. *B. tomentosa, B. variegata,* and *B. purpurea*. Qurecitin-3-rhamnoside was present in *B. purpurea, B. blakeana* and *B. variegata* while Qurecitin-3-arbinoside was specific to *B. racemosa*.

Key words: *Bauhinia Linn.*, Flavonoids, six solvent systems, two-dimensional chromatography.

Analysis of Flavonoids of plants used in food and feed is important because some of these secondary metabolites have nutritional relevance. They constitute an important group of chemotaxonomic parameters in view of their relatively simple extraction and identification, physiological stability and reutilization (Alston *et al.* 1962). They are very stable substance compared to other secondary metabolites and can be detected in herbarium specimens as well (Harborne 1973). Study of flavonoids have attracted the attention of Plant Scientists due to their intense colours and the development of new sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems. The family Caesalpiniaceae is extremely rich in flavonoids. Kaempferol and Quercetin have been reported from flavonol group while Apigenin and Luteolin from flavone group in the members of this family (Harborne 1967). Flavonoids are known antioxidants, of which Quercetin is a potent antioxidant because of its right structural features and free radical scavenging activity.

Bauhinia Linn. is a genus with more than 300 species belonging to the family Caesalpiniaceae. It is the largest genus in the legume tribe Cercideae and subtribe Bauhiniiae (Kajita *et al.* 2001) being distributed throughout the tropical region (Wunderlin 1987). Most of the *Bauhinia* species have great medicinal value (L. D. Kapoor 2005). In the present study, flavonoid patterns of six species have been studied using two dimentional paper chromatography with six solvent systems. Their inter-specific relationship have been discussed.

MATERIALS AND METHODS:

Six species of *Bauhinia* viz. *B. acuminata* Linn, *B. blakeana* Dunn. *B. purpurea* Linn, *B. racemosa* Lam., *B. tomentosa* Linn. *and B. variegata* Linn. were identified from different localities of Patna. 5 grams of mature and healthy leaves were

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Table 1 : Chromatographic and spectral properties of Flavonoid spots of *Bauhinia* species

Spot	Fluorescence			Solvent System : Rf x 100					Forestal	TLC (Silica gel)	Spectral Max. in EtOH	Identity
	Visible Light	UV	$UV+NH_3$	BAW	15% AcOH	30% AcOH	Water	Phenol				
1	Bl	Bl	Bl	38	36	--	--	--	6			?
2	Bl	Bl	Bl	76	51	--	16	34	--		249, 376	Q-3-Rhamnoside
3	Nc	Bl	Yl	66	41	--	17	27	39		251, 373	Quercetin
4	Bl	Bl	Yl	41	19	49	--	--	--			?
5	Bl	Bl	YG	7	49	--	--	--	--			?
6	Bl	Bl	YG	82	37	--	--	58	57		253, 371	Kaempferol
7	Bl	Bl	Yl	15	23	--	31	--	29			?
8	Bl	Bl	Yl	18	6	--	--	--	--			?
9	Bl	Bl	Yl	79	--	13	--	--	27			?
10	Bl	Bl	Yl	49	56	28	--	39	64	10	251, 372	Q-3-rutinoside
11	Bl	Bl	GY	68	10	--	--	26	43		252, 371	Quercetin
12	Yl	GY	GY	74	60	--	13	--	28		224	Glucose ?
13	Yl	GY	Bl	73	--	--	--	69	64		249, 358	Luteolin
14	Yl	GY	GY	71	--	16	--	27	35		218	Glucose ?
15	Yl	Yl	Yl	32	--	--	23	--	--			?
16	Yl	Yl	Yl	59	33	--	11	42	38	33	252, 368	Q-3-glucoside
17	Yl	Yl	Yl	71	44	--	17	--	39	23	249, 368	Q-3-Rhamnoside
18	Yl	Yl	Bl	16	24	--	--	51	29			?
19	Yl	Yl	BY	88	64	--	23	42	49		258, 363	Kaempferol
20	Yl	Yl	Yl	1	29	--	--	33	26			?
21	Yl	Yl	Bl	72	--	36	8	--	29			?
22	Yl	Yl	Bl	82	65	12	31	49	61		259, 364	Kaempferol
23	Yl	Yl	Bl	48	57	29	--	41	63	31	254, 373	Q-3-glucoside
24	Yl	BY	RB	85	67	17	3	53	57		266, 378	Kaempferol
25	BY	BY	BY	84	--	--	51	--	--		--	?
26	Yl	BY	BY	45	49	7	24	27			250, 371	Myricetin
27	GY	BY	BY	48	59	--	13	17	23		257, 372	Q-3-rutinoside

28	Yl	BY	BY	72	64	--	--	39	--		--	?
29	Bl	BY	BY	73	43	8	--	54	59		259, 362	Kaempferol
30	GY	BY	BY	77	47	61	--	69	56		255, 367	Isorhamnetin
31	RB	B	B	36	31	59	--	--	28		--	?
32	Nc	B	B	19	7	--	--	--	--		--	?
33	B	B	B	89	33	--	--	92	84		248,347	Apigenin
34	GY	B	B	86	--	--	28	66	44		--	?
35	RB	B	B	74	47	37	20	31	40	24	253, 369	Q-3-Rhamnoside
36	GY	B	B	93	--	--	40	89			258, 344	Apigenin
37	RB	B	B	56	--	--	18	81	83		272, 343	Isovitexin
38	RB	R	R	30	--	24	--	--	58		220	Glucose ?
39	BY	R	R	7	59	--	--	--	--			?
40	RB	R	R	36	19	47	--	31	29		--	?
41	GY	RB	RB	79	45	67	--	71	57		253, 368	Isorhamnetin
42	Nc	RB	BY	33	21	69	--	25	33		--	?
43	Yl	RB	R	73	35	--	9	--	21	27	252, 368	Q-3-Arbinoside
44	Nc	RB	RB	29	16	--	--	6	--		--	?
45	RB	RB	B	33	--	21	--	--	56		218	Glucose ?

Abbreviation : **Nc =** Indicates no colour in visible light; (--) = Rf values variable, so not included; (?) = Uncertain chemical identity; **BAW =** n– butanol, acetic-acid, water; **AcOH =** aqueous acetic acid; **EtOH=** Ethanol; **TLC**= Thin layer chromatography; **BI** = Bright yellow; **YI** = Yellow; **Gy** = Greenish yellow; **RB** = Reddish brown; **R** = Reddish; **B** = Brown; **By** = Blackish yellow, **Q** = Quercetion.

collected from each species. After air drying, the phenolic compounds were extracted from these leaves at room temperature in different solvents like 70% Ethanol, Petroleum Ether, Acetone and Methanol using standard procedures. Isolation and purification of the compounds were done by repeated chromatography using Whatman 3mm chromatography paper. Characterisation of compound were carried out following standard technique (Markham, 1982). The purified compounds were taken in Ethanol and their UV and visible light spectrum were measured with Spectrophotometer. Further, to know the position of substitution, spectral shifts after the addition of standard diagnostic chemicals were determined.

RESULT AND DISCUSSION:

The chromatograms of flavonoids of the six species of *Bauhinia* investigated here revealed 45 spots. Of these 22 spots could be identified (Table 1). Spot no. 10 was shared by *B. purpurea* x *B. variegata*; Spot no. 11 was shared by *B. blakeana* x *B. purpurea;* Spot no. 19 was shared by *B. blakeana* x *B. variegata* while Spot no. 36 was shared by *B. blakeana* x *B. purpurea.* No other spot was common between any other species under consideration and this feature was useful for delimiting the

Table 2: Characteristic chromatographic spot types of *Bauhinia* species

Species	Spot types
B. acuminata Linn.	4, 6, 14, 18, 23, 37
B. blakeana Dunn.	2, 9, 11, 19, 20, 30, 36, 39, 42, 45
B. purpurea Linn.	5, 10, 11, 12, 17, 22, 32, 36, 41
B. racemosa Lam.	7, 24, 26, 27, 31, 38, 43, 44
B. tomentosa Linn.	1, 13, 15, 16, 21, 25, 29, 34
B. variegat Linn.	3, 8, 10, 19, 28, 33, 35, 40

species (Table 2). The identified spots are four common flavonols viz. Kaempferol, Quercetin, Myrecetin and Isorhamnetin (Spots 2, 3, 6, 10, 11, 16, 17, 19, 22, 23, 24, 26, 27, 29, 30, 35, 41, 43), two flavones viz. Apigenin and Luteolin (Spots 13, 33, 36, 37) and 23 spots were unknown.

Because of uncommon nature of flavonoid spots of six species, the number of spots types on chromatograms of individual species varied from six (*B. acuminata*), eight (*B. racemosa, B. tomentosa* and *B. variegata*), nine (*B. purpurea*) and ten (*B. blakeana*) (Table 3).

The interesting feature of this work was the apparent specialization production of flavonols by the leaves of *Bauhinia* species and this observation recalled the relationship between relative chemical complexity and relative evolutionary advancement (Mabry, 1973a; 1973b). Kaempferol and Quercetin were common in all the six species. Myrecitin was specific to *B. racemosa* while Isorhamnetin to *B. purpurea and B. blakeana. B. variegata* did not exhibit the presence of any specific compound. The presence of Myricetin in a species of *Bauhinia* could be regarded as a plesiomorphic feature, since the presence of this flavonol has been correlated with primitiviness and woodiness. (Harborne, 1977). Luteolin was present only in *B. tomentosa* where as Apigenin in three species viz. *B. blakeana, B. purpurea* and *B. variegata*. Presence of these flavones show advanced nature.

Table 3 : Comparative chart of flavonoid spots of colour of *Bauhinia* species.

Species	Flourescence of spots in UV Light							Total No. of Spots
	Bl	GY	Yl	BY	B	RB	R	
B. acuminata Linn.	2	1	2			1		6
B. blakeana Dunn.	3		2	1	1	2	1	10
B. purpurea Linn.	2	1	2	1	2	1		9
B. racemosa Lam.	1		2	2	1	1	1	8
B. tomentosa Linn	1	1	2	2	1	1		8
*B.variegat*a Linn	3			1	2		1	7

Table 4: Characteristic chromatographic spots types of *Bauhinia* species.

Species	Quercetin	Myricetin	Kaemferol	Isorhamnetin	Apigenin	Luteolin	Isovitexin
B. acuminata Linn.	+		+				+
B. blakeana Dunn.	+		+	+	+		
B. purpurea Linn.	+		+	+	+		
B. racemosa Lam.	+	+	+				
B. tomentosa Linn.	+		+			+	
B. variegata Linn.	+		+		+		

Another distinctive feature among species is the derivatives of Quercetin, viz. Quercetin-3-glucoside (Isoquercitrin) was present in *B. acuminata* and *B. tomentosa*, Quercetin-3-rutinoside (Rutin) was present in *B. racemosa, B. variegata* and *B. purpurea*. Quercetin-3-rhamnoside was present in *B. purpurea, B. blakeana* and *B. variegata* while Quercetin-3-arbinoside was specific to *B. racemosa* (Table 1).

Thus out of the 7 compounds identified, *B. acuminata*, *B. racemosa, B. tomentosa* and *B. variegata* showed the presence of 3 compounds each while *B. blakeana, B. purpurea* revealed the presence of 4 compounds (Table 4). *B. variegata* had no any species-specific flavonoid while myricetin was specific to *B. racemosa.* The presence of myricetin in *B. racemosa* is justified by the characteristic of leaves of woody plants. Isorhamnetin was present in *B. purpurea and B. blakeana*. All the six species showed a close relationship among themselves.

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