Pak.J.Bot., 25(2): 111-117, 1993.

CHEMOTAXONOMIC STUDIES OF THE GENUS *MEDICAGO* L. (PAPILIONOIDEAE) FROM PAKISTAN

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Abstract

The flavonoid chemistry of 7 species belonging to 4 subgenera of the genus *Medicago* L., has been reported for the first time from Pakistan. The flavonoid pattern strongly support the infrageneric classification of the genus. It also seems to be useful to solve the problems at inter and infraspecific levels.

Introduction

The genus *Medicago* L., with over 50 species is widely distributed. The wide interest in the genus is due to its importance as fodder plants, especially *M. sativa* L. (Heyn, 1981). Flavonoid, of the genus *Medicago* L., particularly *M. sativa* L. (Alfalfa) has been reported by Guggolz *et al.*, (1961); Barnes (1966) and Harborne (1971). Furthermore, the relationship between species of the genus *Medicago* on the basis of phenolic constituents have been studied by Simon (1967), Simon & Goodall (1968) and Classen *et al.*, (1982). Isoflavonoid phytoalexins have been reported in 25 *Medicago* species by Ingham (1979). There are differences of opinion among authorities regarding the partition of the genus into major taxa (Heyn, 1981; Lesins & Lesins, 1979; Small *et al.*, 1981a 1981b). In the present study the infrageneric classification of the genus into subgenera and sections by Lesins & Lesins (1979) has been followed. The genus is represented in Pakistan by 7 species belonging to 4 subgenera (Ali, 1977).

Since no chemotaxonomic study on *Medicago* species from Pakistan has been reported, studies were therefore carried out on the flavonoid chemistry of 7 species of the genus *Medicago* viz., *M. sativa* L., *M. falcata* L., *M. lupulina* L., *M. orbicularis* (L.) Bart., *M. polymorpha* L., *M. minima* (L.) Grufb., *M. laciniata* (L.) Mill., var. *laciniata* and var. *brachycantha* Boiss.

Materials and Methods

Mature leaf samples of the genus *Medicago* viz., *M. sativa* L., *M. falcata* L., *M. lupulina* L., *M. orbicularis* (L.) Bart., *M. polymorpha* L., *M. minima* (L.) Grutb., *M. laciniata* (L.) Mill., var. *laciniata* and var. *brachycantha* Boiss, were collected from the herbarium specimens of the Karachi University Herbarium (KUH; Appendix 1). Flavonoids were analysed by 2D-PC, TLC (cellulose) and UV-spectroscopy against authentic markers as described by Husain & Markham (1981) and Harborne (1984).

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Table 1. The characteristics of Flavonoids found in *Medicago*.

Spot No.	Fl. in		Rf (x 100) in				U.V Spectral analysis (Max nm)					Identification
	UV	UV+NH_3	BAW	15%HOAC	PhOH	Forestal	MeOH	MeOH+ACl_3	MeOH+ACl_3	MeOH+NaOAc	MeOH+ NaOAc/HBO	
1.	y	d.ky	65	05	29	41	256.350	276.331	272.353	272.372	260.365	Qu
2.	y	d.ky	42	03	13	28	254.374	271	266	269	258.392	My
3.	dkbr	y.br	92	11	-	84	-	-	-	-	-	km. 3.0 methylether
4.	br	y	51	06	55	49	-	-	-	-	-	My. 7.0 methylether
5.	gr	d.ky	39	78	-	74	-	-	-	-	-	Km. 8.0 methylether
6.	br	y.br	55	35	56	-	257.360	275	268	274.324.380	262.377	Qu. 3 Gal
7.	y	y	50	12	35	-	-	-	-	-	-	Qu. 4. gl
8.	br	y.br	70	51	58	-	-	-	-	-	-	Qu. 3. Rh
9.	br	y.br	74	19	76	-	-	-	-	-	-	Km. 7. Rh
10.	dkbr	dkbr	55	75	68	-	-	-	-	-	-	Km.3.xy.gl
11.	l.br	y	86	01	88	83	267.336	276.301.348	276.299.340	274.301.376	268.336	Ap
12.	y	y	77	01	-	66	253.267	274.328	275.356	269.384	259.371	Lu

Results and Discussion:

Of the 20 flavonoids analysed from 7 *Medicago* species, 17 were identified whereas the spots U_1, U_2 and U_3 could not be identified and are therefore, marked as unknown (Table 1 & 2). The flavonoid pattern investigation in the present study supports the infrageneric classification of the genus proposed by Lesins & Lesins (1979).

A number of subgenus specific compounds have been found such as, Kaempferol 7-rhamnoside, Luteolin 4-glucoside, Tricin 7- glucuronide and U_1 in subgenus *Medicago* L.; Quercetin 3- galactoside, Tricin, Acacetin, U_2 and U_3 in sub-genus *spirocar pos*; Kaempferol 3-0 methylether, Kaempferol 8-0 methylether, Luteolin 3-0 methylether and Myricetin 7-0 methylether, Quercetin 3-0rhamnoside, Kaempferol 3-xylosyl glucoside in subgenus *Lupularia* and *orbicularia*, respectively (Table 2).

Inspite of specific, certain compounds found to be common in subgenera, such as Kaempferol has been found in 3 subgenera and absent from subgenus spirocarpos. Similar results have also been reported by Simon (1967). Like Simon (1967) and Classen *et al.*, (1982), we have also found Quercetin and Luteolin in all the subgenera. Myricetin was absent in subgenus *Lupularia* while present in rest of the three. Tricin has been detected in subgenus *Medicago* and *spirocarpos*, whereas Apigenin was found in subgenus *spirocarpos* and *Orbicularia*.

Apart from infrageneric level, flavonoid pattern has been also useful at inter and infraspecific levels (Table 2). Thus in subgenus *Medicago* (section *Medicago*), *M. sativa* L., and *M. flacata* L. could be easily distinguished by the presence of Myricetin, Quercetin-4-glucoside & Tricin-7-glucuronide in the former and U1 in the later species. Similarly morphological similarity and placement of both the species in the same subgenus could also be confirmed due to the presence of Quercetin, Kaempferol-7-rhamnoside, Luteolin and Luteolin-4-glucoside in both the species. Although both the species often considered as one (Lesins & Lesins, 1979) but many workers have differentiated both of them on pod shape and flower colour. Our data also support the recognition of them as two separate species.

In subgenus *Spirocarpos* (Section Leptospirae) the three species viz., *M. laciniata*, *M. polymorpha* and *M. minima* have been considered to be closely related and often misidentified as *M. laciniata* (Lesins & Lesins, 1979). However these species can be differentiated from each other on the basis of leaflet indumentum, stipules margin, lateral vein pattern on pods and chromosome number (Lesins & Lesins, 1979). Close relationship of the above 3 species have also been reflected in the flavonoid pattern (Table 2). However, the occurence of Quercetin-3- galactoside and U3 in *M. laciniata* only and Apigenin, Luteolin, Tricin and U2 in *M. polymorpha* and *M. minima* distinguished *M. laciniata* from the later two species. *M. polymorpha* and *M. minima* seem to be very closely related and presence of Quercetin-4- glucoside in *M. polymorpha* may be the only distinction between them. In *M. laciniata* var. *laciniata* Quercetin-3-galactoside and Quercetin-4-glucoside and in *M. laciniata* var. *brachycantha* U3 differentiate both the varieties.

The present investigation of flavonoid pattern revealed that flavonoids could be useful in classification of the genus *Medicago* L., not only at subgeneric but also at inter and infraspecific levels.

Table 1 (Cont'd)

Spot No.	Fl. in		Rf (x 100) in				U.V Spectral analysis (Max nm)					Identification
	UV	UV+NH_3	BAW	15%HOAC	PhOH	Forestal	MeOH	MeOH+ACl_3	MeOH+ACl_3	MeOH+NaOAc	MeOH+ NaOAc/HBO	
13.	br.y	y	72	-	86	73	349 244.269 350	275.303	277.302.306	262.321	270.350	Tricin
14.	br	y	91	00	88	91	269.327	277.302.344	279.300.338	276.357	269.331	Acacetin
15.	br	y	82	02	-	84	-	-	-	-	-	Lu.3.0 methylether
16.	br	dkbr	65	36	-	-	-	-	-	-	-	Lu.4.gl
17.	l.y	y	29	11	71	-	-	-	-	-	-	Tr.7.glu
U_1.	br	dkbr	62	70	-	-	-	-	-	-	-	Unknown
U_2.	br	y.br	67	13	-	-	-	-	-	-	-	Unknown
U_3.	br	y	16	11	-	-	-	-	-	-	-	Unknown

Key: y = Yellow, br = Brown, gr = Green, dk = Dark, l = Light, UV = Ultraviolet, Fl = Flourescence, Qu = Quercetin, My = Myricetin, Km = Keemoferol, Ap = Apigenin, Lu = Luteolin, Tr = Tricin, xy = Xylosyl, gl = Glucoside, Gal = Galactoside, Rh = Rhamnoside, Glu = Glucuronide.

Table 2. The flavonoids present in species of *Medicago*.

Spot number Subgenus	Section	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	U1	U2	U3
Medicago	Falcago	*M.Sativa*	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-	+	+	-	-	-
		M.falcata	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-
Lupularia	Suffruticosae	*M.lupulina*	+	-	+	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Orbicularia	Orbiculares	*M.orbicularis*	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-
Spirocarpos	Leptospirae	*M.laciniata* var. *brachycantha*	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
		M.laciniata var. *laciniata*	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
		M.minima	-	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-
		M.polymorpha	-	+	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-	-	+	-

1, Quercetin; 2, Myricetin; 3, Kaempferol 3.Omethylether; 4 Myrcetin-7.Omethyl ether; 5, Kaempferol 8.Omethyl ether 6; Quercetin 3. Galactoside; 7; Quercetin, 4. gl; 8, Quercetin.3.Rhamnoside; 9, Kaempferol.7. Rhamnoside; 10, Kaempferol.3.Xylosyle glucoside; 11, Apigenin; 12, Luteolin; 13, Tricin; 14, Acacetin; 15, Luteolin.3.Omethyl ether; 16, Luteolin.4.glucoside; 17, Tricin.7. Glucuronide; U1, U2, U3 Un known.

Key:- - = absent; + = Present.

Acknowledgements

We are grateful to Pakistan Science Foundation (PSF) for financial support. Thanks are also due to Dr. Attaur-Rehman (H.E.J. Post Graduate Institute of Chemistry) for UV spectrophotometery. Thanks are also due to the Curator of Karachi University Herbarium (KUH), Mr. M. Ahmed and Mr. A. Hafeez for their help during the collection trips.

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(Received for Publication 6 December 1992)

Appendix-1. List of specimen studied.

Species	Locality	Collector	No.
Medicago sativa L.	c. 40-45 miles from Juglote on way to Astore	S.Omer & M. Qaiser	2303
M. sativa L.	Chitral Dist. c. 45 Km from Chitral on way to Booni	A. Ghafoor & S. Omer	2985
M. sativa L.	c. 10 miles from Nasirabad on way to Larkana	Nazim, S. Abedin & M. Qaiser	122
M. sativa L.	Near Bangla Hussain Shah, between Shahpur and Sahiwal	A. Ghafoor & Tahir Ali	3842
M. sativa L.	Chautair, Loralai Dist.	Tahir Ali & Tufail Ahmed	1271
M. sativa L.	c. 8 miles from Mahmori on way to Kaghan	M. Qaiser & A. Ghafoor	5236
M. sativa L.	Bot Garden P.C.S.I.R., Karachi	Sultan Abedin	5756
M. falcata L.	Gilgit, Rama Rest House	S.Omer & M. Qaiser	2341
M. lupulina L.	Murree	S. Abedin	2868
M. lupulina L.	c.30 Km to Ziarat, Kach road	S. Khatoon, E. Ahmed & Mola Baksh	548
M. lupulina L.	Shahezan Hills, Parachinar	S. Nazimuddin- & S. Abedin	1112
M. lupulina L.	Utror, Swat	S. Abedin	8442
M. orbicularis (L.) Bart.	Mazaffarabad Dist. Neelam valley, Between Shadi and Nari Noor	M. Qaiser & R.Y.Hashmi	8086
M. laciniata (L.) Mill. var. *brachycantha* Boiss	Dir Dist. c. 4 Km from Chakdara on way to Dir.	A. Ghafoor & Tahir Ali	4096
M. laciniata (L.) Mill. var. *laciniata*	Parachinar	M. Qaiser & S. Abedin	5904
M. minima (L.) Grufes	c. 2 miles from Mingora towards Medayan	S.I. Ali 38798 (H.B.No.)	
M. minima (L.) Grufes	National Agricultural Research Centre, Chak Shahzad, Islamabad	R.Y. Hashmi	155
M. polymorpha	Aainabad c. 65 Km from Sost, on way to Aliabad	S.I. Ali, *et al.*,	3451
M. polymorpha	c. 1 mile before Daulet pur Hyderabad rd.	S.A. Farooqi	509
M. polymorpha	Teejahban 12 miles from Hushab on way to Turbat	S.Abedin, A. Hussain	6019