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GENERIC LIMITS IN *GENTIANA* L. (s.l) FROM PAKISTAN AND KASHMIR: A CHEMOTAXONOMIC APPROACH

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Abstract

Generic limits in *Gentiana* L. (s.l.) from Pakistan and Kashmir was evaluated chemotaxonomically. Forty four taxa belonging to 8 genera were delimited into three broad groups on the basis of their flavonoid patterns where Rutin and Quercetin were the most widely distributed compounds present in 79 % and 76 % of the taxa respectively. The observations and results also suggested that the genera *Aliopsis, Gentianopsis, Aloitis, Comastoma* and *Jaeschkea* are advanced than *Ciminalis, Gentianodes* and *Qaisera* in evolutionary sequence.

Introduction

The genus *Gentiana* L. (s.l.) is represented in Pakistan and Kashmir by 44 taxa (Stewart, 1972). Because of heterogenity it has been split into a number of smaller genera (Smith, 1936; Ma, 1951; Toyokuni, 1961; Löve & Löve, 1972; 1975; Löve, 1986; Omer, 1992; Omer *et al.*, 1988 a & b; Omer & Qaiser, 1989; 1992) and hitherto 22 splits are known (Love, 1986). Flavonoid pattern has been extensively used in solving the taxonomic problems in several taxa viz., *Dahlia* (Giannasi, 1975); *Baptisia* (Alston & Turner, 1963; Markham *et al.*, 1970), and members of the Oleaceae (Harborne & Green, 1980); Liliaceae (Williams, 1975). There does not appear to be any report on the use of flavonoid patterns in the taxonomy of the genus *Gentiana* L. (s.l.). The studies have indicated that *Gentiana* L. (s.str.) does not occur in Pakistan and Kashmir (Omer & Qaiser, 1992) whereas 9 different genera are present and of these 55 taxa are encountered. The present paper is a supporting evidence for the recognition of the genera.

Materials and Method

Dried leaves (c. 10 g) of 44 taxa of 8 genera viz. *Ciminalis* Adans., *Gentianodes* Löve & Löve, *Qaisera* Omer, *Aliopsis* Omer & Qaiser, *Gentianopsis* Ma, *Aloitis* Rafin., *Comastoma* Toyok., and *Jaeschkea* Kurz from herbarium specimens obtained from B, BM, CAL, E, G, K, KUH, LE, NA, NY, O, P, PPFI-B, RAW, RNG, W were used. Extraction was carried out with ethanol. Paper chromatography was used for the separation of flavonoids following Harborne (1973). BAW (Butanol: Acetic acid: Water) was used as a solvent, the spots were located and their color recorded with and without ammonia (NH_3) treatment in UV light. Rf value was calculated and compounds identified by comparing with the standard Rf values in the literature (Harborne, 1973) in relation to Rf value of Rutin used as a marker.

Results and Discussion

An analysis of 44 taxa from Pakistan and Kashmir showed the following three groups recognized on the basis of flavones and flavonol aglycones.:

Group I. Three genera i.e., *Ciminalis* Adans., *Gentianodes* Löve & Löve and *Qaisera* Omer characterized by the absence of Apigenin (Table 1).

Group II. Four genera i.e., *Aliopsis* Omer & Qaiser; *Gentianopsis* Ma; *Aloitis* Rafin., and *Comastoma* Toyok., characterized by the presence of both Apigenin and Luteolin (Table 1).

Group III. Single genus i.e., *Jaeschkea* Kurz is the representive of this group in which only Apigenin is present and Luteolin is absent (Table 1).

Of the 44 species examined from which 11 flavonoids were identified on the basis of Rf value and colour reactions, 7 were flavones whereas 4 were flavonols (Table 1). The unidentified spots and different number of compounds encountered in different genera are given in Table 2 where 7 unidentified spots are classified as flavones and flavonols.

The genus *Ciminalis* Adans. showed 3 unidentified spots with spot no 2 as the exclusive among the genera (Table 2), with a Rf value of 32-35 and yellow/yellow color reaction. Similarly in the genus *Gentianodes* Löve & Löve, spot no. 5 was exclusive of the genus. The genus *Gentianopsis* Ma has spot no. 7 as the exclusive. The genus *Qaisera* Omer is characterized by the presence of Kaempferol, which is absent in the other two related genera i.e., *Ciminalis* Adans., and *Gentianodes* Löve & Löve with the exception of 2 species in *Gentianodes* Löve & Löve. Spot no. 6 is exclusive of the genus *Qaisera* Omer.

Table 1. Comparative account of the flavone aglycone present in the different genera.

Genera	Apigenin	Luteolin	Tricin	Vitexin	Isovitexin	Orientin	Isoorientin	Kaempferol
Ciminalis Adans.	-	(+)	+	-	(+)	+	-	-
Gentianodes Love & Love	-	+	-/+	-	-/+	+	(+)	-/+
Qaisera Omer	-	-	-	-	+	+	+/-	+
Aliopsis Omer & Qaiser	+	+	-	-	+	-	+	-
Gentianopsis Ma	+	+	-	-	+	+	-	-
Aloitis Rafin.	+	+	-	(+)	(+)	+/-		(+)
Comastoma Toyok.	+	(+)	-	-	-	+	-	-
Jaeschkea Kurz	+	-	-	-	+	+	-	-

Table 2. Different types of compounds in the species of the group.

Species	Apigenin	Luteolin	Tricin	Vitexin	Isovitexin	Orientin	Isoorientin	Kaempferol	Quercetin	Myricetin	Rutin	Unidentified (Rf in BAW)
Ciminalis												
C.aquatica	-	-	-	-	-	+	-	-	+	-	+	34.16,Y/Y
C.prostrata	-	-	+	-	-	+	-	-	+	-	+	32.08,Y/Y
C.riparia	-	-	+	-	-	+	-	-	+	-	+	33.3,Y/Y
C.pseudoaquat ica	-	-	-	-	-	+	-	-	-	-	+	-
C.squarrosa	-	-	-	-	-	+	-	-	+	-	+	-
C.leucomalaena	-	-	+	-	+	+	-	-	-	-	+	14.58,Y/Y
C.capitata	-	+	+	-	+	+	-	-	-	-	+	25,Dk/y; 14.5,Y/Ygrn
C.karelinii	-	-	-	-	-	-	-	-	-	-	+	21.22,Dk/Ygrn
Gentianodes												
G.huxleyi	-	+	-	-	-	-	+	-	-	-	+	14.58,Y/Y
G.argentea	-	-	-	-	-	+	-	-	-	-	+	-
G.alii	-	-	-	-	-	+	+	-	+	+	-	16.25,Y/Y
G.burkillii	-	+	-	-	+	-	-	-	+	-	+	-
G.marginata	-	-	-	-	+	+	-	-	-	-	+	17.91,Y/Y
-var.harr-issii	-	-	-	-	+	-	-	-	+	-	-	6.2,Y/Y
-var.scab-romarginata	-	-	-	-	-	+	-	-	-	+	+	18.75,Y/Y
G.nasirii	-	-	-	-	+	+	-	-	+	-	-	-
G.intermedia	-	-	-	-	-	+	-	-	+	-	+	-
G.pedicellata	-	-	-	-	-	-	-	-	+	-	+	40.74,Dk/Y; 26.66, Ygrn/Ygrn

Table 2 (Cont'd.)

Species	Apigenin	Luteolin	Tricin	Vitexin	Isovitexin	Orientin	Isoorientin	Kaempferol	Quercetin	Myricetin	Rutin	Unidentified (Rf in BAW)
G.aprica	-	+	-	-	-	-	-	-	+	-	-	-
G.venusta	-	-	-	-	+	+	-	-	+	-	+	-
G.algida												
G.cachemerica	-	+	-	-	-	+	-	-	+	-	+	48.14, Ygrn/Ygrn
-var.nubi-gena	-	+	-	-	+	+	-	-	+	-	+	-
G.kaufmanniana	-	+	-	-	+	-	-	+	+	+	-	-
G.kurroo	-	-	-	-	-	+	-	-	+	-	+	19.25,Y/Y
G.olivieri	-	+	-	-	-	-	-	-	+	-	+	-
G.tianschanica	-	+	+	-	-	-	-	+	-	-	-	-
Qaisera												
Q.carinata	-	-	-	-	+	+	-	+	+	+	+	17.54,Y/Y
Q.hugellii	-	-	-	-	+	+	-	+	+	-	+	17.89,Y/Y
Q.coronata	-	-	-	-	+	-	+	+	+	-	+	15.78,Y/Y, 60.35,Dk/Ygrn
Aliopsis												
A.pygmaea	+	+	-	-	+	-	+	-	=	-	-	16.45,Y/Y
Gentianopsis												
G.contorta	+	+	-	-	+	-	-	-	+	-	+	20,Y/Y
G.vedenskeyi	+	+	-	-	-	+	-	-	+	-	+	79.13,Dk/ Ygrn;6.5,Y/Y
G.paludosa	+	+	-	-	+	+	-	-	+	-	-	77.82,Dk/ Ygrn;19.56,Y/Y
Aloitis												

Table 2 (Cont'd.)

Species	Apigenin	Luteolin	Tricin	Vitexin	Isovitexin	Orientin	Isoorientin	Kaempferol	Quercetin	Myricetin	Rutin	Unidentified (Rf in BAW)
A.azurea	+	-	-	-	-	-	-	-	+	-	+	-
A.maddeni	+	+	-	-	+	-	-	-	+	-	+	19.56,Y/Y
A.moorcroftiana	+	+	-	-	+	+	-	-	+	+	+	19.56,Y/Y
A.umbellata	+	+	-	+	-	-	-	-	-	-	+	-
A.holosteoides	+	+	-	-	-	-	-	-	+	-	+	-
A.stoliczkai	+	-	-	-	-	-	-	+	-	+	-	
Comastoma												
C.falcatum	+	+	-	-	-	-	-	-	-	-	+	-
C.pulmonarium	+	-	-	-	-	+	-	-	+	-	+	-
C.borealis	+	-	-	-	-	-	-	-	+	-	-	25.41,Y/Y
Jaeschkea												
J.oligosperma	+	-	-	-	+	+	-	-	+	-	+	48.97,Y/Y
J.canaliculata	+	-	-	-	+	+	-	-	+	-	+	51.02,Y/Y; 38.77,Dk/Ygrn

Table 3. Different number of compounds and their classification breakup.

Genera	Flavones	Flavanols	Unidentified spots	Total	% of flavones	% of flavanols
Ciminalis	4	2	3	9	55.55	44.44
Gentianodes	5	3	4	12	58.33	41.66
Qaisera	3	4	2	9	44.44	55.55
Aliopsis	4	1	1	6	66.66	33.33
Gentianopsis	4	2	3	9	66.66	33.33
Aloitis	5	4	1	10	50.00	50.00
Comastoma	3	4	1	8	66.66	33.33
Jaeschkea	3	0	0	3	100.00	00.00

The present study indicate that the broad grouping of plicate corolla and the gynobasal nectaries (lacks Apigenin) against the non-plicate corolla and epipetalous nectaries (Apigenin and Luteolin present, except *Jaeschkea* Kurz) are also substantiated by the chemical analysis (For detailed group analysis and characters, see Omer, 1995; & Omer & Qaiser, 1992).

The evolutionary studies indicate that flavonol glycosides (Kaempferol, Quercetin and Myrecetin) are present in the primitive dicots and flavone-O-glycosides (Luteolin and Apigenin) are present in advanced dicots, whereas C-glycosyl flavones (Orientin, Vitexin, Isorientin and Isovitexin) are an intermediate stage between flavonols and flavone-O-glycosides (William & Harborne, 1971; Harborne, 1977; Crawford, 1978). The results indicate that *Aliopsis* Omer & Qaiser, *Gentianopsis* Ma, *Aloitis* Rafin., and *Comastoma* Toyok., are advanced than *Ciminalis* Adans., *Gentianodes* Löve & Löve and *Qaisera* Omer as the former group bear both Apigenin and Luteolin, whereas the 3 later genera lack Apigenin and only Luteolin is present with the exception of perennial species of *Gentianodes* Löve & Löve (Table 2). *Jaeschkea* Kurz may lie in between as it lacks Luteolin and only Apigenin is present.

The genus *Qaisera* Omer seems to be primitive as it lacks both the flavones (Luteolin and Apigenin) whereas the 3 basic flavonols (Kaempferol, Quercetin and Myrecetin) are present. Rutin (Quercetin-3-rutinoside) which is widespread among angiosperms was found absent in *Aliopsis* Omer & Qaiser and *Jaeschkea* Kurz. It is considered important taxonomically (Harborne, 1973; 1984a) since it is an interesting condition among non-plicate corolla and epipetalous nectaries (Tables 1 & 2).

The percentage of different flavones and flavonols (Table 3) gives an idea as to which genera are more advanced than the other (William & Harborne, 1971; Harborne, 1977; Crawford, 1978). On the basis of flavones and flavonols the position of evolution in different genera also becomes evident (Fig. 1) although it is only a tentative and hypothetical indication for the evolutionary sequence.

The results of the present studies shows that flavonoids and their systematic values give an indication of the differences in all the recognized genera. It also suggests that

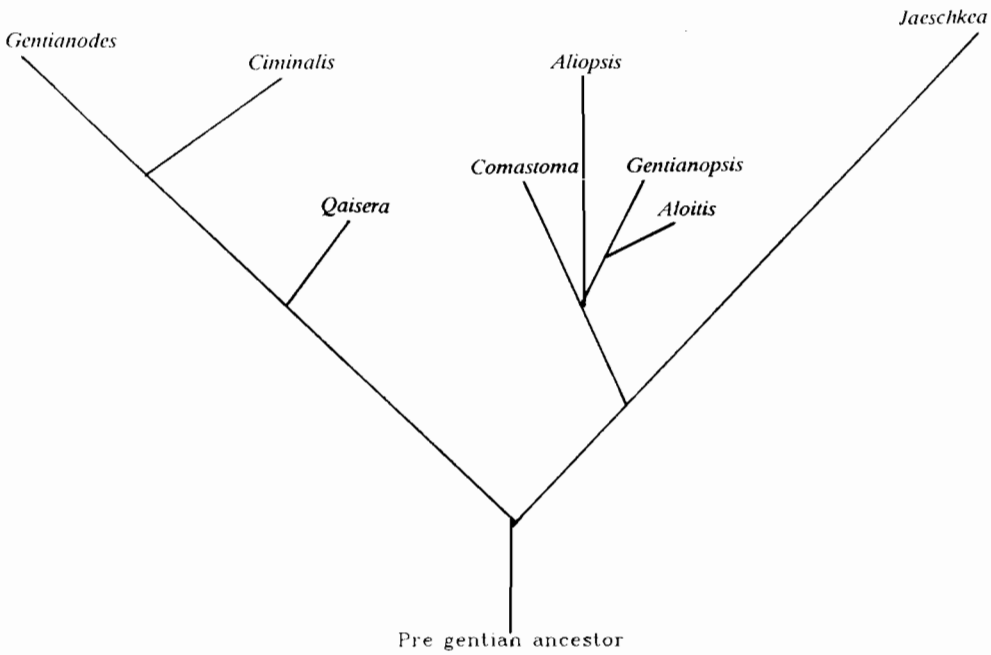

Fig. 1. Evolutionary sequence of genera on flavonoid basis.

phylogenetic affinities indicated elsewhere (Omer, 1995; Omer & Qaiser, 1992) is in conformity with the present findings.

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