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CHEMOTAXONOMIC STUDY OF *INULA* L. (S.STR.) AND ITS ALLIED GENERA (INULEAE - COMPOSITAE) FROM PAKISTAN AND KASHMIR

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Abstract

Leaves of 21 taxa belonging to *Inula* L. (s.str.) and its allied genera viz., *Pentanema* Cass., *Duhaldea* DC., *Dittrichia* Greuter and *Iphiona* Cass., have been analyzed from Pakistan and Kashmir for their phenolic compounds. Analysis was carried out by two dimensional chromatography. Spectral analysis was performed on Shimadzu UV-spectrophotometer. The chemical data of this intricate group also support the generic delimitation of *Dittrichia* by having O-coumaric acid and Sakuranetin, while tricin 5-glucoside is exclusive for *Iphiona* and *Duhaldea* is distinctive by the presence of Vitexin and Isovitexin. However, *Inula* and *Pentanema* shared a mix pattern of compounds which points out the close relationship of both genera as compared to other genera of this group.

Introduction

There are several reports on the chemotaxonomy of the family Compositae. Most of the workers gave the attention to the flavonoids, sesquiterpene lactones and some other compounds (Crawford, 1970; Geissman & Irwin, 1973; Bohlmann *et al*., 1981; Valant-Vetschera & Wollenweber, 1981; Ates *et al*., 1982; Shukla & Gupta, 1985; Ling, 1992; Iwashina *et al*., 1995; Tzakao *et al*., 1995 and Akkal *et al*., 1997). Although some of the individual species of *Inula* and its allied genera were also chemically investigated but the chemical data was not utilized in the taxonomy of the genus. Dombrowicz & Greiner (1968) isolated quercetin and isoquercitrin from the aerial parts of *Inula britannica* L., Baruah *et al*., (1979) reported three new flavonoids from the aerial parts of *Inula cappa* (=*Duhaldea cappa*), Oksuz & Topcu (1987) reported a new Kaempferol derivative from the aerial parts of *Inula britannica*, Ahmed & Ismail (1991) isolated the sterols and a new flavonol from the aerial parts of *Inula grantioides* (=*Iphiona grantioides*). Oksuz & Topcu (1992) analyzed the extract of aerial parts of *Inula graveolens* (=*Dittrichia graveolens*) and isolated sesquiterpene lactones, flavonoids, dihydroflavonols and flavones. It is therefore evident from the preceding literature that some chemical informations are available on few taxa of *Inula* L., and its allied genera but no attempt has ever been made to utilize this data for taxonomical purposes. The present study was carried out to utilize the chemical data as a taxonomic evidence.

Materials and Methods

Leaves of 21 taxa belonging to *Inula* L. (s.str.) and its allied genera were analyzed for their phenolic compounds. For extraction, approximately 1 gm of dried leaves from herbarium specimens were extracted with 70% ethanol at room temperature. Extracts were concentrated and chromatographed two dimensionally on Whatman no.1 paper using two solvent combinations, i.e., BAW (n-butanol: acetic acid: water, 4:1:5) versus 15% acetic acid and BAW versus distilled water, following standard procedure of Harborne (1973). A list of voucher specimens is given in appendix-I.

Phenolic compounds were identified by comparing with authentic markers along with the Rf values and colour in ultra-violet light before and after fuming with ammonia vapours.

Compounds were repeatedly purified by paper chromatography, till the absorption properties became constant. Hence an elute of a paper blank in 95% ethanol (usually about $150cm^2$) was taken and applied (spotted) to the paper, and run in BAW and 15% HOAc separately. After the purification of compounds, the spots of chromatogram were cut and shaken in 95% ethanol for 30 minutes. The solution was filtered and allowed to concentrate, and directly used for spectral analyses on Shimadzu UV-240 spectrophotometer.

Results and Discussion

Paper chromatography of the aqueous ethanolic extracts from the leaves of 21 taxa of *Inula* L., and its allied genera led to the isolation of 42 (including 11 unknown compounds) different phenolic compounds (phenolic acids, flavonols, flavones, glycosylflavones, flavanones and chalcones) (Table 1.1-1.5, Fig. 1.1-1.7).

Generic key based on chemical characters

1. + Vitexin and iso-vitexin both present .... ***Duhaldea***
   - No such compounds present **2**

2. + Tricin 5-glucoside present .... ***Iphiona***
   - Tricin 5-glucoside absent **3**

3. + Sakuranetin present .... ***Dittrichia***
   - Sakuranetin absent .... ***Inula, Pentanema***

The results suggested that the chemical data of flavonoids is not always correlated with the classification. This contention was also supported by Heywood (1973), as he pointed out that the flavonoids themselves could not be taken as independent evidence relative to supporting a particular classification. However, it may be useful in taxonomic delimitation at generic or specific level. *Iphiona* and *Dittrichia* are chemically related to *Inula* (s.str.) as all of these genera share a number of flavonoids, although the presence of O-coumaric acid along with sakuranetin in *Dittrichia*, and tricin 5-glucoside in *Iphiona*, keep them distinct from the other genera. *Duhaldea* is chemically separated from the other genera due to the occurrence of vitexin, iso-vitexin and quercetin 4-glucoside.

The flavonoid patterns of the genera *Inula* (s.str.) and *Pentanema* are similar to the other genera as they share number of flavonoids and do not possess any specific compounds of their own. Although the presence of certain specific compounds in other genera, make them separate. However, the rarer occurrence of ferulic acid, aesculetin, aesculin, some glycosides of quercetin, kaempferol, luteolin and hesperitin in *Inula* (s.str.) further make it distinct from all the other genera.

Table 1.1. Phenolic acids in *Inula* L. (s.str.) and its related genera.

Name of taxa	Ferulic Acid	Caffeic acid	Chlorogenic acid	O-coumaric acid	Aesculetin	Aesculin
Inula koelzii	+	+	-	-	+	-
I. royleana	-	+	-	-	-	-
I. racemosa	-	+	+	-	-	-
I. stewartii	-	+	-	-	+	-
I. orientalis	-	+	-	-	-	-
I. clarkei	+	+	-	-	-	-
I. obtusifolia	+	+	-	-	-	-
I. britannica	-	-	-	-	-	-
I. acuminata	-	+	+	-	-	-
I. falconeri	-	+	+	-	-	-
I. rhizocephala	-	+	-	-	-	+
Pentanema glanduligerum	-	-	-	-	-	-
P. indicum	-	-	-	-	-	-
P. divaricatum	-	+	-	-	-	-
P. vestitum	-	+	-	-	-	-
Duhaldea cappa	-	+	-	-	-	-
D. eupatorioides	-	+	+	-	-	-
D. cuspidata	-	+	+	+	-	-
Dittrichia graveolens	-	+	-	+	-	-
Iphiona aucheri	-	+	-	+	-	-
I. grantioides	-	+	-	-	-	-

Key: + = Present, - = Absent

Table 1.2. Flavonols of *Inula* L. (s.str.) and its related genera.

Name of taxa	1	2	3	4	5	6	7	8	9	10	11	12
Inula koelzii	-	-	-	-	-	-	-	-	-	+	+	-
I. royleana	-	-	-	-	-	-	-	-	-	-	+	-
I. racemosa	-	-	+	-	-	-	-	-	-	-	+	-
I. stewartii	-	+	+	-	-	-	-	+	-	-	+	-
I. orientalis	-	+	-	-	-	-	-	-	-	+	-	+
I. clarkei	-	-	-	-	-	-	+	+	+	-	-	-
I. obtusifolia	+	-	-	-	-	-	+	+	+	-	-	-
I. britannica	-	+	-	-	-	-	-	+	-	-	-	+
I. acuminata	-	+	-	-	+	+	-	+	-	-	+	-
I. falconeri	-	+	-	-	+	+	-	+	-	-	+	-
I. rhizocephala	-	-	-	-	-	-	-	-	-	-	-	-
Pentanema glanduligerum	+	-	-	-	-	-	-	-	-	-	+	-
P. indicum	-	+	-	-	-	-	-	-	-	-	-	-
P. divaricatum	-	+	-	-	-	-	-	+	-	-	+	-
P. vestitum	-	+	-	-	-	-	-	+	-	-	-	-
Duhaldea cappa	-	-	-	+	-	-	-	-	-	-	-	-
D. eupatorioides	-	-	-	-	-	-	-	+	-	-	-	-
D. cuspidata	-	-	-	+	-	-	-	+	-	-	-	-
Dittrichia graveolens	-	+	-	-	-	-	-	-	+	-	+	-
Iphiona aucheri	-	+	-	-	-	-	-	-	-	-	-	-
I. grantioides	-	+	-	-	-	-	-	+	-	-	-	-

Key: 1 = Quercetin, 2 = Quercetin 3-rutinoside, 3 = Quercetin 3-rutinoside 7-glucuronide, 4 = Quercetin 4-glucoside, 5 = Quercetin 7-glucoside, 6 = Quercetin 7-4 diglucoside, 7 = Quercetin 3-sophoroside 7-glucoside, 8 = Quercetin 6-OH, 9 = Kaempferol 3-rutinoside 7-glucuronide, 10 = Kaempferol 3-sophoroside 7-rhamnoside, 11 = Kaempferol 3-sophorotrioside 7-rhamnoside, 12 = Kaempferol 3-lathyroside 7-rhamnoside, - = Absent, += Present.

Table 1.3. Flavones, glycosylflavones, flavanones and chalcones in *Inula* L. (s.str.) and its related genera.

	Flavones					Glycosylflavones			Flavanones			Chalcones	
Name of taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Inula koelzii	-	-	+	-	-	-	-	+	-	-	-	-	-
I. royleana	-	+	+	-	-	-	-	+	-	-	-	+	-
I. racemosa	-	-	-	-	-	-	-	+	+	-	-	+	-
I. stewartii	-	+	-	-	-	-	-	+	-	+	-	-	-
I. orientalis	-	-	-	+	-	-	-	-	-	-	-	+	-
I. clarkei	-	-	+	-	-	-	-	+	-	-	-	-	-
I. obtusifolia	-	-	+	-	-	-	-	-	-	-	-	-	-
I. britannica	+	-	-	+	-	-	-	-	-	-	-	+	-
I. acuminata	+	-	-	-	-	-	-	-	+	-	-	-	+
I. falconeri	+	-	-	-	-	-	-	-	+	-	-	-	+
I. rhizocephala	-	+	+	-	-	-	-	-	+	-	-	-	+
Pentanema glanduligerum	-	-	-	-	-	-	-	-	-	-	-	+	-
P. indicum	-	+	-	-	-	-	-	-	-	-	-	+	-
P. divaricatum	-	+	-	-	-	-	-	-	-	-	-	+	-
P. vestitum	-	-	-	-	-	-	-	-	-	-	-	-	-
Duhaldea cappa	-	+	-	-	-	+	+	+	-	-	-	-	-
D. eupatorioides	-	-	-	-	-	+	-	-	+	-	-	-	-
D. cuspidate	-	+	-	-	-	+	+	+	+	-	-	-	-
Dittrichia graveolens	+	-	-	-	-	-	-	-	+	-	+	+	-
Iphiona aucheri	-	-	-	-	+	-	-	-	+	-	-	-	-
I. grantioides	-	-	-	-	+	-	-	-	+	-	-	-	-

Key: 1 = Apigenin 6-OH, 2 = Apigenin 7-glucoside, 3 = Apigenin 8-rhamnosyl glucosyl, 4 = Luteolin 7-glucoside, 5 = Tricin 5-glucoside, 6 = Vitexin, 7 = Iso-vitexin, 8 = Iso-orientin, 9 = Hesperidin, 10 = Hesperitin 7-glucoside, 11 = Sakuranetin, 12 = Isoliquiritigenin 4-glucoside, 13 = Butein 4-glucoside, - = Absent, + = Present.

Table 1.4. Total number of flavonol, flavone glycoside and c-glycosyl flavones in *Inula* L. (s.str.) and its related genera.

Name of genera	Total no. of compounds		
	Flavonol glycosides	Flavone glycosides	C-glycosyl flavones
Inula	32	12	5
Pentanema	9	2	-
Duhaldea	4	2	7
Dittrichia	3	1	-
Iphiona	3	2	-

Table 1.5. Unidentified compounds of *Inula* L. (s.str.) and its related genera.

Name of taxa	Rf values		Colour in UV	
	BAW	15% HOAc	With ammonia	Without ammonia
Inula koelzii	54.96	74.40	l. yell.	yell. br.
	60.02	99.33	yell.gr.	yell. gr.
I. royleana	55.38	74.84	l. yell.	Yell. br.
	62.92	100.02	yell.gr.	yell. gr.
I. racemosa	0.0	92.0	b.bl.	l. bl.
	19.38	97.74	b. purp.	purp. bl.
	61.88	99.81	yell. gr.	yell. gr.
I. stewartii	22.22	100.0	b. purp.	purp. bl.
	0.0	97.50	d. br.	d. br.
I. orientalis	92.91	86.90	Yell.	Yell.
I. clarkei	28.05	17.20	b. yell.	b. yell.
I. obtusifolia	94.28	86.88	yell.	yell.
I. britannica	95.66	87.05	yell.	yell.
I. acuminata	20.50	45.25	bl.	bl.
I. falconeri	-	-	-	-
I. rhizocephala	70.75	26.30	b. purp.	purp. bl.
	19.28	39.99	bl.	bl.
Pentanema glanduligerum	0.0	91.66	b. bl.	l. bl.
	24.80	99.87	b. purp.	purp. bl.
P. indicum	19.99	98.20	b. purp.	purp. bl.
P. divaricatum	-	-	-	-
P. vestitum	-	-	-	-
Duhaldea cappa	59.25	93.06	b. bl.	l. bl.
D. eupatorioides	-	-	-	-
D. cuspidata	-	-	-	-
Dittrichia graveolens	69.21	92.42	yell.	d. yell.
Iphiona aucheri	-	-	-	-
I. grantioides	82.05	99.12	yell.	yell. gr.

Key: yell. = yellow; gr. = green; br. = brown; purp. = purple; bl. = blue; b. = bright; d. = dull; l. = light.

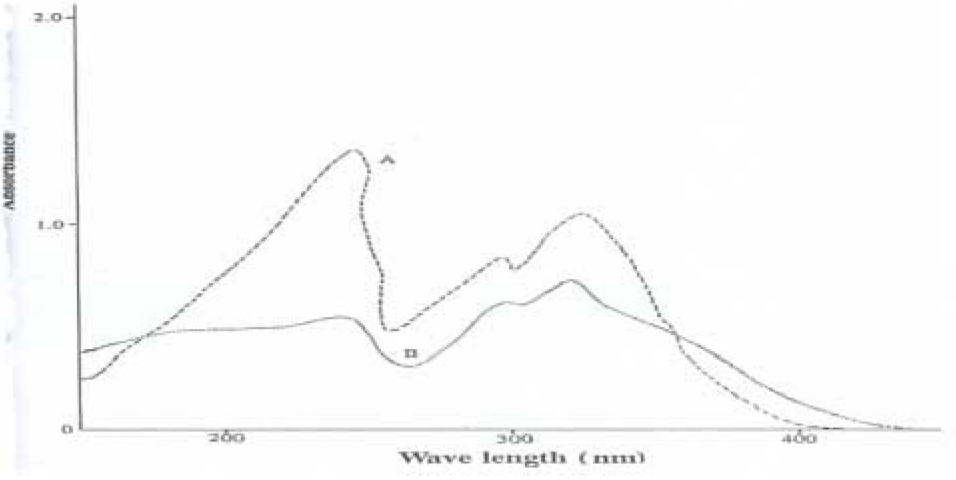

Fig. 1.1. Absorption spectra of Caffeic acid (curve A) and Chlorogenic acid (curve B) in 95% EtOH.

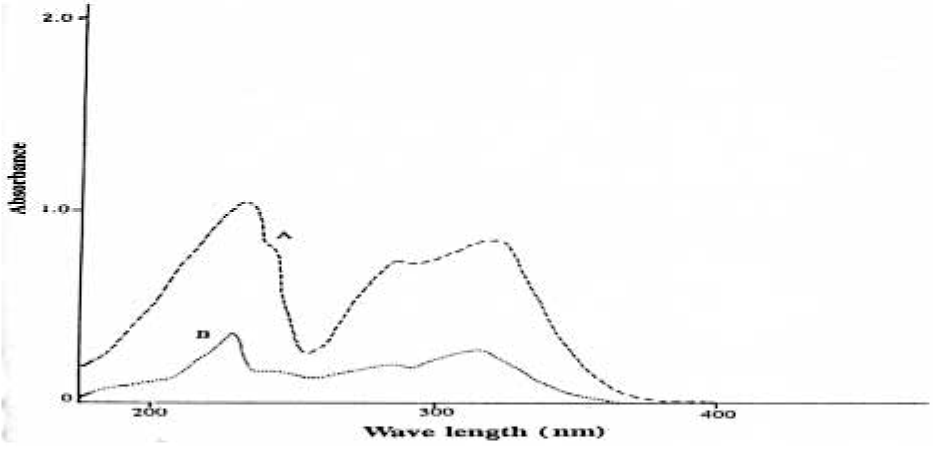

Fig. 1.2. Absorption spectra of Ferulic acid (curve A, marker; curve B, isolated compound) in 95% EtOH.

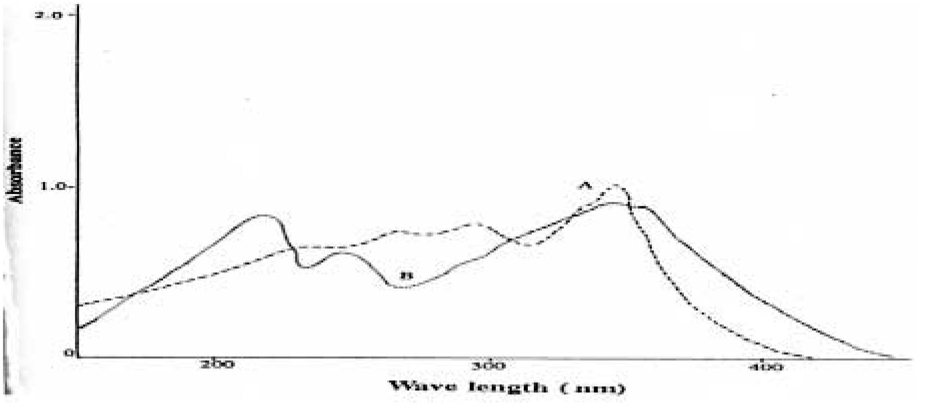

Fig. 1.3. Absorption spectra of Aesculetin (curve A) and Aesculin (curve B) in 95% EtOH.

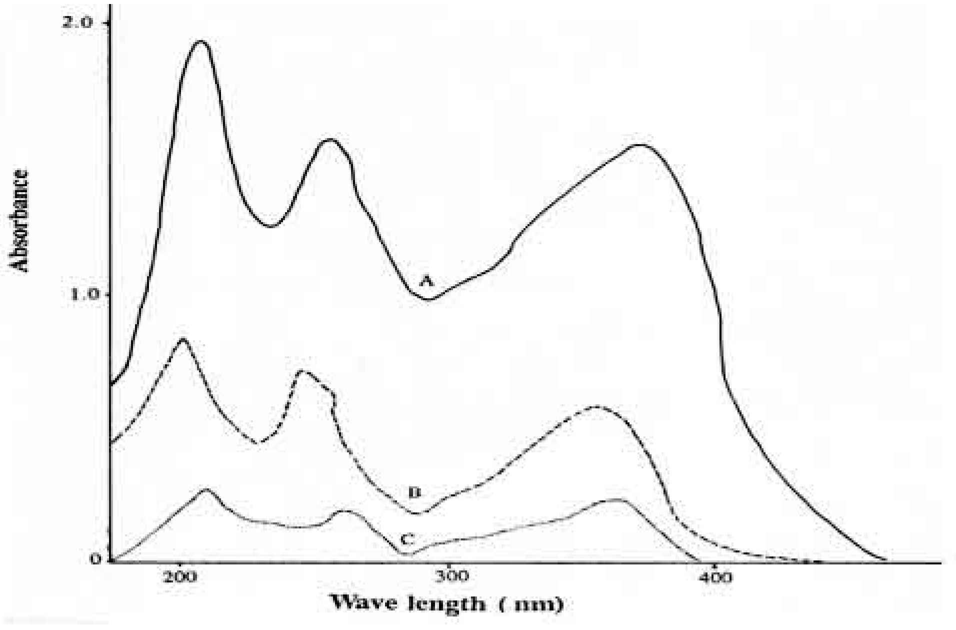

Fig. 1.4. Absorption spectra of Rutin (curve A, marker; curve B isolated compound) and Quercetagetin (curve C) in 95% EtOH.

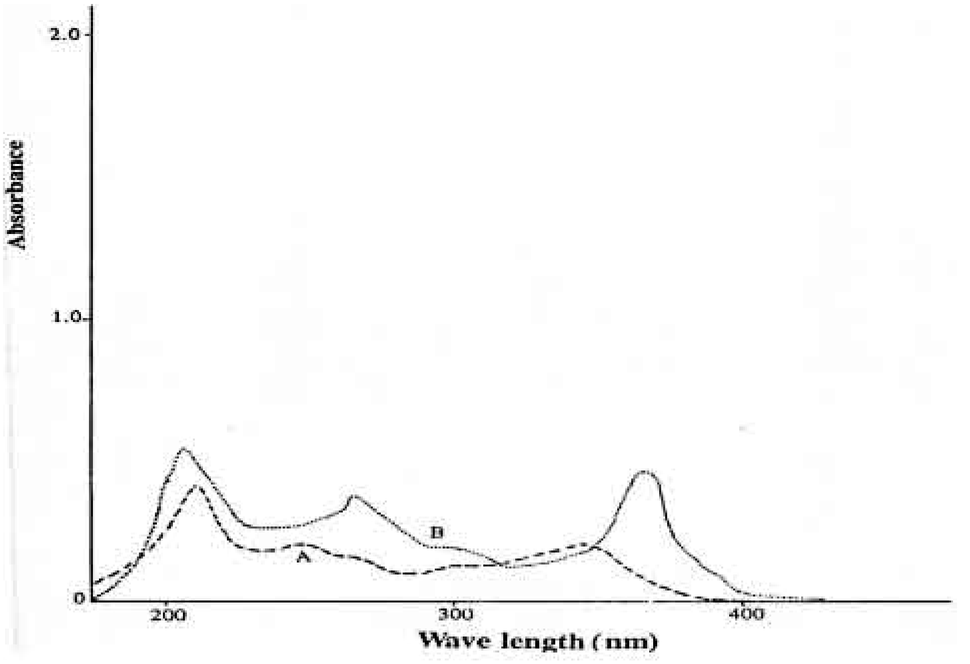

Fig. 1.5. Absorption spectra of Luteolin 7-glucoside (curve A) and Kaempferol 3-sophorotrioside 7-rhamnoside (curve B) in 95% EtOH.

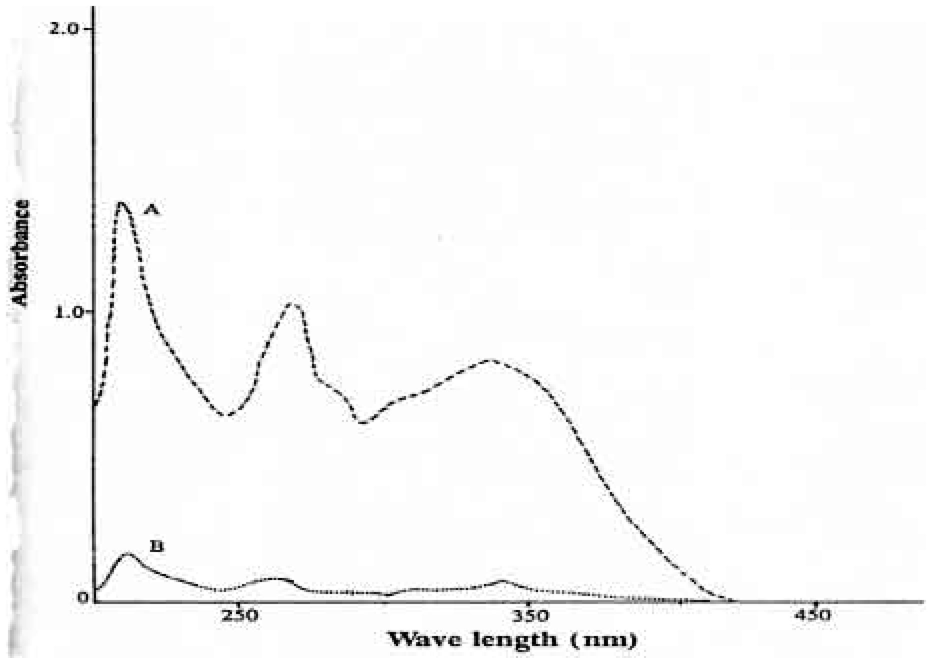

Fig. 1.6. Absorption spectra of Apigenin 7-glucoside (curve A, marker; curve B isolated compound) in 95% EtOH.

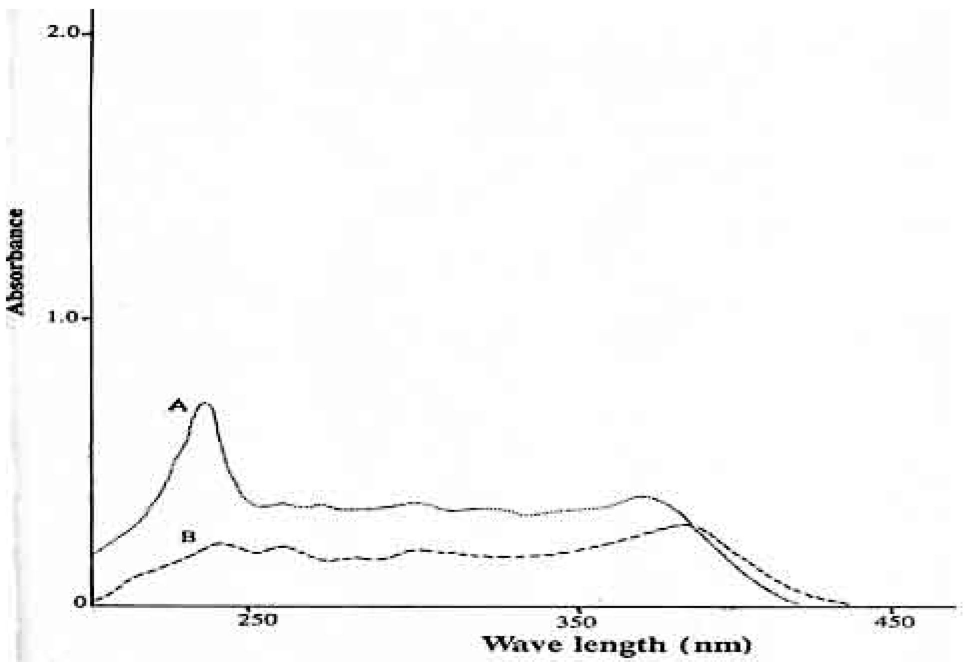

Fig. 1.7. Absorption spectra of Isoliquiriteginin 4-glucoside (curve A) and Butein 4-glucoside (curve B) in 95% EtOH.

Key to the species of *Inula* (s.str.) based on chemical characters

1. + Kaempferol 3-sophoroside 7-rhamnoside and kaempferol 3-sophorotrioside 7-rhamnoside both present .... ***I. koelzii***
  - No such compounds present **2**

2. + Iso-orientin present .... ***I. clarkei***
  - Iso-orientin absent .... ***I. obtusifolia***

3. + Kaempferol 3-lathyroside 7-rhamnoside and luteolin 7-glucoside both present. **4**
  - Both the compounds absent **5**

4. + Kaempferol 3-sophoroside 7-rhamnoside present .... ***I. orientalis***
  - No such compound present .... ***I. britannica***

5. + Butein 4-glucoside present .... ***I. acuminata, I. falconeri, I. rhizocephala***
  - Butein 4-glucoside absent **6**

6. + Quercetagetin present .... ***I. stewartii***
  - Quercetagetin absent **7**

7. + Apigenin 7-glucoside and apigenin 8-rhamnosyl glucosyl both present .... ***I. royleana***
  - Both the compounds absent .... ***I. racemosa***

In the genus *Inula* L. (s.str.), *I. koelzii* Dawar & Qaiser, *I. royleana* DC., *I. racemosa* Hook.f., and *I. stewartii* R. Abid & Qaiser are generally related in their morphology as they have upper sessile and semiamplexicaul, while lower winged petiolate leaves and 3-4 mm long and usually glabrous cypselas, similarly, the chemical data of these species also strengthen the above morphological correlation due to the presence of iso-orientin, kaempferol 3-sophorotrioside 7-rhamnoside and caffeic acid. Some other glycosides of kaempferol, quercetin, apigenin, luteolin and tricin are absent from all the four species. However, the four species can still be differentiated on the basis of flavonoid pattern, *I. koelzii* and *I. stewartii* have aesculetin but lacks in *I. racemosa* and *I. royleana*, while *I. koelzii* and *I. stewartii* differ from each other by the presence of ferulic acid and rutin respectively. On the other hand, *I. royleana* and *I. racemosa* differ due to occurrence of apigenin glucoside in *I. royleana* and hesperidin in *I. racemosa* respectively.

Inula britannica L., *I. acuminata* Royle ex DC., and *Inula falconeri* Hook.f., are more or less morphologically related species which share several compounds viz., rutin, quercetagetin and apigenin 6-OH but *I. britannica* can be delimited from both the species by the presence of kaempferol 3-lathyroside 7-rhamnoside, luteolin 7-glucoside and isoliquiritigenin 4-glucoside. *I. acuminata* and *I. falconeri* could not be distinguished chemically from each other and share all the compounds with the exception of an unidentified compound with Rf values 20.5 (BAW), 45.25 (15% HOAc) and with unchanged blue colour, that is exclusive for *I. acuminata*. On the other hand, the specific compounds of *I. britannica* are also shared with *I. orientalis* Lamk., although both are morphologically dissimilar furthermore, both the species are also delimited due to the occurrence of kaempferol 3-sophoroside 7-rhamnoside in *I. orientalis* and apigenin 6-OH in *I. britannica*. Likewise, *Inula obtusifolia* Kern., and *Inula clarkei* (Hook.f.) Stewart are also closely related species as they share ferulic acid, caffeic acid, quercetin 3-sophoroside 7-glucoside, quercetagetin, kaempferol 3-rutinoside 7-glucuronide and apigenin 8-rhamnosyl glucosyl. However, these 2 species can be differentiated from each other as the quercetin aglycon and iso-orientin are present in *I. obtusifolia* and *I. clarkei* respectively. However, *Inula rhizocephala* Schrenk is morphologically distinct from rest of the species due to stemless habit, similarly the presence of aesculin in *I. rhizocephala* also makes it chemically distinct from rest of the species of *Inula* L.

Key to the species of *Pentanema* based on chemical characters

1. + Rutin present **2**
 - Rutin absent .... ***P. glanduligerum***

2. + Quercetagetin present **3**
 - Quercetagetin absent .... ***P. indicum***

3. + Apigenin 7-glucoside and kaempferol 3-sophorotrioside 7-rhamnoside both present .... ***P. divaricatum***
 - No such compounds present .... ***P. vestitum***

Species of *Pentanema* Cass., could be delimited on the basis of flavonoid pattern. On the other hand morphologically unrelated species share the similar compounds for instance, kaempferol 3-sophorotrioside 7-rhamnoside is present in 2 morphologically different species viz., *Pentanema glanduligerum* (Krasch.) Gorschk., and *Pentanema divaricatum* Cass. Similarly apigenin 7-glucoside is found in *Pentanema indicum* (L.) Ling and *P. divaricatum* both of them are morphologically dissimilar to some extent. However *P. glanduligerum* is distinguished from all of the other species of *Pentanema* by having the quercetin aglycone while other 3 species have rutin. *P. vestitum* (Wall. ex DC.) Ling and *P. divaricatum* are separated from *P. indicum* by the presence of caffeic acid and quercetagetin but absent from *P. indicum*. *P. divaricatum* is splitted from *P. vestitum* due to the presence of apigenin 7-glucoside and kaempferol 3-sophorotrioside 7-rhamnoside which are exclusive for *P. divaricatum*.

Key to the species of *Duhaldea* based on chemical characters

1. + Apigenin 7-glucoside and iso-vitexin both present **2**
 - No such compounds present .... ***D. eupatorioides***

2. + Quercetagetin present .... ***D. cuspidata***
 - Quercetagetin absent .... ***D. cappa***

Chemical variation is not so pronounced within all the species of *Duhaldea* DC., as *Duhaldea eupatorioides* (Wall.ex DC.) A.Anderb., shares all its compounds with *D. cuspidata* (Wall.ex DC.) A.Anderb., and no specific compound is found in *D. eupatorioides*. However, the *D. cuspidata* can be separated from *D. cappa* (Ham.ex D.Don) A.Anderb., due to the occurrence of chlorogenic acid and quercetagetin which are exclusive for *D. cuspidata*.

Key to the species of *Iphiona* based on chemical characters

1. + Quercetagetin present .... ***I. grantioides***
   \- Quercetagetin absent .... ***I. aucheri***

Both the species of *Iphiona* Cass., are chemically distinguished from each other due to the presence of quercetagetin in *I. grantioides* (Boiss.) A. Anderb., but absent from *I. aucheri* (Boiss.) A. Anderb.

Apart from the known compounds several unidentified compounds are also found within the genus *Inula* L. (s.str.) and its allied genera (Table 1.5). Those spots which have more or less same Rf-values and colours are tentatively grouped in 11 different compounds. Morphologically dissimilar taxa share same unknown compounds. For instance a compound with Rf-values 0.0 and 91.66-94.93 (blue coloured) respectively in BAW and 15% HOAc is common in *Inula racemosa* and *Pentanema glanduligerum*. Another compound which is purplish blue in colour having the Rf-values from 19.38-24.8 (BAW) and 97.74-100 (15% HOAc) is found in *Inula stewartii*, *I. racemosa, Pentanema glanduligerum* and *Pentanema indicum*. *Inula rhizocephala* and *Inula acuminata* also share a compound, which has blue colour with Rf-values from 19.28-20.5 (BAW) and 39.99-45.25 (15% HOAc). On the other hand *Dittrichia graveolens* (L.) Greuter has an exclusive unknown compound with the Rf-values 69.21 (BAW) and 92.42 (15% HOAc) (colour change from yellow to dull yellow after ammonia fumigation). Similarly, *Inula rhizocephala* has an exclusive unknown compound with purplish shade and 70.75 (BAW) and 26.30 (15% HOAc) Rf-values. However, *Inula clarkei* and *I. obtusifolia are* closely related but an unknown compound with the Rf-values 28.05 (BAW) and 17.20 (15% HOAc) with bright yellow colour is found only in *I. clarkei*. Likewise, *Iphiona grantioides* has an unknown compound which is yellow / yellowish green in colour with the Rf-values 82.05 and 99.12 in BAW and 15% HOAc respectively is not shared by closely related taxon *Iphiona aucheri*.

It is generally agreed that flavonol glycosides (kaempferol, quercetin, particularly myricetin) are present in the supposedly more primitive dicotyledons and flavone o-glycosides (apigenin and luteolin) occur in more highly advanced dicotyledons, c-glycosyl flavones (vitexin, iso-vitexin, orientin and iso-orientin) are considered as an intermediate state between flavonols and flavone o-glycosides (William & Harborne, 1971; Harborne, 1977; Crawford, 1978; Omer *et al*., 1996). The present studies are also in accordance with this contention. All the genera have shared a mix pattern of flavonol, flavone o-glycoside and c-glycosyl flavone. Although the flavonols predominated over flavone. Likewise, *Inula* L. (s.str.) shared some morphologically primitive and advance characters so it is paraphyletic in origin and this paraphyletic condition of the genus was earlier reported by Anderberg (1991). However, primitive characters are quite dominant in this genus with always radiate capitula and pappus bristles in large number, while on the other hand it is also characterized by the presence of herbaceous nature of plants and conspicuously ribbed cypselas. So this morphological evolution in *Inula* (s.str.) also support the above mentioned generalizations as in this genus flavonol glycosides predominated over flavone glycosides by the presence of 32 flavonol glycosides and 10 flavone glycosides and the number of flavonol gradually reduced to 9, 4, 3, and 3 in the remaining genera viz., *Pentanema, Duhaldea, Dittrichia* and *Iphiona* respectively (Table 1.4). This clearly indicated gradual co-evolution of flavonoids and morphological characters. This predominant pattern of flavonols over flavones was also observed by Seelingmann (1996) in certain tribes of Compositae. The mix pattern of flavonol, flavone o-glycoside and c-glycosyl flavone in all the genera is also in agreement with a number of instances given by Crawford (1978) and Averett *et al*., (1986) in which all three types (flavonols, flavone o-glycoside and c-glycosyl flavones) have been observed.

Appendix-I

No.	Taxon	Collector, number and herbarium
1.	*Inula koelzii*	W. Koelz 2900a (KUH); W. Koelz 2827a (NY)
2.	*I. royleana*	Y. Nasir & Rubina Akhtar 12996 (RAW); M. Qaiser & Rizwan Y. Hashmi 7868 (KUH)
3.	*I. racemosa*	R.R. Stewart 14052 (KUH); R.R. Stewart 19550 (RAW)
4.	*I. stewartii*	R.R. Stewart s.n. (RAW)
5.	*I. orientalis*	S. Abedin & M. Qaiser 8887 (KUH); Tahir Ali, M. Qaiser & M. Ajmal 503 (KUH).
6.	*I. clarkei*	Hans Hartmann s.n. (RAW); E. Nasir & G.L. Webster 5804 (RAW)
7.	*I. obtusifolia*	M. Qaiser, S. Omer & S.Z. Hussain 8414 (KUH); R.R. Stewart 18803 (RAW)
8.	*I. britannica*	R.R. Stewart 54 (RAW)
9.	*I. acuminata*	Stainton 3077 (RAW); R.R. Stewart 26356 (RAW)
10.	*I. falconeri*	R.R. Stewart 20484 (KUH); M.A. Siddiqui, Y. Naisr & Zaffar 4182 (K)
11.	*I. rhizocephala*	R.R. Stewart 18859 (RAW); S. Omer & M. Qaiser 2360 (KUH)
12.	*Pentanema glanduligerum*	G.R. Sarwar & S. Omer 256 (KUH); Stainton 2944 (RAW)
13.	*P. indicum*	A. Rashid 26985 (RAW); Farrukh Hussain s.n. (RAW)
14.	*P. divaricatum*	S. Abedin & Abrar Hussain 6232 (KUH); S.M.H. Jafri 2854 (KUH)
15.	*P. vestitum*	Y. Nasir & Rubina Akhter 11863 (RAW); S. Abedin 2659 (KUH)
16.	*Duhaldea cappa*	A. Ghafoor & Tahir Ali 4005 (KUH); S.A. Farooqui & M. Qaiser 3172 (KUH)
17.	*D. eupatorioides*	R.R. Stewart & I.D. Stewart 4145 (RAW)
18.	*D. cuspidata*	Tahir Ali, M. Qaiser & M. Ajmal 367 (KUH); Y. Nasir & Nazir 10519 (RAW)
19.	*Dittrichia graveolens*	J.L. Stewart 245 (K)
20.	*Iphiona aucheri*	Tahir Ali & G.R. Sarwar 2868 (KUH); Tahir Ali 1478 (KUH)
21.	*I. grantioides*	S. Omer & Rizwan Y. Hashmi 2003 (KUH); A. Ghafoor & S. Omer 1825 (KUH)

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