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Root Flavonoids of Some Iranian *Scirpus* L. (Cyperaceae) Members

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Abstract: Root flavonoids of 5 *Scirpus* L. species: *Cypereae* Colla. Tribe, *Cyperaideae* Kostel. subfamily and Cyperaceae family (*S. holoschenus* L., *S. lacustris* L., *S. littoralis* Kuntze, *S. maritimus* L. and *S. multicaule*) from different parts of Markazi Province, Iran area were studied using 2-dimentional Paper Chromatography (2-DPC) and Thin Layer Chromatography (TLC). Flavonoids are as one set of the polyphenolic compounds among secondary metabolites in different organs of plants that are used in chemotaxonomy. Also many flavonoids are active principles of medicinal plants, exhibit pharmacological effects and contribute to human health. Voucher samples were prepared for reference as herbarium vouchers. Results showed all of studied taxa contain flavonoid sulphates, flavone C and C-/O-glycosides and aglycones in their roots while Rutin, Myricetin and Vitexin were just found in *S. maritimus*. Also presence of Morin, Tricin and Loteulin in all of the species roots with the exception of *S. maritimus* are more valuable tools for taxa separation. Kaempferol was found in *S. lacustris* and *S. littoralis* species, where as others lack.

Key words: *Scirpus*, *Cyperaceae*, flavonoid compounds, *Chemotaxonomy*, chromatography

INTRODUCTION

Plant chemosystematics is the application of chemical data to systematic problems. It is a rapidly expanding interdisciplinary field concerned with using chemical constituents for explaining relationships between plants and inferring phylogeny (Jones and Luchsinger, 1987). Secondary metabolites specially flavonoids are valuable and widely and effectively used in chemosystematics (Noori, 2002). Flavonoids occur widely in plants and are a biologically major and chemically diverse group of secondary metabolites that are popular compounds for chemotaxonomic surveys of plant genera and families (Harborne, 1994). Today, flavonoids are used for making antitumoure, anticancer, antibacterial, antiviral, antifungal drugs and insecticides. There are some studies in this connection from mosses and liverworts (Mues and Zinsmeister, 1988), ferns (Harborne, 1994) to the angiosperms (Williams and Harborne, 1977; Harborne, 1994; Williams *et al*., 1986; Saleh *et al*., 1988). Their phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e.g., at the species and genus level (Harborne, 1994; Moore and Giannasi, 1994; Noori *et al*., 2009).

Some flavonoid compounds have been reported from *Scirpus* L. (rush grass): *Cypereae* Colla. tribe, *Cyperaideae* Kostel. subfamily, Cyperaceae. Clifford and Harborne, 1969) studies showed identification of the flavonoid pigment aureusidin from *Scirpus nodosus*. Quercetin, kaempferol, apigenin and luteolin were reported from *S. wichurai* (Abdel-Mojib *et al*., 2001). Nassar *et al*. (2000) identified lupeol betulin, betulinalaldehyde and apigenin from *Scirpus tuberosus*. Also β-sitosterol, quercetin 3-β-glucoside, quercetin 3, 7-β-diglucoside and isorhamnetin 3, 7-β-glucoside were identified from *Scirpus litoralis* using spectroscopic analyses (Nassar *et al*., 2000). Spectral measurements of separated tuber extract of the *Scirpus holoschoenus* species showed existing stilbenes (Abdel-Mojib *et al*., 2001). Yang *et al*. (2010) used a developed capillary electrophoresis with amperometric detection method for the determination of some phenolic compounds in the rhizome of *Scirpus yagara* Ohwi. Their work determined existing four phenolic compounds: transresveratrol, scirpusin A, scirpusin B and p-hydroxycinnamic acid in the species rhizome. Studies on 5 Iranian *Scirpus* leaf flavonoids showed presence of Vitexin, Luteolin, Rutin and Rhamnetin. Quercetin was not found in *S. maritimus* and *S. littoralis* where as three other species had. Also *S. lucustris* had not chrysin and naringenin (Noori, 2012).

The aim of this study was to compare the root flavonoids profiles of 5 *Scirpus* species from central of Iran.

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MATERIALS AND METHODS

Collection of plant material and preparation: Mature fresh roots of 5 *Scirpus* species (*S. holoschenus* L., *S. lacustris* L., *S. littoralis* Kuntze, *S. maritimus* L. and *S. multicaule*) were collected from Markazi Province, Iran area during 2011 as described in Table 1. Plants identified using available references (Mobayen, 1979; Ghahreman, 1977, 1994). Specimens of each sample were prepared for reference as herbarium vouchers that were deposited at the Arak University Herbarium. Samples were air dried for detection and identification of flavonoids.

Extraction of the plant material: For a comparative analysis of the flavonoids, small extracts of all the accessions were prepared by boiling 200 mg of powdered air dried root material for 2 min in 5 mL of 70% EtOH. The mixture was cooled and left to extract for 24 h. The extract was then filtered, evaporated to dryness by rotary evaporation at 40°and taken up in 2 mL of 80% MeOH for analysis by 2DPC.

Flavonoid analysis by 2-DPC: For the detection of flavonoids, ca 20 μL of each of the small extracts was applied to the corner of a quarter sheet of Whatman No 1 chromatography paper as a concentrated spot (10 applications of 2 μL). The chromatogram for each sample was developed in BAW (n-BuOH: HOAc: H_2O = 4:1:5; V/V; upper layer), 1st direction and HOAc (=15% aqueous acetic acid), 2nd direction, with rutin (= quercetin 3-O-rutinoside) as a standard. After development, the chromatograms were viewed in longwave UV light (366 nm) and any dark absorbing and fluorescent spots were marked. R_f-values in BAW and 15% HOAc were calculated.

Methods of identification of the flavonoids: After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from 5 *Scirpus* species, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids (Mabry *et al*., 1970; Markham, 1982) and by acid hydrolysis to identify the aglycone and sugar moieties. Cochromatography with standards was also performed where possible. Flavonoid standards available for comparison during the study were Apigenin, Chrysin, Isorhamnetin, Kaempferol, Luteolin, Morine, Myricetin, Narengenin, Quercetin, Rhamnetin, Rutin, Tricine and Vitexin (all obtained commercially, Rutin from Merck, Apigenin and Luteolin from Sigma and the rest from Fluka).

Acid hydrolysis and identification of flavonoid aglycones: A small amount of each purified flavonoid (ca 0.5 mg) was dissolved in 0.5 mL of 80% MeOH in a test tube. To this sample 2 mL of 2 M HCl were added and the mixture was heated in a water bath at 100°C for 0.5 h. The solution was cooled, 2 mL of EtOAc were added and thoroughly mixed with the aqueous layer using a whirley mixer. The upper EtOAc layer was removed with a pipette, evaporated to dryness, dissolved in 0.5 mL of MeOH and applied as spots on thin layer chromatograms (cellulose). The TLC plates were run in three solvents alongside standards to identify the aglycone moiety (Harborne, 1998).

RESULTS

All studied *Scirpus* species contained flavonoid compounds in their roots. Their flavonoid profiles show a wide variety between the species. Data in Table 1 and 2 show the sampling and also 2-dimentional paper and thin layer chromatographical data of 5 studied *Scirpus* species from central of Iran. Figure 1 shows stacked column with

Table 1: Collection information, 2-dimentional paper chromatography data of 5 studied *Scirpus* species from Markazi Province, Iran area

					Flavonoid type			
Voucher data	Taxon	Latitude	Longitude	Altitude (m)	No. of total flavonoids	No. of flavonoid sulphates	No. of flavone C-and C-/O-glucosides	No. of aglycones
*CNM4	*S. holoschenus* L.	49°24′N	33°98′E	1700	5	1	1	3
CNM23	*S. lacustris* L.	49°41′N	34°29′E	1809	6	2	3	1
CNM8	*S. littoralis* Kuntze	49°60′N	34°02′E	1100	5	1	2	2
CNM6	*S. maritimus* L.	49°24′N	34°31′E	1800	5	1	3	1
CNM19	*S. multicaule*	50°01′N	34°41′E	1980	6	1	3	2

*CNM: Negar mehrdoost collection number

Table 2: Thin layer chromatography data of 5 studied *Scirpus* species from Markazi Province, Iran area

	Flavonoids identification												
Voucher data	Apegenin	Chrysin	Isorhamnetin	Kaempferol	Luteolin	Morine	Myricetin	Narengenin	Quercetin	Rhamnetin	Rutin	Tricine	Vitexin
*CNM4	-	-	-	-	+++	++	-	-	-	-	-	+++	-
CNM23	+++	-	-	+++	+++	+++	-	-	+++	-	-	++	-
CNM8	+	+	-	+	+	+++	+	-	+	-	-	++	-
CNM6	-	-	-	-	-	-	+++	-	+	-	+++	-	+++
CNM19	-	-	-	-	+++	+	-	-	+	-	-	++	-

Scored characters: -: Non flavonoid, +: Few flavonoid, ++: Middle concentration of flavonoid, +++: High concentration of flavonoid

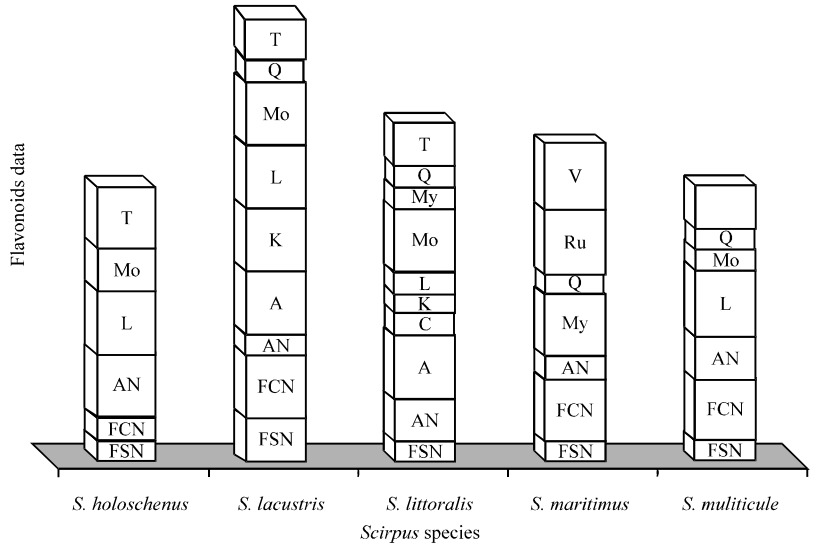

Fig. 1: Stacked column with a 3-D visual effect histogram for comparing root flavonoids data of studied *Scirpus* L. species of Markazi Province, Iran, using 2-DPC and TLC methods, scored characters for drawing 3-D column histogram in excel based on Table 2 data: -: Non flavonoid, +: few flavonoi, ++: Middle concentration of flavonoid, +++: High concentration of flavonoid

a 3-D visual effect histogram for comparing root flavonoids data (number of flavonoid sulphate, number of flavone C-and C-/O-glucosides, number of aglycones and occurrence and concentrations of Apigenin, Chrysin, Kaempferol, Luteolin, Morine, Myricetin, Quercetin, Rutin, Tricine and Vitexin) in the species. As Table 1 and Fig. 1 show all of studied *Scirpus* species have flavonoid sulphate, flavone C-and C-/O-glucosides and aglycones in their roots. The most flavonoids number and variety was observed in *Scirpus littoralis species* and *S. holoschenus* showed the lowest. Vitexin, Rutin and Myricetin were just found in *S. maritimus* and other lack.

DISCUSSION

As Harborne *et al*. (1982) studies on 92 Australian *Cyperus* species showed phytochemical studies of the Cyperaceae have been extremely useful in clarifying systematic relationships within the family members (Harborne *et al*., 1982). Flavonoids occur widely in plants and are a biologically major and chemically diverse group of secondary metabolites that are popular compounds for chemotaxonomic surveys of plant genera and families (Harborne, 1994). The phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e.g., at the species and genus level. Turning to the angiosperms, a chemotaxonomic survey of 255 species of the family Iridaceae has been carried out by Williams *et al*. (1986), who found that flavone *C*-glycosides were present in 66% of the samples (Harborne, 1994). Studying flavonoid pattern can be used for chemosystematic and lower taxonomic levels. 12 species of the genus *Ephedra* have been surveyed (Porter and Wallace, 1988). 25 *Avena* species (Poaceae) were investigated for the flavonoid content of leaf tissue (Saleh *et al*., 1988). Diploid *triticum* species could be divided into two groups depending on the presence or absence of two major di-C-glycosyl flavones (Harborne *et al*., 1982). Several studies indicated that flavonoids occurred in various species of *Cyperaceae* (Glennie and Harborne, 1971; Williams and Harborne, 1977; Harborne, 1971; Noori, 2012). As (Harborne, 1971) studies showed flavonoids may be useful taxonomic markers within the family. The presence of the characteristic leaf flavonoids (glycoflavones, tricin) of the grasses in this family shows that the Cyperaceae and the Gramineae are more closely linked chemically than a previous study of their inflorescence pigments suggested (Harborne, 1971). Also *cyperaceae* flavonoids are very important for their different potential clinical applications such as their toxicity, antidiarrhoeal, antibacterial, antiflogestic, tonic and stimultant effects (Cronquist, 1981; Mirheidar, 1993; Joungduk and Hong, 2010). *S. lacustris* is used as Local medicinal plant in Canada (Arnason *et al*., 1981), its stem known as antibacterial drug and is effective on *E. coli* (Villars and Delvigne, 2001).

Results showed all studied *Scirpus* species contained flavonoid compounds in their roots that their flavonoid profiles show a wide variety between the taxa. There are flavonoid sulphate, flavone C and C-/O-glycosides and aglycones in all species. *S. lacustris* had the highest number of total flavonoid compounds and the most aglycones were found in *S. holochenus* (Table 1). Two stilbene dimers, scirpusin A and B, together with resveratrol, 3, 3', 4, 5'-tetrahydroxy stilbene and triterpenoids were isolated from the rhizomes of *S. fluviatilis*. From the tubers of *S. fluviatilis* species, four stilbene trimers have been isolated, one of which has been formulated as an antiallergic and anti-inflammatory agent (Abdel-Mojib *et al*., 2001). A hydroxystilbene dimer has been isolated from the seeds of *S. maritimus* (Powell *et al*., 1987). Yang *et al*. (2010) used a developed capillary electrophoresis with amperometric detection method for the determination of some phenolic compounds in the rhizome of *Scirpus yagara* Ohwi. Their work determined existing four phenolic compounds: transresveratrol, scirpusin A, scirpusin B and p-hydroxycinnamic acid in the species rhizome. Spectral measurements of separated tuber extract of the *Scirpus holoschoenus* species showed existing stilbenes (Abdel-Mojib *et al*., 2001). Identification of flavonoids by standards showed all of studied *Scirpus* species root contain Luteoloin, Morin and Tricin with the exception of *S. maritimus*. Also Rutin were just found in *S. maritimus*. Quercetin was found in all species with the exception of *S. holoschenus* species. Myercetin found in both *S. littoralis* and *S. maritimus* species while others lack (Table 2, Fig. 1). Chrysin was just found in *S. littoralis* which among the studied taxa had the most variation and number of flavonoids. Noori (2012) studies on 5 Iranian *Scirpus* leaf flavonoids showed presence of Vitexin, Luteolin, Rutin and Rhamnetin. Quercetin was not found in *S. maritimus* and *S. littoralis* where as three other species had. Also *S. lucustris* had not chrysin and naringenin (Noori, 2012). Clifford and Harborne (1969) studies showed identification of the flavonoid pigment aureusidin from *Scirpus nodosus* (Clifford and Harborne, 1969) and quercetin, kaempferol, apigenin and luteolin from *S. wichurai* (Abdel-Mojib *et al*., 2001).

Nassar *et al*. (2000) identified lupeol betulin, betulinalaldehyde and apigenin from *Scirpus tuberosus*. Also β-sitosterol, quercetin 3-β-glucoside, quercetin 3, 7-β-diglucoside and isorhamnetin 3, 7-β-glucoside were identified from *Scirpus litoralis* using spectroscopic analyses (Nassar *et al*., 2000).

Finally the presence of Tricin, Morin and Loteuline in all of studied species exceptional *S. maritimus* species and absence of Myrestin, Vitexin and Rutin in all of them with the exception of *S. maritimus* are taxonomic characters for separation of the species. Also presence of Quercetin just in *S. holoschenus* and its absence in others is a separator character for the species from other species. Among the many functions of flavonoids at the interface between plant and environment, their activity as signals was intensively studied. Flavonoids are also beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents and in general, by their significant role in plant resistance (Treutter, 2006). But, further work is needed using high performance liquid chromatography with diode array detection, atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy to evaluate all flavonoid profiles in studied and other *scirpus* species.

ABBREVIATIONS

TFN = Total flavonoids number
FSN = Flavonoid sulphates number
FCN = Flavone C-and C-/O-glucosides number
AN = Aglycons number
C = Chrysin,
I = Isorhamnetin
K = Kaempferol
Q = Quercetin
RU = Rutin
T = Tricine
A = Apegenin
Mo = Morrine
My = Myricetin
V = Vitexin
L = Luteolin

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