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Root and Aerial Parts Flavonoids of 3 Iranian *Carex* L. (Cyperaceae) Species

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Abstract Root and aerial parts flavonoids of 3 *Carex* L. species: *Cariceae* Pax. Tribe *Cyperaideae* Kostel. subfamily and Cyperaceae family [*C. divisa* Huds., *Carex melanostachya* M. Bieb. ex Willd. (syn.: *C. nutans* Host) and *C. stenophylla* Wahlenb] from Iran were studied using two-dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC). *Carex* plants are characterized by the production of stilbene derivatives and other bioactive polyphenols including lignans and flavonoids. By this reason they have attracted recent attention as potential food additives. Flavonoids are as one set of the polyphenolic compounds among secondary metabolites that are active principles of medicinal plants, exhibit pharmacological effects and contribute to human health. Collected plants were identified using available references and voucher samples were prepared as herbarium vouchers. Results showed all 3 studied species contain flavone *C* and *C-/O*-glycosides in their roots and aerial parts. Flavonoid sulphates were found in all of roots and aerial parts of the studied species with the exception of *C. stenophylla* root and aerial parts. Aglycones was not found in ***C. melanostachya*** aerial parts, where as other samples had. The studied taxa showed variety in their root and aerial parts flavonoids compounds. Rutin, Myricetin, Kaempferol, Loteulin, Narengenin, Apigenin, Morin, Rhamnetin and Chrysin were found in their root or aerial parts, while all of samples lacked Quercetin, Isorhamnetin, Tricin and Vitexin.

Keywords *Carex, Cariceae*, Cyperaceae, Flavonoid compounds, Chromatography

1. Introduction

Carex L. from *Cariceae* Pax. Tribe, *Cyperaideae* Kostel. Subfamily and Cyperaceae family includes sedges that dominate wetlands, pastures, prairies, tundra, and the herb layer of temperate forests [1]. More than 1800 species were recorded for the genus in the world that about 43 species are in Iran [2]. They have attracted recent attention as potential food additives because they contain high levels of bioactive polyphenols commonly found in plant foods. Despite there being as many as 2000 *Carex* species worldwide, only a few members including *C. fedia, C. kobomugi, C. pumila, C. humilis, C. pendula,* and *C. distachya* have been previously investigated for their phytochemical constituents. This is unfortunate because *Carex* plants are characterized by the production of stilbene derivatives and other bioactive polyphenols including lignans and flavonoids [3]. Feizbakhsh et al (2012) identified essential oils composition of *C. pseudofoetida* aerial parts from Iran [4]. Manhart (1986) reported foliar flavonoids of the North

American members of the *Carex* section Laxiflorae [5]. Four metabolites, named carexanes I-L, have been isolated from the roots of *C. distachya* Desf, an herbaceous plant living in the Mediterranean Maquis, together with three known compounds, already isolated from the aerial part of the plant [6]. Also, they (2008) isolated 16 polyphenols, identified on the basis of spectroscopic data as 7 lignans, 4 phenylethanoids, 3 resveratrol derivatives, a monolignol, and a secoiridoid glucoside in *C. distachya* root. The species roots extract contained high quantities of polyphenols, most of them reported as constituents of edible plants, such as grape and olive, suggesting that the methanol root extract of this plant could be used as a source of natural antioxidants useful as potential food additives [1]. Seven compounds, which included two resveratrol oligomers and five flavonoids, were isolated from seeds of *C. folliculata* L. (northern long sedge), a forage prevalent in the northern United States. The flavonoids were isoorientin, luteolin, quercetin, 3-*O*-methylquercetin, and rutin [3]. Five flavonoids: tricin, tricin *O*-(erythro-b-guaiacylglyceryl) ether, apigenin-6-C-b-D-xylopyranosyl-8-C-b-D-glucopyranoside, apigenin-6-C-b-D-glucopyranosyl-8-C-b-D-xylopyranoside, luteolin-6-b-D-glucopyranosyl-8-C-b-D-xylopyranoside were reported from *C. distachya* Desf. fresh leaves [1]. Flavonoids as secondary metabolites are valuable and widely and effectively used in medicinal ingredients and chemosystematics [7]. The flavonoid work consists mostly of broad scale comparisons of aglycone distributions in a number of sedge genera to include some members of *Carex*. These comparisons proved to be of limited use in understanding phylogenetic relationships within the genus

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Carex. However, detailed flavonoid analyses of sections *Laxiflorae* and *Acrocystis* demonstrated the utility of flavonoid surveys in *Carex* at infrasectional levels and possibly at intersectional levels [8]. Catling et al (1989) used flavonoids for separating two hybrid *C. lacustris* × *C. trichocarpa*. Luteolin glycosides were found only in *C. lacustris*, whereas tricin glycosides were restricted to *C. Trichocarpa* [9]. Therefore, depth study of *Carex* L. medicinal ingredients and flavonoids can provided the basis for further development and utilization. In this study, root and aerial parts flavonoids of 3 Iranian *Carex* L. (Cyperaceae) species aqueous-ethanolic extracts are reported.

2. Materials and Methods

2.1. Collection of Plant Material and Preparation

Mature fresh roots and aerial parts of 3 *Carex* species [*C. divisa* Huds., *Carex* ***melanostachya*** M. Bieb. ex Willd. (syn.: *C. nutans* Host) and *C. stenophylla* Wahlenb] were collected from Iran area during 2013 as described in Table 1. Plants identified using available references [2, 10, 11, 12]. Specimens of each sample were prepared for reference as herbarium vouchers that were deposited at the Arak University Herbarium. Root and aerial parts samples were separately air dried for detection and identification of flavonoids.

2.2. 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, and aerial parts 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 2-Dimensional Paper Chromatography (2-D PC).

2.3. Flavonoid analysis by 2-Dimensional Paper Chromatography (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 2µl). 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.

2.4. Methods of Identification of the Flavonoids

After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from 3 *Carex* species roots and aerial parts, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids and by acid hydrolysis to identify the aglycone and sugar moieties [13, 14]. 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).

2.5. 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 2M 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 [15].

3. Results

All studied *Carex* species contained flavonoid compounds in their roots and aerial parts. Their flavonoid profiles showed a wide variety between the species. Data in Tables 1 and 2 show the sampling and also 2-dimentional paper and thin layer chromatographical data of 3 studied *Carex* species from Iran. Figure 1 shows stacked column with a 3-D visual effect histogram for comparing root and aerial parts flavonoids data (number of flavonoid sulphate, number of flavone *C*-and *C-/O*-glucosides, number of aglycones and occurrence of Apigenin, Chrysin, Isorhamnetin, Kaempferol, Luteolin, Morine, Myricetin, Narengenin, Quercetin, Rhamnetin, Rutin, Tricine and Vitexin) in the species. The studied taxa showed variety in their root and aerial parts flavonoids compounds. As Table 1 and Figure 1 show all 3 studied species contain flavone *C* and *C-/O*-glycosides in their roots and aerial parts. Flavonoid sulphates were found in all of roots and aerial parts of the studied species with the exception of *C. stenophylla* root and aerial parts. Aglycones was not found in ***C. melanostachya*** aerial parts, where as other samples had. Rutin, Myricetin, Kaempferol, Loteulin, Narengenin, Apigenin, Morin, Rhamnetin and Chrysin were found in their root or aerial parts, while all of samples lacked Isorhamnetin, Tricin and Vitexin. *C. divisa* root and *C. stenophylla* aerial parts had the most flavonoids number. At least flavononoid number was observed in aerial parts of ***C. melanostachya***.

Table 1. Collection information and 2-Dimentional Paper Chromatography flavnoids data of 3 studied *Carex* species roots and aerial parts from Iran

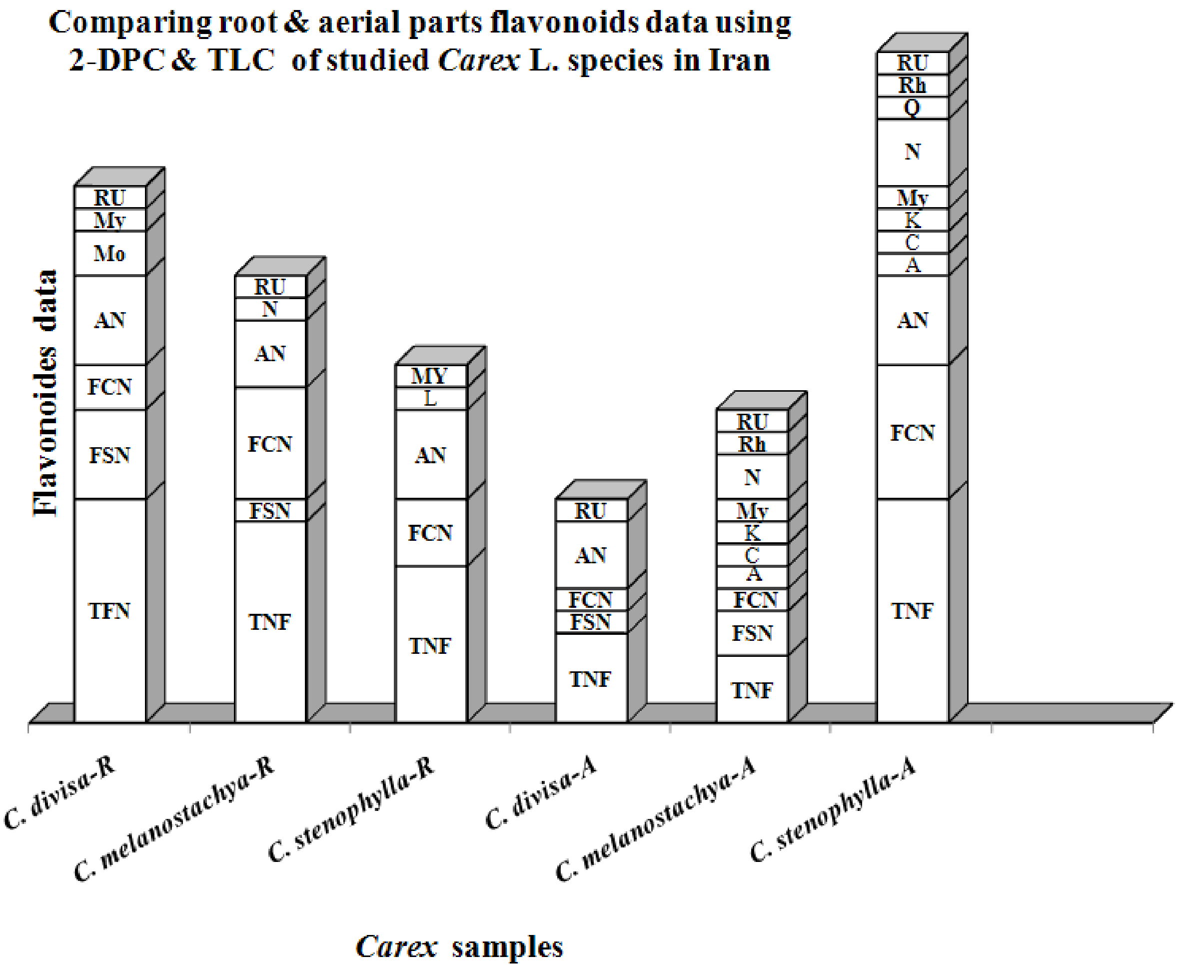
Voucher data	Taxon	Latitude	Longitude	Altitude (m)	Flavonoid type: Total flavonoids Number	Flavonoid sulphates Number	Flavone *C*-and *C*-/*O*-glucosides Number	Aglycones Number
* CMNR$_1$	*C. divisa* Huds.	34° 03´ N	49° 23´ E	1800	10	4	2	4
CMnR$_2$	*C.* ***melanostachya*** M. Bieb. ex Willd. (syn.: *C. nutans* Host	34° 08´ N	49° 01´ E	1300	9	1	5	3
CMNR$_3$	*C. stenophylla* Wahlenb	34° 03 □ N	49° 23´ E	1800	7	0	3	4
CMNA$_1$	*C. divisa* Huds.	34° 03´ N	49° 23´ E	1800	4	1	1	3
CMNA$_2$	*C.* ***melanostachya*** M. Bieb. ex Willd. (syn.: *C. nutans* Host	34° 08´ N	49° 01´ E	1300	3	2	1	0
CMNA$_3$	*C. stenophylla* Wahlenb	34° 03 □ N	49° 23´ E	1800	10	0	6	4

❋ CMN= Mitra Noori *Carex* Collection Number, R=root, A=aerial parts.

Table 2. Thin Layer Chromatography data of 3 studied *Carex* species roots and aerial parts from Iran

Voucher data	Flavonoids Identification: Apegenin	Chrysin	Isorhamnetin	Kaempferol	Luteolin	Morine	Myricetin	Narengenin	Quercetin	Rhamnetin	Rutin	Tricine	Vitexin
* CMNR$_1$	-	-	-	-	-	++	+	+++	-	-	+	-	-
CMNR$_2$	-	-	-	-	-	-	-	++	-	-	+	-	-
CMNR$_3$	-	-	-	-	+	-	+	-	-	-	-	-	-
CMNA$_1$	-	-	-	-	-	-	-	-	-	-	+	-	-
CMNA$_2$	+	+	-	+	-	-	+	+	-	+	+	-	-
CMNA$_3$	+	+	-	+	-	-	+	-	+	+	+	-	-

Scored characters: -0 (non flavonoid), +1 (few flavonoid), ++2 (middle concentration of flavonoid), +++3 (high concentration of flavonoid).



Abbreviation: NTF=number of total flavonoids, FS=number of flavonoid sulphate, FC=number of flavone *C*-and *C-/O*-glucosides, Agl=number of aglycones, A=apigenin, C=chrysin, K=kaempferol, L=luteulin, R=rutin, Q=quercetin, Mo=morin, My=myricetin, N=narigenin, R=rhamnetin, -R=root, -A=aerial part.

Figure 1. Stacked column with a 3-D visual effect histogram for comprising root and aerial parts flavonoids data using 2-Dimentional Paper and Thin Layer Chromatography of studied *Carex* L. species from Iran. Scored characters for drowing 3-D column histogram in Excel based on Table 1 data: -0 (non flavonoid), ± 1 (UV absorbance < 0.1), + 2 (few flavonoid), ++ 3 (high concentration of flavonoid) and +++ 4 (very high concentration of flavonoid)

4. Discussion and Conclusions

Flavonoids are as one set of the polyphenolic compounds among secondary metabolites in different organs of plants that are popular compounds for chemotaxonomic surveys of plant genera and families [16]. Also many flavonoids are active principles of medicinal plants, exhibit pharmacological effects and contribute to human health [16-18]. Several studies indicated that flavonoids occurred in various species of Cyperaceae [19-21]. 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 [22]. 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 and flavonoids may be useful taxonomic markers within the family [22, 23]. These results show that phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e. g. at the species and genus level [16]. Noori (2014) compared 10 populations root and leaf flavonoids profiles of 5 *Scirpus* species from Markazi Province, Iran for introducing chemotypes. Her results showed all of studied Scirpus populations contain vitexin, luteolin, rutin and rhamnetin in their aerial parts and roots. Also morin and tricin are two separator phytochemical characters for studied samples [24].

As we know Cyperaceae flavonoids are very important for their different potential clinical applications such as their toxicity, antidiarrhoeal, antibacterial, antiflogestic, tonic and stimultant effects [25-27]. *S. lacustris* is used as local medicinal plant in Canada [28], its stem known as antibacterial drug and is effective on *E. coli* [29]. Studying root flavonoids of 5 *Scirpus* L. species from Cyperaceae (*S.*

holoschenus L., S. lacustris L., S. littoralis Kuntze, S. maritimus L. and *S. multicaule*) from different parts of Markazi Province, Iran area 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 [30]. Plants of the *Carex* genus have attracted recent attention as potential food additives because they contain high levels of bioactive polyphenols commonly found in plant foods [3]. As Fiorentino et al (2008) studies showed high quantities of polyphenols in *C. distachya* species root methanol extract that could be used as a source of natural antioxidants useful as potential food additives [1]. Li et al (2009) isolated five flavonoids (isoorientin, luteolin, quercetin, 3-*O*-methylquercetin, and rutin) from C. folliculate seeds [3]. Also Manhart (1986) reported foliar flavonoids of the North American members of the *Carex* section Laxiflorae [5].

Our results showed existing flavonoid sulphates in all of roots and aerial parts of the studied species with the exception of *C. stenophylla* root and aerial parts. Aglycones was not found in ***C. melanostachya*** aerial parts, where as other samples had. The studied taxa showed variety in their root and aerial parts flavonoids compounds. Rutin, Myricetin, Kaempferol, Loteulin, Narengenin, Apigenin, Morin, Rhamnetin and Chrysin were found in their root or aerial parts, while all of samples lacked Quercetin, Isorhamnetin, Tricin and Vitexin (Tables 1 and 2, Figure 1)). Based on these results it is concluded that the quantities and presence of important metabolites such as flavonoids depend on the various parts of the plant used. Therefore, depth study of *Carex* L. medicinal ingredients and flavonoids can provided the basis for further development and utilization.

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