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Leaf flavonoids of *Convolvulus* L. species in Markazi Province, Iran

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Abstract

In order to study the leaf flavonoids pattern of *Convolvulus* leaves in 12 populations from four species (*C. arvensis*, *C. commutatus*, *C. lineatus* and *C. pilosellaefolius*) originated from Markazi Province, Iran, two-dimensional paper chromatography (2-DPC) and thin layer chromatography (TLC) were used. Results indicated that the leaves contained flavonoid sulfates and flavones *C* and *C*-/*O* glycosides, quercetin, kaempferol, isorhamnetin, myricetin, rhamnetin, rutin, apigenin, chrysin, luteolin, vitexin, genistein, hesperidin and naringenin. All of *C. arvensis* populations and one *C. commutus* population (CBB_9) had morin while the rest lacked. Tricine was not found in any of the taxa and Quercetin was the most found flavonoid. There was not any aglycone in the studied populations with the exception of *C. lineatus*. It seems that the aglycone pattern is useful for the separation of *C. lineatus* species from the rest.

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Introduction

Flavonoids are found in fruits, vegetables, grains, bark, roots, stems, leaves and flowers (Middleton 1998, Robles *et al*., 2003). Flavonoid compounds are taxonomically important. They have popular characters for chemosystematics studies because the almost universal presence of flavonoids in vascular plant, their structural diversity, the fact that each species usually contains several flavonoids and chemical stability of many flavonoids in dried plant material. Flavonoid profiles using different chromatographic techniques are easily obtained and are reasonably easy to identify using published UV spectra data and available standards. They often show correlations with existing classifications at these levels, and support revisions of existing classifications at the family, genus and species level (Noori, 2012). Plant 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, Moor and Giannasi 1994, Noori *et al.* 2009). Noori (2014) compared root and leaf flavonoids profiles from 10 populations of five *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.

The Convolvulaceae (Morning Glory family) is a beautiful family which is widely cultivated as ornamentals (Rajurkar *et al*., 2011). *Convolvulus* from *Convolvuleae* (Convolvulaceae) has about 250 species worldwide (Perveen *et al*., 1989) and 60 species in Iran (Ghahreman, 1994). The family is widely distributed in cold regions, temperate, subtropical and tropical areas all over the world. There are several chemical studies both in the family Convolvulaceae and the genus *Convolvulus*. Alkaloids were reported from *Convolvulus* (Mothes and Romeike, 1958), while acylated anthocyanins have been identified in many genera e.g. *Ipomoea* (Pomilio and Sproviero, 1972), *Convolvulus* (Tronchet, 1966) and *Calystegia* (Uneo *et al*., 1969). Rutin (quercetin 3-rutinoside), isoquercetin and kaempferol, 3-rhamnoglucoside and the coumarins scopoletin and umbelliferone have been recorded in the family (Tronchet, 1966). Rizk (1982) and El- Nasr (1983) studied *Convolvulus* species and showed the presence of flavonoids but they did not identify their constituents. Some species of Convolvulaceae produce resin where glycosides from which D-glucose, D-rhamnose, D-fucose and D-quinovose have been isolated (Anthonsen *et al*., 1979). Studies on phenolic constituents of *Ipomoea eriocarpa* and *I. sindica* using 2D paper chromatography and TLC methods showed seven flavonoid glycosides in *I. eriocarpa* and four of these compounds in *I. sindica* (Khatoon and Husain, 1992). Mann *et al.* (1999) isolated flavonoid sulfates from some convolvulaceae members such as *Argyreia mollis*, *A. capitata*, *Ipomoea reticulate* and *I. regnellii*. Menemen *et al.* (2002) studies on 20 *Convolvulus* taxa showed that aglycone pattern was useful for separation of some species in the genus. They found quercetin, kaempferol, isorhamnetin, luteolin and cichorin (hydroxycoumarin) in their studied species leaves. Isorhamnetin 3-gucoside, quercetin 3-glucoside and 3-galactoside and luteolin 5-glucoside were identified in *C. mazicum*. Atta *et al.* (2007) isolated kaempferol and quercetin from *C. fatmensis* Ktz. Mojab *et al.* (2003) showed presence of flavonoids in *C. arvensis*. Kaur and Kalia (2012) reported 7-*O*-β-D-glucoside, 3-*O*-β-D-galactorhamnoside, 7-*O*-rutinoside, 3-*O*-α-L-rhamnosyl, 3-*O*-α-L-rhamnoside, Kaempferol -3-*O*-β-D-glucoside and quercetin -3-*O*-α-L-rhamnoside in root, aerial parts and flower of *C. arvensis*. In addition, they found, kaempferol in the species aerial parts. Madhavan *et al.* (2008) carried out physico-chemical analysis on *C. microphyllus* and *Evolvulus alsinoides* and identified their phenolic compounds, but not the type of them. Bhowmik *et al.* (2012) showed the presence of alkaloids, glycosides, coumarins and flavonoids in *C. pluricaulis*. Moreover, Andrade *et al.* (2012) found kaempferol in *C. pluricaulis*.

This study presents the leaf flavonoid patterns of 12 collected *Convolvulus* (*C. arvensis, C. commutatus, C. lineatus and C. pilosellaefolius*) populations from

different parts of Markazi Province, Iran for understanding flavonoids role in Convolvulaceae chemotaxonomy. This is a novel report on *Convolvulus* leaf flavonoid patterns. In addition, some of flavonoid types in *C. arvensis* were identified for the first time.

Materials and methods

Plant collection and preparation

Mature fresh leaves of 12 *Convolvulus* populations were collected from different parts of Markazi Province, Iran during 2013 spring as described in Table 1. Samples were identified using available references (Rechinger, 1963; Ghahreman, 1979-2006, 1993; Nowroozi, 2002). Voucher specimens of each sample were prepared for reference as herbarium vouchers. Samples were air dried for detection and identification of their flavonoids.

Plant extract preparation

For a comparative analysis of the flavonoids, small extracts of all the accessions were prepared by boiling 200 mg of powdered air dried leaf 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°C, and taken up in 2 ml of 80% MeOH for analysis by 2-Dimensional Paper Chromatography (2-DPC) (Markham 1982).x

Two-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-AcOH-H_2O=4:1:5; V/V; upper layer), 1st direction, and AcOH (=15% aqueous acetic acid), 2nd direction, with rutin (quercetin 3-*O*-rutinoside) as a standard. After development, the chromatograms were viewed in long wave UV light (366 nm) and any dark absorbing and fluorescent spots were marked. R_f values in BAW and 15% AcOH were calculated.

Flavonoids identification

After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from leaf of the population, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids (Mabry *et al.*, 1970, Markham *et al.*, 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, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, morin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine and vitexin (all obtained commercially from Merck, apigenin, luteolin and hesperidin 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 2M HCl was 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 was 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 and discussion

Results showed all of studied *Convolvulus* samples contained flavonoid compounds in their leaves. Data in Tables 1 and 2 show the collection information and also 2-dimentional paper and thin layer chromatographical data of 12 studied *convolvulus* populations from Markazi Province.

Fig. 1 shows stacked column with a 3-D visual effect histogram for comparing leaf flavonoids data (number of total flavonoids, flavonoid sulphates number, flavone *C*-and *C*-/*O*-glucosides number,

aglycones number and occurrence of apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, morin, myricetin, naringenin, quercetin, rhamnetin, rutin and vitexin in the populations).

As indicated in Table 1 and 2 and also Fig. 1 quercetin was detected in all of the populations whereas tricin was not found. Morin was not detected in all taxa with the exception of *C. arvensis* (CBB_4, CBB_5, CBB_7 and CBB_{19}) and *C. commutatus* (CBB_9). Chrysin and naringenin were identified in all of the studied populations. Furthermore, apigenin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, myricetin, rhamnetin, rutin and vitexin were found in all of the studied taxa. Total flavonoids and flavonoid sulfates number in *C. commutatus* populations were higher than the others. Only one flavonoid aglycone was found in *C. lineatus* and others lacked.

Table 1. Collection information and leaf 2-Dimentional Paper Chromatography data for 12 studied *Convolvulus* populations from Markazi Province, Iran.

Code	Taxon	Flavonoids type								
		Date	Sampling locality	Latitude N	Longitude E	Altitude (m)	Total flavonoids number	Flavonoid sulfates number	Flavonoid C- & *C-/O*-glucosides number	Number of aglycones
*CBB4	*C. arvensis*	24.05.2013	Arak - Shahsavaran	34° 09'	49° 59'	1680	9	5	4	0
CBB5	*C. arvensis*	24.05.2013	Arak - Shahsavaran	34° 09'	49° 59'	1680	8	3	5	0
CBB7	*C. arvensis*	24.05.2013	Tafresh	34° 41'	50° 01'	1948	9	3	6	0
CBB19	*C. arvensis*	13.06.2013	Delijan - Naragh	34° 00'	50° 50'	1848	8	6	2	0
CBB2	*C. commutatus*	17.05.2013	Arak - Sardasht	34° 04'	49° 37'	1870	9	4	5	0
CBB8	*C. commutatus*	27.05.2013	Arak - Nazmabad	34° 03'	49° 44'	1884	11	7	4	0
CBB9	*C. commutatus*	30.05.2013	Arak - Gerdo	34° 02'	49° 41'	1886	9	5	4	0
CBB13	*C. lineatus*	04.06.2013	Arak - Zaloo	33° 51'	49° 56'	2026	10	6	3	1
CBB6	*C. pilosellaefolius*	24.05.2013	Saveh - Saft	34° 37'	50° 23'	1350	9	4	5	0
CBB10	*C. pilosellaefolius*	04.06.2013	Delijan - Hajiabad	34° 15'	50° 32'	1332	8	5	3	0
CBB11	*C. pilosellaefolius*	04.06.2013	Delijan - Dodahak	34° 07'	50° 35'	1377	7	3	4	0
CBB12	*C. pilosellaefolius*	04.06.2013	Delijan -15 Khordad Park	34° 02'	50° 40'	1507	8	4	4	0

*CBB: Batoul Bahrami collection numbers.

More than 4000 varieties of flavonoids have been identified in different higher and lower plant species (De Groot and Rauen, 1998). The main flavonoid groups are flavones (e.g. luteolin), flavanone (e.g. naringenin), flavonols (e.g. kaempferol), anthocyanidins (e.g. pelargonidin) and chalcones (e.g. butein) (Harborne *et al.*, 1975).

In this research, the presence of three types of flavonoids including flavonols (quercetin, kaempferol, isorhamnetin, morin, myricetin, rhamnetin and rutin), flavones (apigenin, chrysin, luteolin, vitexin), isoflavones (genistein and tricine) and flavanone (hesperidin and naringenin) were reported in all of the studied species leaves (ILDIS). *C. arvensis* populations (CBB_4, CBB_5, CBB_7 and CBB_{19}) and *C. commutatus* (CBB_9) had morin while the rest lacked. In addition, tricine (syn: zwitterionic amino acid) as an isoflavone was not found in any population. Quercetin was the most common flavonoids in the studied taxa, then rutin and vitexin, but loteolin and

kaempferol were least in comparison with the above named.

Populations of *C. commutatus* had the most total flavonoid and flavonoid sulfate numbers (Table 1 and Fig. 1). It seems that the flavonoid number pattern would be useful for the separation of *C. commutatus* from the other *Convolvulus* species. However, it is believed that aglycones absence in all of the studied populations with the exception of *C. lineauts* would be a key element for the delimitation of *C. lineatus* from other *Convolvulus* species. Kaur and Kalia (2012) reported 7-*O*-β-D-glucoside, 3-*O*-β-D-galactorhamnoside, 7-*O*-rutinoside, 3-*O*-α-L-rhamnosyl, 3-*O*-α-L-rhamnoside, Kaempferol -3-*O*-β-D-glucoside and quercetin -3-*O*-α-L-rhamnoside in root, aerial parts and flower of *C. arvensis*. In addition, they found, kaempferol in the species aerial parts. Furthermore, Menemen *et al.* (2002) detected quercetin 3-mono- or di glycosides in all of 20 studied *Convolvulus* species, while kaempferol 3-mono-glycosides was just found in four taxa (*C. arvensis*, *C. sabatius* ssp. *sabatius*, *C. sabatius* ssp *mauritanicus* and *C. siculus* ssp. *elongatus*). They reported presence of quercetin 3-mono- or di glycosides and cichorin in *C. lineatus*. The flavonoid was found in addition to other flavonoid compounds in this species.

Table 2. Thin Layer Chromatographical data of 12 studied *Convolvulus* populations' leaf flavonoid from Markazi Province, Iran.

Code	Taxon	Flavonoids Identification														
		A	C	G	H	I	K	L	Mo	My	N	Q	Rh	Ru	T	V
*CBB_4	*C. arvensis*	+	+	+	+	+	++	++	+	++	+	+++	+	++	-	++
CBB_5	*C. arvensis*	++	+	++	++	++	++	++	+	++	+	+++	++	++	-	++
CBB_7	*C. arvensis*	+	+	+	+	+	+	++	+	++	+	+++	+	++	-	++
CBB_{19}	*C. arvensis*	+	+	+	+	+	+	++	+	+	+	+++	+	++	-	++
CBB_2	*C. commutatus*	+	+	+	+	+	++	++	-	+	+	+++	+	++	-	++
CBB_8	*C. commutatus*	+	+	+	+	+	+	++	-	+	+	++	+	+	-	+
CBB_9	*C. commutatus*	+	+	+	+	+	++	++	+	+	+	+++	+	++	-	++
CBB_{13}	*C. lineatus*	+	+	+	+	+	+	+	-	+	+	+++	+	++	-	++
CBB_6	*C. pilosellaefolius*	+	+	+	+	+	+	+	-	+	+	+++	+	++	-	++
CBB_{10}	*C. pilosellaefolius*	+	+	+	+	+	+	+	-	++	+	+++	+	++	-	++
CBB_{11}	*C. pilosellaefolius*	+	+	+	+	+	+	+	-	+	+	+++	+	++	-	++
CBB_{12}	*C. pilosellaefolius*	+	+	+	+	+	+	+	-	+	+	++	+	+	-	+

*CBB: Batoul Bahrami collection numbers.

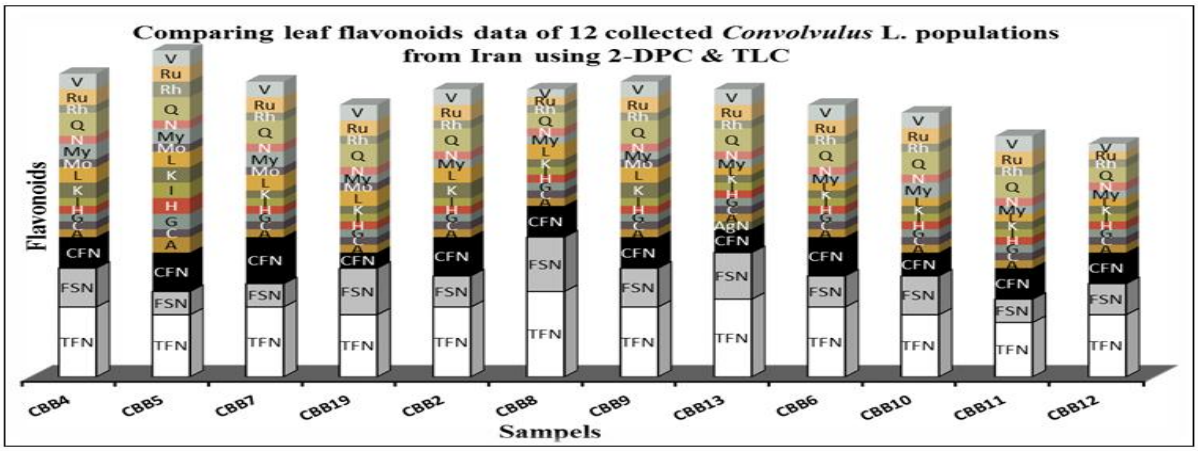

Fig. 1. Stacked column with a 3-D visual effect histogram for comparing leaf flavonoids data of *Convolvulus* populations from Markazi Province, Iran using 2-dimensional paper and thin layer chromatographically methods. Scored characters for drawing 3-D column histogram in Excel based on Table 2 data: -0 (none

flavonoid), +1 (few concentration of flavonoid), ++2 (middle concentration of flavonoid), +++3 (high concentration of flavonoid).

CBB: Batoul Bahrami collection number.

Although flavonoid compounds are taxonomically important and often show correlations with existing classifications at the family, genus, and species but rarely provide key characters since the flavonoid may be absent in one or more members of the taxon and the same flavonoid may occurs in an unrelated taxon (Harbone and Turner, 1984). These studies show that plant phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e. g. at the species and genus level as Harborne (1994), Moor and Giannasi (1994), Noori *et al.* (2009) and Noori (2014) found in their works. As we know that plant flavonoid pattern depends on genetics factors and ecological conditions and these parameters are effective on flavonoid production, it is believed that flavonoid patterns cannot always reveal the taxa differences.

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Recommendation

It is suggested that for more subtle results, studying other biosystematics characters would be required. In addition, molecular marker application along with the current research strategies could be useful and is recommended.

Abbreviations

Apigenin (A), Chrysin (C), Genistein (G), Hesperidin, (H), Isorhamnetin (I), Kaempferol (K), Luteolin (L), Morin (Mo), Myricetin (My), Naringenin (N), Quercetin (Q), Rhamnetin (Rh), Rutin (Ru), Tricine (T), Vitexin (V).data: -0 (none flavonoid), +1 (few concentration of flavonoid), ++2 (middle concentration of flavonoid), +++3 (high concentration of flavonoid).

Abbreviations

NTF=Number of total flavonoids, FSN= Flavonoid sulphates number, FCN= Flavone *C* & *C*-/*O* glucosides number, AgN= Aglycones number, Apigenin (A), Chrysin (C), Genistein (G), Hesperidin (H), Isorhamnetin (I), Kaempferol (K), Luteolin (L), Morin (Mo), Myricetin (My), Naringenin (N), Quercetin (Q), Rhamnetin (Rh), Rutin (Ru), Tricine (T), Vitexin (V).

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