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Research Article
Flower Flavonoids of *Convolvulus* L. species in Markazi Province, Iran

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Abstract
Background: Flavonoids are taxonomically important and have popular characters for chemosystematics studies by the reason universal presence in vascular plants. Also they are active principles of medicinal plants, exhibit pharmacological effects and contribute to human health. Flavonoids have roles in plant ecological adaptation, pollination, distribution and as attractant/deterrent factor for pollinator or pests. This study shows the flower flavonoid patterns of *Convolvulus* L. populations from Markazi Province, Iran for revealing flavonoids role in Convolvulaceae chemotaxonomy. This is a novel report on *Convolvulus* flower flavonoid patterns and also some of flavonoid types in *C. arvensis* were identified for the first time. **Materials and Methods:** Phytochemical studies on 12 collected *Convolvulus* populations from 4 species (*C. arvensis*, *C. commutatus*, *C. lineatus* and *C. pilosellaefolius*) were done using two-dimensional paper chromatography (2-DPC) and Thin Layer Chromatography (TLC). Voucher specimens of each species were prepared for reference as herbarium vouchers. **Results:** Results indicated that the flowers contained flavonoid sulfates and flavones C and C-/O glycosides, apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine and vitexin. Morin was not found in any of the taxa and kaempferol, hesperidin and naringenin was the most found flavonoids in order. There were not any aglycones in the studied populations. Populations of *C. commutatus* had the most number of total flavonoids and *C. lineatus* had the least one. Tricine was the rare flavonoid in all of *C. arvensis* populations and rhamnetin was the rare flavonoid in all of *C. commutatus* populations, too. **Conclusion:** It seems that kaempferol concentration pattern would be useful for separation of *C. arvensis* from the other *Convolvulus* species and also, naringenin concentration pattern would be useful for the separation of *C. commutatus* from rest.

Key words: *Convolvulus*, chemotaxonomy, chromatography, flavonoid, flower

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Flavonoids are synthesized in all parts of the plant. They play a role in providing color, fragrance and taste to the fruits, flowers and seeds, which makes them attractants for insects, birds or mammals, which aid in pollen or seed transmission[1]. Plants release various chemicals both to deter and attract insects, in some cases natural predators of herbivores feeding on a plant. One of the best established functions of flavonoid pigments is in the production of flower color and the provision of colors attractive to plant pollinators[2]. Plants that are insect-pollinated generally have flowers with large, brightly coloured petals, in contrast to most wind-pollinated plants for which flowers are small, dull and often apetalous. Pigmentation presumably acts as a signal to attract pollinating insects or birds[3]. By contrast with the very visible flavonoids in flower petals, the flavonoids present in leaves are completely hidden by the ubiquitous green of the chlorophylls. Nevertheless, there is increasing evidence that these flavonoids, particularly when they are located at the upper surface of the leaf or in the epidermal cells have a role to play in the physiological survival of plants[4]. Hosaka *et al.*[5] isolated three flavonols, myricetin 3-O-glucoside, kaempferol 3-O-rutinoside, kaempferol 3-O-glucoside and a flavone, luteolin 4-O-glucoside from the black flowers of *Alcea rosea nigra*. Also nine anthocyanins consist of delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyaniding 3-O-glucoside, cyanidin 3-O-rutinoside, petunidin 3-O-glucoside, petunidin 3-O-rhamnosylglucoside, malvidin 3-O-glucoside, malvidin 3-O-rhamnosylglucoside and malvidin 3-O-malonylglucoside was isolated from the species[5].

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[6]. Plant phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e.g., at the species and genus level[7-9].

The Convolvulaceae (Morning Glory family) is a beautiful family which is widely cultivated as ornamentals[10]. *Convolvulus* from *Convolvuleae* (Convolvulaceae) has about 250 species worldwide[11] and 60 species in Iran[12]. 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*[13], while acylated anthocyanins have been identified in many genera e.g., *Ipomoea*[14], *Convolvulus*[15] and *Calystegia*[16]. Rutin (quercetin 3-rutinoside), isoquercetin and kaempferol, 3-rhamnoglucoside and the coumarins scopoletin and umbelliferone have been recorded in the family[15]. Rizk[17] and El-Nasr[18] studied *Convolvulus* species and showed the presence of flavonoids but they did not identify their constituents[17,18]. Some species of Convolvulaceae produce resin where glycosides from which D-glucose, D-rhamnose, D-fucose and D-quinovose have been isolated[19]. 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*[20]. Mann *et al.*[21] isolated flavonoid sulfates from some convolvulaceae members such as *Argyreia mollis*, *A. capitata*, *Ipomoea reticulate* and *I. regnellii*[21]. Menemen *et al.*[22] 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[22]. Isorhamnetin 3-gucoside, quercetin 3-glucoside and 3-galactoside and luteolin 5-glucoside were identified in *C. mazicum*. Atta *et al.*[23] isolated kaempferol and quercetin from *C. fatmensis* Ktz.[23]. Mojab *et al.*[24] showed presence of flavonoids in *C. arvensis*[24]. Kaur and Kalia[25] 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*[25]. In addition, they found kaempferol in aerial parts of the species. Madhavan *et al.*[26] carried out physicochemical analysis on *C. microphyllus* and *Evolvulus alsinoides* and identified their phenolic compounds, but not the type of them[26]. Bhowmik *et al.*[27] showed the presence of alkaloids, glycosides, coumarins and flavonoids in *C. pluricaulis*[27]. Moreover Andrade *et al.*[28] found kaempferol in *C. pluricaulis*[28]. This study presents the flower 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 in addition our previous work[29]. This is a novel report on *Convolvulus* flower 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 flowers of 12 *Convolvulus* populations were collected from different parts of Markazi Province, Iran during 2013 as described in Table 1. Samples were identified using available references[12,30-32]. Voucher specimens of each sample were prepared for reference as herbarium vouchers. Samples were air dried for detection and identification of their flavonoids.

Flowers 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 flower 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)[33].

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 2 µL). 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. The 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 flower of the population, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids[34,35] and by acid hydrolysis to identify the aglycone and sugar moieties. Cochromatography with standards was also performed where its 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 2 M 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[35].

RESULTS

Results showed all of studied *Convolvulus* flowers contained flavonoid compounds. Data in Table 1 and 2 show the collection information and also 2-dimentional paper and thin layer chromatographical data of 12 studied *convolvulus* flower populations from Markazi Province, Iran. Figure 1 shows stacked column with a 3-D visual effect histogram for comparing flower flavonoids data (total flavonoids, flavonoid sulphates, flavone C- and C-/O-glucosides numbers and

Table 1: Collection information and flower 2-dimentional paper chromatographical data for 12 studied *Convolvulus* populations from Markazi province, Iran

							Flavonoid types		
Code	Taxon	Date	Sampling locality	Latitude N	Longitude E	Altitude (m)	Total flavonoids number	Flavonoid sulfates number	Flavonoid C- and C-/O-glucosides number
*CBB[4]	*C. arvensis*	24.05.2013	Arak-Shahsavaran	34°09′	49°59′	1680	8	3	5
CBB[5]	*C. arvensis*	24.05.2013	Arak-Shahsavaran	34°09′	49°59′	1680	7	4	3
CBB[7]	*C. arvensis*	24.05.2013	Tafresh	34°41′	50°01′	1948	10	6	4
CBB[19]	*C. arvensis*	13.06.2013	Delijan-Naragh	34°00′	50°50′	1848	7	5	2
CBB[2]	*C. commutatus*	17.05.2013	Arak-Sardasht	34°04′	49°37′	1870	9	6	3
CBB[8]	*C. commutatus*	27.05.2013	Arak-Nazmabad	34°03′	49°44′	1884	11	8	3
CBB[9]	*C. commutatus*	30.05.2013	Arak-Gerdo	34°02′	49°41′	1886	11	7	4
CBB[13]	*C. lineatus*	04.06.2013	Arak-Zaloo	33°51′	49°56′	2026	6	3	3
CBB[6]	*C. pilosellaefolius*	24.05.2013	Saveh-Saft	34°37′	50°23′	1350	9	6	3
CBB[10]	*C. pilosellaefolius*	04.06.2013	Delijan–Hajiabad-Raveh	34°15′	50°32′	1332	9	6	3
CBB[11]	*C. pilosellaefolius*	04.06.2013	Delijan-Dodahak	34°07′	50°35′	1377	6	3	3
CBB[12]	*C. pilosellaefolius*	04.06.2013	Delijan-15 Khordad Park	34°02′	50°40′	1507	9	5	4

*CBB: Batoul Bahrami collection number

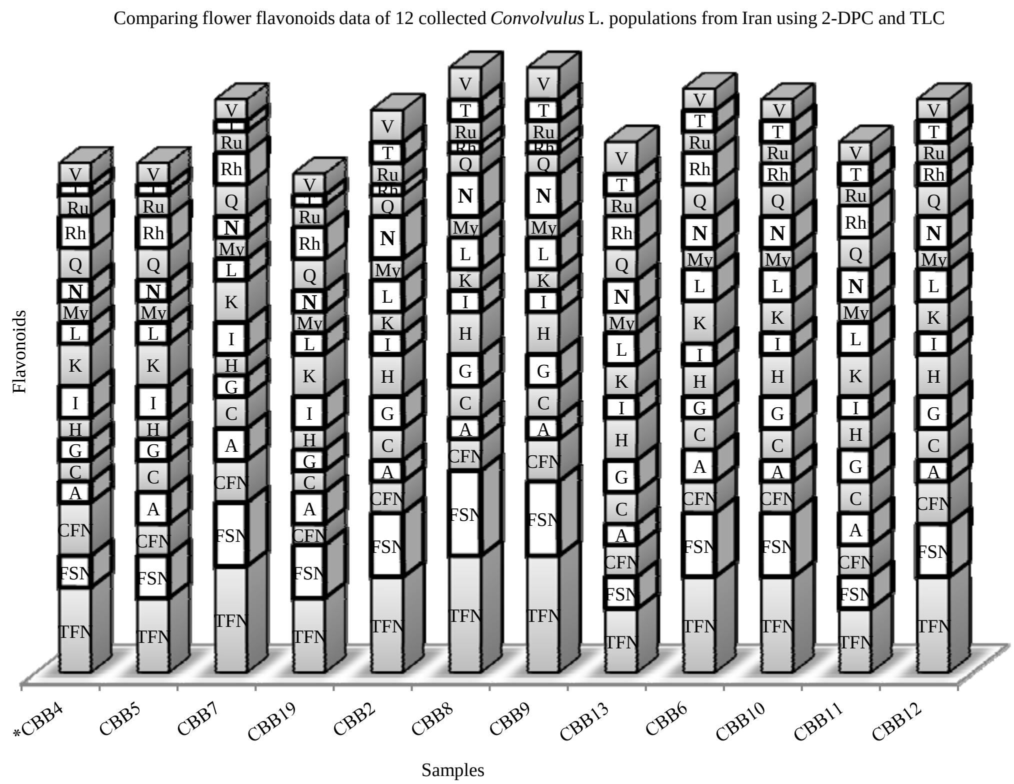

Fig. 1: Stacked column with a 3-D visual effect histogram for comparing flower flavonoids data of *Convolvulus* populations from Markazi Province, Iran using 2-dimensional paper and thin layer chromatographically methods. Scored characters for drawing 3-D column histogram in excel based on Table 2 data: -: None flavonoid, +: Few concentration of flavonoid, ++: Middle concentration of flavonoid, +++: High concentration of flavonoid, NTF: Number of total flavonoids, FSN: Flavonoid sulphates number, FCN: Flavone C and C-/O glucosides, AgN: Aglycones number, A: Apigenin, C: Chrysin, G: Genistein, H: Hesperidin, I: Isorhamnetin, K: Kaempferol, L: Luteolin, Mo: Morin, My: Myricetin, N: Naringenin, Q: Quercetin, Rh: Rhamnetin, Ru: Rutin, T: Tricine, V: Vitexin, ±: Rare flavonoid

Table 2: Thin layer chromatographical data of 12 studied *Convolvulus* populations flower flavonoids from Markazi province, Iran

		Flavonoids identification														
Code	Taxon	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*	+	++	++	+++	+	++	++	-	+	++	++	+	+	+	+

NTF: Number of total flavonoids, FSN: Flavonoid sulphates number, FCN: Flavone C and C-/O glucosides, AgN: Aglycones number, A: Apigenin, C: Chrysin, G: Genistein, H: Hesperidin, I: Isorhamnetin, K: Kaempferol, L: Luteolin, Mo: Morin, My: Myricetin, N: Naringenin, Q: Quercetin, Rh: Rhamnetin, Ru: Rutin, T: Tricine, V: Vitexin, -: None flavonoid, ±: Rare flavonoid, +: Few concentration of flavonoid, ++: Middle concentration of flavonoid, +++: High concentration of flavonoid

occurrence of apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine and vitexin in the populations). Table 1 and 2 and also Fig. 1 show that morin was not found in any of the taxa. Kaempferol, hesperidin and naringenin were the most found flavonoids, in order. There were not any aglycones in the studied populations. Populations of *C. commutatus* had the most number of total flavonoids and *C. lineatus* had the least one.

DISCUSSION

More than 4000 varieties of flavonoids have been identified in different lower and higher plant species[36]. 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)[37]. In this study, the presence of three types of flavonoids including flavonols (quercetin, kaempferol, isorhamnetin, 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 flowers (ILDIS)[38]. Rhamnetin was the rare flavonoid in *C. commutatus* (CBB_2, CBB_8 and CBB_9) populations. In addition, tricine (Syn: zwitterionic amino acid) as an isoflavone was rare in *C. arvensis* populations (CBB_4, CBB_5, CBB_7 and CBB_{19}), while was plentiful in the rest. Kaempferol was the most flavonoid in *C. arvensis* and naringenin was the most flavonoid in *C. commutatus* populations. It seems that kaempferol concentration pattern would be useful for the separation of *C. arvensis* from the other *Convolvulus* species and also, naringenin concentration pattern would be useful for the separation of *C. commutatus* from rest. Also *C. commutatus* species had the most total flavonoids and flavonoid sulfates numbers (Table 1, Fig. 1). It seems that the flavonoids number pattern would be useful for the separation of *C. commutatus* from the other *Convolvulus* species. As Bahrami *et al.*[29] showed aglycones were not found in 12 populations of four *Convolvolus* species (*C. arvensis*, *C. commutatus*, *C. lineatus* and *C. pilosellaefolius*) leaves. Their study confirmed that the aglycones pattern is useful for the separation of *C. lineatus* species from the rest[29]. Kaur and Kalia[25] 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 aerial parts of the species[25]. Kaempferol, hesperidin and naringenin were the most found flavonoids in order in this study (Table 2). Furthermore, Menemen *et al.*[22] 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*)[22]. They reported presence of quercetin 3-mono- or di-glycosides and cichorin in *C. lineatus*. These flavonoids were found in addition to other flavonoid compounds in this species.

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[39]. 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 found in the previous studies[7-9,29,40]. As we know that plant flavonoid pattern depends on genetics factors and ecological conditions and these parameters are effective on flavonoids production, it is believed that one organ flavonoid patterns cannot always reveal the taxa differences. More work on flavonoids profiles of other species organs that collected from different regions is needed.

CONCLUSION AND RECOMMENDATION

It is suggested that for more suitable 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. Further work is needed using other techniques such as 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 *Convolvulus* species. Finally depth study of *Convolvulus* L. medicinal and food additive potentials and their flowers flavonoids can provide the basis for further development and utilization.

SIGNIFICANT STATEMENTS

- Reporting flavonoid compounds existence, number and diversity in some Iranian *Convolvulus* L. species flower for first time
- Showing flavonoids importance in *Convolvulus* chemotaxonomy, phytochemistry, pollination, specially their function in flower color agent and plant reproduction and as attractant/deterrent for pollinators or pests
- Flavonoids chemical stability in herbarium samples can help to study these compounds in many of herbarium *Convolvulus* species flower and support revisions of

existing classifications at the lower genus and species levels. Flavonoids presence/absence and their kind can be more useful for studying relationships within relatively narrow taxonomic limits, e.g., at the lower than species level (sub species, variety, cultivar or chemotype)

- *Convolvulus* flower flavonoids can be used as key and marker compounds in ecological adaptations, plant defending, plant resistance and plant chemo-diversity studies

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