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Flavonoid Constituents in Some Species of *Salvia* L. (Lamiaceae) in Iran

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Abstract

Eight *Salvia* L. species including *S. spinosa* L., *S. reuterana* Boiss., *S. macrosiphon* Boiss., *S. syriaca* L., *S. nemorosa* L., *S. virgata* Jacq., *S. sharifii* Rech. f. & Esfand. and *S. mirzayanii* Rech. f. & Esfand. were studied for flavonoid compounds. These constituents were analyzed using two-dimensional maps on silica gel 60F thin layer chromatography. The flavonoid compounds of each species were purified using column chromatography with sephadex LH20 and the type of flavonoid compounds was determined using UV spectra. Based on the findings, the highest flavonoid variations were related to hydroxylation and methoxylation patterns. Five flavonoid classes namely flavones, flavanones, flavonols, isoflavones and chalcones were determined. The flavones (92%) and isoflavones (15.6%) were the highest and the lowest flavonoid classes the eight *Salvia* species. In addition, a total of 60 flavonoid compounds were identified. Some flavonoid compounds in studied *Salvia* species were first reported for Iran. The amount of flavonoid compounds in *S. reuterana, S. nemorosa* and *S. mirzayani* (27, 24, 22 compounds, respectively) was more than the other *Salvia* species. In conclusion, the flavonoid compounds appear to be an appropriate marker in taxonomic status of *Salvia*.

Keywords: flavonoid, *Salvia*, Lamiaceae, Iran, flavones.

Introduction

Salvia L. belonging to the Lamiaceae family and Nepetoideae subfamily distributes throughout the world, in subtropical, tropical, temperate, sub- arctic and arctic areas [1, 2]. Some of the *Salvia* species are herbaceous, perennial, suffruticose, fruticose and subshrubby [1, 3]. This genus exhibits a tremendous and cosmopolitan distribution and displays an outstanding variation with nearly 1000 species worldwide and 55-61 species in Iran [1, 4]. The east of Mediterranean regions, south-west, western, eastern and central regions of Asia, south of Africa, and south and central regions of America are considered to be the main speciation centers of this taxon [2, 5, 6]. It is recognized that *Salvia* species are used in biological activity and traditional medicines such as antioxidative, antitdiabetic, antiviral, etc. [7].

Salvia is an abundant reservoir of phenolic glycosids, anthocyanins, coumarins, sterols, flavonoid and

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phenolic acids, essential oils and polysaccharides [8, 9, 10, 11, 12, 13]. Based on the literatures, studies on chemical constituents of this genus were mainly confined to the phenols (flavonoid), phenolic acids, essential oils and terpenoids [9, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]. Consequently, the flavonoid compounds were mostly identified as flavones, flavonols and flavone glycosides [9, 24]. In *Salvia*, variation patterns on the A-ring in *c*6 and/or *c*6 plus *c*8 positions, A-ring substitutions, mono-substituted and di-substituted B-ring were identified [25]. Assessment of the chemical constituents of *Salvia* extracts can possibly facilitate to better recognize the biological prospective and the taxonomic relations among the studied species [26].

Since the flavonoid constituents of this genus have not been exactly determined in Iran, there is a need for clarifying this significant pool of the taxa. In the present study, the aims are to recognize the flavonoid compounds from eight *Salvia* species including *S. spinosa* L., *S. reuterana* Boiss., *S. macrosiphon* Boiss., *S. syriaca* L., *S. nemorosa* L., *S. virgata* Jacq., *S. sharifii* Rech. f. & Esfand. and *S. mirzayanii* Rech. f. & Esfand., and explain the chemotaxonomic significance of these compounds. Some of the flavonoid constituents in this study are first reported for Iran.

Materials and Methods

Plant material

The location of eight *Salvia* species and accessions collected from natural habitats in Iran are shown in Table 1. The voucher specimens were deposited in the Herbarium of Shahrekord University (HSU).

Sample extraction

Extraction of flavonoids was based on the protocol suggested by *Rahman* (2005) [27]. The flavonoid solution was extracted from air-dried leaves (10.5 g) of eight *Salvia* species using crude 85% MeOH at 60°C. The extract was dissolventized using a rotary evaporator at 70°C for total solvent removal. Purification of flavonoids from carotene and chlorophyll was provided using n-BuOH and subsequently analyzed by two-dimensional maps (2DM) on Silica gel 60F 254 (15 mg, 67.5 ml H_2O) thin layer chromatography (TLC; 3 µm, 20 ×20 cm). The chromatogram was developed in BuOH-$C_2H_4O_2$-H_2O (BAW 3:1:1) representing an organic system and H_2O-$C_2H_4O_2$ (85:15) representing an aqueous system. Spots' detection with natural product identifiers (H_2SO_4 in MeOH) was performed under UV-366 nm [28, 29]. The purification of flavonoid compounds of each species was carried out using column chromatography (65 × 3 cm) with sephadex LH20 Sigma- Aldrich (Sephadex and MeOH 20% mixture) in 100 ml MeOH solution (with increasing MeOH content 20%, 40%, 60%, 80%, 100% and Acetone) and extracted in fractions (the amount of packing material is 50 ml for each MeOH content 20%, 40%, 60%, 80%, 100% and Acetone). The fractions were subjected to one dimensional map (1DM) on Silica gel plates (3µm). Identification of purified compounds was performed on the basis of their UV spectra (366 nm), MeOH solution and shift reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/H_3Bo_3 and MeOH. Based on TLC profiles, the retention time (Rf) of each spot was estimated for each *Salvia* species [29].

Results and Discussion

The flavonoid contents of crude extract from each *Salvia* species were investigated. Coloured spots on chromatography plates were detected. The numbers of spots observed for each species were found to be: 1) *S. macrosiphon* 27 spots, 2) *S. spinosa* 34 spots, 3) *S. reuterana* 29 spots, 4) *S. virgata* 16 spots, 5) *S. syriaca* 50 spots, 6) *S. nemorosa* 28 spots, 7) *S. sharifii* 23 spots and 8) *S. mirzayanii* 22 spots. The yellow, blue and violet spots were frequent in *Salvia* species (Table 2a). White-yellow, dark yellow, white-blue, orange, brown, yellow fluorescent, blue fluorescent, pale yellow and

Table 1. The locality of *Salvia* species in natural habitats from Iran

Species	Locality	Height (m)
S. macrosiphon	Isfahan- Hojat abad, Kharazian (128)	2189
S. reuterana	Tehran- Firouzkouh, Kharazian (132)	1791
S. syriaca	Chaharmahal-e Bakhtiari- 30 km to Ardal, Amir abad, Kharazian and Kakaeian (98)	1987
S. spinosa	Isfahan- Shams abad, Kharazian and Kakaeian (111)	1788
S. nemorosa	Chaharmahal-e Bakhtiari- Gandoman, Kharazian and Kakaeian (109)	1867
S. virgata	Lorestan- Oshtoran kouh, Kharazian (13)	1980
S. sharifii	Kerman- Sirjan, Kharazian (60)	1700
S. mirzayani	Fars- Marvdasht, Fatahi (25)	1850

Table 2a. Presence and absence of each spot in *Salvia* species before and after detection of natural products

Species	1	2	3	4	5	6	7	8	9	10	11	12
S. macrosiphon	+, +a	+	+, +a	+, +a	-	-	-, +a	-, +a	-	-	-	-
S. spinosa	+, +a	-	+	+	-	+	+	+, +a	+	+	-	-
S. reuterana	+, +a	-	+, +a	-	+	-	-	-, +a	-	-, +a	+a	-
S. syriaca	+, +a	-	+, +a	+	-	+	+	+	-, +a	+	-	-
S. nemorosa	+	-	+	+	+	+	-	-	-	-	-	+a
S. virgata	+	-, +a	+, +a	+	-	-	-	-, +a	-	-	-	+a
S. sharifii	+	+, +a	+, +a	+	-	+	-	-	-	-	-	+a
S. mirzayanii	+	-	+	-	-	-	-	+	-	-, +a	-	-

a: the spots after detection of natural product. 1: Yellow, 2: white-yellow, 3: blue, 4: violet, 5: dark yellow, 6: white-blue, 7: orange, 8: fluorescent yellow, 9: brown, 10: fluorescent blue, 11: pale yellow, 12: pale blue.

Table 2b. Rf value of each spot from eight *Salvia* species based on TLC chromatogram.

Species	Rf
S. macrosiphon	1= 0.21, 2= 0.3, 3= 0.6, 4= 0.49, 5= 0.38, 6= 0.13, 7= 0.21, 8= 0.16, 9= 0.3, 10= 0.53, 11= 0.62, 12= 0.62, 13= 0.6, 14= 0.59, 15= 0.68, 16= 0.64, 17= 0.66, 18= 0.66, 19= 0.68, 20= 0.84, 21= 0.83, 22= 0.86, 23= 0.98, 24= 1, 25= 1, 26= 0.97, 27= 0.95
S. spinosa	1= 1.2, 2= 1.1, 3= 1.1, 4= 0.88, 5= 0.77, 6= 0.64, 7= 0.65, 8= 0.59, 9= 0.56, 10= 0.76, 11= 0.86, 12= 0.83, 13= 0.77, 14= 0.83, 15= 0.56, 16= 0.56, 17= 0.89, 18= 0.88, 19= 0.81, 20= 0.81, 21= 0.15, 22= 0.99, 23= 0.8, 24= 0.68, 25= 0.6, 26= 0.5, 27= 0.54, 28= 0.36, 29= 0.97, 30= 0.84, 31= 0.75, 32= 0.83, 33= 0.62, 34= 0.45
S. reuterana	1= 0.24, 2= 0.27, 3= 0.28, 4= 0.5, 5= 0.7, 6= 0.6, 7= 0.55, 8= 0.53, 9= 0.54, 10= 0.75, 11= 0.67, 12= 0.82, 13= 0.7, 14= 0.7, 15= 0.72, 16= 0.82, 17= 0.83, 18= 0.89, 19= 1, 20= 1.03, 21= 0.92, 22= 0.86, 23= 0.91, 24= 0.81, 25= 0.92, 26= 0.97, 27= 0.95, 28= 1.04, 29= 1.4
S. syriaca	1= 1, 2= 1, 3= 0.74, 4= 0.77, 5= 0.54, 6= 1, 7= 0.85, 8= 0.76, 9= 0.67, 10= 0.57, 11= 1.04, 12= 0.77, 13= 1.04, 14= 0.83, 15= 0.78, 16= 0.68, 17= 0.61, 18= 0.56, 19= 0.85, 20= 1.05, 21= 1.02, 22= 0.85, 23= 0.75, 24= 0.67, 25= 0.51, 26= 0.53, 27= 0.68, 28= 0.7, 29= 0.8, 30= 0.9, 31= 1.06, 32= 0.92, 33= 0.68, 34= 0.64, 35= 0.81, 36= 1.08, 37= 1.02, 38= 0.82, 39= 0.65, 40= 0.42, 41= 0.53, 42= 0.45, 43= 0.65, 44= 0.77, 45= 1.01, 46= 1.05, 47= 1, 48= 0.84, 49= 0.72, 50= 0.61
S. nemorosa	1=0.11, 2= 0.25, 3= 0.35, 4= 0.45, 5= 0.53, 6= 0.48, 7= 0.61, 8= 0.45, 9= 0.67, 10= 0.4, 11= 0.35, 12= 0.51, 13= 0.69, 14= 0.72, 15= 1.06, 16= 0.87, 17= 0.83, 18= 0.96, 19= 1.03, 20= 0.94, 21= 1, 22= 1.06, 23=1.09, 24= 1.12, 25= 1.12, 26= 1.22, 27=1.22, 28= 1.25
S. virgata	1= 0.69, 2= 1.6, 3= 0.94, 4= 1.2, 5= 1.5, 6= 1.8, 7= 2.1, 8= 1.8, 9= 1.8, 10= 1.7, 11= 1.7, 12= 1.3, 13= 1.1, 14= 0.86, 15= 0.86, 16= 1.3
S. sharifii	1= 0.59, 2= 0.63, 3= 0.76, 4= 0.93, 5= 0.81, 6= 0.78, 7= 0.81, 8= 0.9, 9= 0.96, 10= 0.96, 11= 0.93, 12= 0.84, 13= 0.81, 14= 0.84, 15= 1.04, 16= 1.07, 17= 1.3, 18= 1.3, 19= 1.1, 20= 1.3, 21= 1.2, 22= 1.25, 23= 1.2
S. mirzayanii	1= 0.15, 2= 0.23, 3= 0.3, 4= 0.37, 5= 0.44, 6= 0.5, 7= 0.62, 8= 0.7, 9= 0.82, 10= 0.76, 11= 0.9, 12= 0.95, 13= 0.95, 14= 0.95, 15= 0.92, 16= 1.1, 17= 1.1, 18= 1.1, 19= 1.1, 20= 1.1, 21= 1.1, 22= 1.1

pale blue spots were observed in some of these species (Table 2a).

After detection of natural products, we observed colour variations and new colour spots including yellow, white-yellow, yellow fluorescent, blue fluorescent, orange, violet, brown, pale yellow, blue and pale blue (Table 2a). In addition, the highest Rf value was observed in *S. virgata* (Rf= 2.1) and the lowest was in *S. nemorosa* (Rf= 0.11) (Table 2b).

The percentage of each substitution was 71.25% hydroxylation, 50% methoxylation, 25% rhamnoglucosylation, glucosylation, glucoronosylation, 3' and 4'-methylenedioxidation, 18.75% rhamnosylation and 12.5% rutinosylation, rhamnogalactosylation and galactosylation. B-ring*ortho*-dihydroxylation observed in *S. spinosa*, *S. macrosiphon, S. reuterana, S. syriaca, S. mirzayanii* and *S. nemorosa* and A-ring*ortho*-dihydroxylation observed in *S. spinosa, S. reuterana, S. mirzayanii, S. syriaca* and *S. nemorosa* (Table 3).

In this study, the five flavonoid classes such as flavones, isoflavones, flavanones, flavonols and chalcones were identified and 60 flavonoid compounds were found from the leaves of eight *Salvia* species (Table 4). The highest flavonoid compounds in eight *Salvia* species were flavones (28 derivatives) and the lowest were isoflavones and chalcones (6 derivatives) (Table 3). The quantities of flavonoid compounds in *S. reuterana*, *S. mirzayanii* and *S. nemorosa* were considerably higher than the other species: 27 compounds in *S. reuterana*, 24 in *S. nemorosa*, 22 in *S.*

Table 3. The flavonoid variation patterns (oxidation) in eight *Salvia* species

Variation patterns/ species	*spinosa*	*macrosiphon*	*reuterana*	*syriaca*	*nemorosa*	*virgata*	*sharifii*	*mirzayanii*
A-ring*ortho*-dihydroxyltion	+	-	+	+	+	-	-	+
B-ring*ortho*-dihydroxylation	+	+	+	+	+	-	-	+
2-hydroxylation	-	+	-	-	+	-	+	-
3-hydroxylation	+	+	-	-	+	+	+	+
5-hydroxylation	+	+	+	+	+	+	+	+
6-hydroxylation	+	-	+	-	+	-	-	+
7-hydroxylation	+	+	+	+	+	+	+	+
8-hydroxylation	-	+	-	+	+	+	+	+
2'-hydroxylation	-	+	+	-	+	-	+	+
3'-hydroxylation	+	+	+	+	+	+	+	+
4'-hydroxylation	+	+	+	+	+	+	+	+
5'-hydroxylation	-	-	-	-	+	-	-	-
5-methoxylation	-	+	+	-	+	-	-	-
6-methoxylation,	-	+	+	-	+	+	+	+
7-methoxylation	-	+	+	+	+	+	+	+
8-methoxylation	-	-	-	-	-	-	-	+
2-methoxylation	+	-	-	-	-	-	-	-
3'-methoxylation	-	-	-	-	+	+	-	+
4'-methoxylation	-	+	+	+	+	+	+	+
7-*o*-rhamnoglucosylation	+	-	+	-	+	+	-	-
8-*c*-rhamnoglucosylation	-	-	-	-	+	-	-	-
3'-methylenedioxidation	-	-	-	-	-	+	-	+
4'-methylenedioxidation	-	-	-	-	-	+	-	+
5-*o*-glucosylation	-	-	-	+	+	-	-	-
8-*c*-glucosylation	-	-	-	-	-	+	-	-
6-*c*-glucosylation	-	-	-	+	-	-	-	+
7-*o*-rhamnosylation	-	-	+	-	+	-	-	-
3-*o*-rhamnosylation	-	-	-	-	+	-	-	-
3-*o*-rhamnogalactosylation	-	-	+	-	-	-	-	-
7-*o*-glucuronosylation	-	-	+	+	-	-	-	-
3-*o*-galactosylation	-	-	-	-	+	-	-	-
7-*o*-rutinosylation	-	-	-	-	-	+	-	-

mirzayanii, 16 in *S. macrosiphon*, 13 in *S. virgata*, 10 in *S. sharifii* and 8 in *S. syriaca* and *S. spinosa* which varied from 66.7%-3.3% (Table 4).We observed 92% flavone, 31.25% flavonol, 29.6% flavanone, 21.8% chalcone and 15.6% isoflavone.

Based on our results, the colour spots in some of *Salvia* species are based on the Nakiboglu (2002) and Kharazian's (2013) results [24, 28]. The presence of yellow fluorescent in *S. virgata* is supported by the chemotaxonomy of Nakiboglu's (2002) results [28]. Noticeably, Nakiboglu (2002) reported only four spots for this species which is not in agreement with our research (16 spots) [28].

According to previous explanations, the highest flavonoid variation concerns to hydroxylation. These variation patterns are based on the reports of Ulubellen and Topcu (1979), Ulubellen et al. (1981), Abdalla et al. (1983), Gonzalez et al.(1988), Tomas-Barberan and Wollenweber (1990), Wollenweber et al. (1992), Lu and Foo (2000), Valant-Vetschera et al. (2003), Nikolova et al. (2006), Ciesla et al. (2010), Shirsat et al. (2012) and Kharazian (2013) on *Salvia* species [8, 14, 17, 20, 22, 24-26, 30-33]. Based on the UV absorption, 2-hydroxylation, 3-hydroxylation, 6-hydroxylation, 8-hydroxylation, 2'-hydroxylation and 5'-hydroxylation were observed in some of studied species. Also, further flavonoid variations such as 5-methoxylation, 6-methoxylation, 7-methoxylation, 8-methoxylation, 2'-methoxylation, 3'-methoxylation and 4'-methoxylation were observed in some of studied species. However, a disposition towards 5-hydroxylation, 7-hydroxylation, 3'-hydroxylation, 4'-hydroxylation and a considerable degree of methoxylation was observed in eight *Salvia* species which is in accordance with our previous results [24] (Table 3). These results were supported by the reports of *Ullubelen* et al. (1981), Tomas-Barberan and Wollenweber (1990), Lu and Foo (2000), Nikolova et al. (2006) and Kharazian (2013) [8, 22, 24, 25, 31].

3-*o*-rhamnogalactosylation, 3-*o*-rhamnosylation, 3-*o*-galactosylation, 5-*o*-glucosylation, 7-*o*-glucosylation, 7-*o*-rhamnosylation, 7-*o*-rutinosylation, 7-*o*-rhamnoglucosylation, 7-*o*-glucuronosylation, 6-*c*-glucosylation, 8-*c*-rhamnoglucosylation, 8-*c*-glucosylation, 3' and 4'-methylenedioxidation

Table 4. Percentage of flavonoid constituents in *Salvia* species.

Compounds/ Species	*spinosa*	*macrosiphon*	*reuterana*	*syriaca*	*nemorosa*	*virgata*	*sharifii*	*mirzayanii*
7-hydroxyflavone (flavones)	-	-	7.1	-	7.1	-	-	4.3
3',4',7-trihydroxyflavone-7-*o*-rhamnoglucoside (flavones)	16.6	-	7.1	-	39.1	14.2	-	-
4',7-dihydroxyflavone (flavones)	-	-	-	-	-	-	22.2	-
3',4'-dihydroxyflavone (flavones)	-	-	3.5	-				4.3
5,7-dihydroxy-2'-methoxyflavone (flavones)	16.6	-	-	-	-	-	-	-
5,7-dihydroxyflavone (chrysin) (flavones)	-	-	-	-		7.1	33.3	4.3
3',4',7-trihydroxyflavone (flavones)	-	-	-	-	7.1	-	-	4.3
3,3',4'-trihydroxyflavone (flavones)								4.3
4'-methoxyflavone (flavones)	-	6.25	-	-	-	-	-	-
5,7,8-trihydroxyflavone (norwogonin) (flavones)	16.6	25	32.1	66.6	25	42.8	33.3	52.1
5,7-dihydroxy-6,8,4'trimethoxyflavone (nevadensin) (flavones)	-	12.5	-	-	-	-	-	-
Apigenin (flavones)	-	-	7.1	-	3.5	21.4	-	8.6
6-methoxyapigenin (hispidulin) (flavones)	-	6.25	-	-	-	-	-	-
Apigenin-8-*c*-glucoside (vitexin) (flavones)	-	-	-	-	7.1	7.1	-	-
Isovitexin (flavones)	-	-	-	11.1	-	-	-	4.3
5,7,3'-trihydroxy-4'-methoxyflavone (Diosmetin) (flavones)	-	-	-	11.1	-	-	-	-
Salvigenin (flavones)	-	11.76	10	-	-	-	10	-
5,4'-dihydroxy-6,7-dimethoxyflavone (Cirsimaritin) (flavones)	-	-	3.3	-	-	7.69	-	-
5,7,8-trihydroxyflavone-7-*o*-glucoronide (flavones)	-	-	-	11.1	-	-	-	-
Himenoxin (flavones)	-	12.5	28.5	-	-	-	-	8.6
Luteolin (flavones)	-	-	-	-	3.5	-	-	-
Luteolin-7-*o*-glycoside (flavones)	-	6.25	-	-	-	-	-	-
Tectochrysin (flavones)	-	6.25	-	-	-	-	22.2	-
5,6,7-trihydroxyflavone (baicalein) (flavones)	-	-	3.5	33.3	17.8	-	-	-
3',4',7-trihydroxyflavone (flavones)	-	-	7.1	-	28.5	7.1	-	-
3-hydroxy-4'-methoxyflavone (flavones)	-	-	-	-	-	-	-	4.3
7-hydroxy-3',4'-dimethoxyflavone (flavones)			3.5					
Herbacetin-8-methylether (flavonols)		6.25						
Quercetin (flavonols)			3.5					4.3

substitutions were encountered in our results (Table 3). Some of the variations are accorded with the literature reports on flavonoid exudates of some *Salvia* species [8, 13, 15, 24, 34]. In our results, rutinosylation, rhamnogalactosylation and galactosylation were found in the lowest contents.

In our results, the substituted B-ring and A-ring, 5,7-dihydroxy-2'-methoxyflavone, 5,7-dihydroxy-6,8,4'-

Table 4. (continued)

Compounds/ Species	*spinosa*	*macrosiphon*	*reuterana*	*syriaca*	*nemorosa*	*virgata*	*sharifii*	*mirzayanii*
Quercetin- 3',4',5,7-teramethylether (flavonols)			3.5					
Kaempferol (flavonols)								4.3
Kaempferol-4'-methylether (flavonols)		18.75	3.5					4.3
Kaempferol-3-*o* robinoside-7-*o* rhamnoside (robinin) (flavonols)	-	-	3.5	-	3.5	-	-	-
5,7-dihydroxy-3',4'-dimethoxyflavone (ermanin) (flavonols)	-	-	3.5	-	-	-	22.2	-
6-hydroxyluteolin-6,3'-dimethylether (jaceosidin) (flavonols)	-	-	6.45	-	3.5	-	-	-
3-hydroxy-4'-methoxyflavone (flavonols)	-	-	10.7	-	-	-	-	-
3,3',4'-trihydroxyflavone (flavonols)	-	-	-	-	7.1	50	33.3	-
3-hydroxy-3,'4'-dimethoxyflavone (flavonols)	-	-	-	-	-	7.1	-	-
3,4'-dihydroxyflavone (flavonols)	-	-	-	-	-	21.4	-	-
5,7-dimethoxyisoflavone (isoflavones)	-	6.25	-	-	-	-	-	-
Tectorigenin (isoflavones)	-	-	10.7	-	3.5	21.4	-	-
Tectorigenin 7-o-glucoside (isoflavones)	-	-	-	11.1	-	-	-	8.6
5,7-dihydroxyisoflavone (isoflavones)	-	-	3.5	-	-	-	-	-
Pseudobaptigenin (isoflavones)	-	-	-	-	32.1	57.1	-	43.4
4,5-dihydroxy-7-methoxyisoflavone (isoflavones)	-	-	-	-	-	-	-	4.3
Pomiferin (flavanones)	-	37.5	39.2	-	7.1			-
5,7-dihydroxyflavanone (flavanones)	8.3	6.25	10.7	-		6.6	33.3	4.3
Taxifolin (flavanones)	8.3	-	-	-	3.5			-
5,6,7-trihydroxyflavanone (flavanones)	16.6	12.5	3.5	-	14.2	-	-	8.6
5,6,7-trihydroxyflavanone-7-*o*-glucuronide (flavanones)	-	-	7.1	-	-	-	-	-
Naringenin (flavanones)	8.3	-	-	11.1	-	-	-	4.3
Hesperidin (flavanones)	-	-	-	-	3.5	-	-	-
Sakuranin (flavanones)	-	-	-	66.7	-	-	-	-
2,2'-dihydroxychalcone	-	6.25	3.5	-	10.7		22.2	-
2',3',4'-trihydroxychalcone	-	6.25	3.5	-	7.1	-	-	-
3,4-dihydroxychalcone	-	-	3.5	-	7.1	-	-	4.3
4'-hydroxychalcone	8.3			-	3.5			-
2',3,4,4'-tetrahydroxychalcone	-	-	-	-	10.7	-	-	-
2',3,4-trihydroxychalcone	-	-	-	-	-	-	-	4.3

trimethoxyflavone and 5,7-dihydroxy-3',4'-dimethoxyflavone were found (Table 4). 5,7-dihydroxy-6-methoxyflavone with a substituted B-ring was moderately found which is an attribute of this genus. *Tomas-Barberan* and Wollenweber (1990) and Kharazian (2013) accounted that the substituted B-ring, A-ring and Mono-substituted (4'-) or di-substituted (3', 4'-) B-rings are common in *Salvia* species [24, 25] which is based on our results.

Consistent with Lu and Foo (2000, 2002) and Kharazian's (2013) results, the 6-hydroxylated of luteolin and apigenin (flavone glycoside) were observed in *Salvia* species [8, 9, 24]. In addition, 6-methylated derivatives of apigenin and 6-hydroxylated derivatives of luteolin have all been found in *Salvia* species [14, 15]. In this research 6-methoxyapigenin was encountered but the derivatives of luteolin are related to **7**-*o*-glycoside (cinaroside) which is in accordance with the reports of Ullubelen et al. (1981) in S. tomentosa, Lu and Foo (2002), Matloubi Moghaddam et al. (2008) and Gohari et al. (2011) in S. macrosiphon (Table 3) [9, 11, 13, 31]. Takeda et al. (1994), Lu and Foo (2002), Gohari et al. (2011), Shirsat et al. (2012) and Kharazian (2013) reported that flavone-*o*-glycoside, 7-*o*-rhamnoglucoside, 5-*o*-glucoside, 8-*c*-glucoside, 6-*c*-glucoside, 3-*o*-galactoside and 3'-methylendioxide are

apparently frequent in *Salvia* which is based on our results [9, 13, 24, 33, 35]. Moreover, the 7-*o*-rhamnoglycosyle, trihydroxyflavones with 7-*o*-glucuronides and 7-*o*-rhamnosyle (flavanol glycoside) positions were observed in *Salvia* species. Also, kaempferol-3-*o*-robinoside-**7**-*o*-rhamnoside (flavonol compounds) was observed in some of *Salvia* species (Table 3). Whereas, kaempferol derivatives such as **3**-robinoside were reported in previous researches [25, 36]. Consistently, the flavonoid compounds such as quercetin were in accordance with the reports of Abdalla (1984) and Tomas-Barberan and Wolleweber (1990) in S. glutinosa, Wollenweber et al. (1992) in S. triloba L., Tsimogiannis et al. (2007) in some genera of Lamiaceae and Kharazian (2013) in *Salvia multicaulis* Vahl. and *S. sclarea* L. species [24, 25, 32, 37, 38].

In our results, the flavonoid variations such as 6-*c*-glucosyl, 8-*c*-glucosyl and 8-*c*-rhamnoglucosyl were generally identified (Table 3). The flavonoid derivatives in *Salvia* contain *c*-6 and *c*-8-substitutions [24, 25].

The hydroxylation in the 5 and 7 positions in flavanone derivatives were considerable which supports the *Kharazian*'s (2013) reports in S. hydrangea Dc. ex Benth. and S. sclarea L. [24] and Pereda-Miranda's (1986) study in *Salvia sapinae* Epling. [39]. The etherified positions in quercetin were reported in the 3, 7, 3', and 4' positions in *Salvia* species [22, 24, 40]. Moreover, the quercetin substitutions in the 3, 5, 7, 4' positions were found. Other etherified positions (Table 4) also agreed with the results of *Lu* and *Foo* (2000), *Nikolova* et al. (2006) and *Qia* et al. (2009) in some *Salvia* species [8, 22, 41].

Valant-Vetschera et al. (2003) and Kharazian (2013) reported that there is a great inclination toward gathering of 6-hydroxyflavone and their methyl ethers [20, 24]. The flavone derivatives such as 7-hydroxyflavone or 5,7-dihydroxy-2'-methoxyflavone were verified in *S. texana* [18]. Other flavone derivatives such as 5,7-dihydroxyflavone (chrysin), 5,6,7-trihydroxyflavone (Baicalein), 6-methoxyapigenin (hispidulin), tectochrysin, norwogonin, nevadensin, himenoxin, taxifolin, vitexin (apigenin-8-*c*-glucoside), isovitexin, diosmetin (5,7,3'-trihydroxy-4'-methoxyflavone), salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone), cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone), 5,7,8-trihydroxy-7-*o*-glucoronide, flavonol derivatives such as jaceosidin (6-hydroxyluteolin-6,3'-dimethylether), ermanin, herbacetin-8-methylether, flavanone derivatives such as pomiferin, hesperidin, naringenin, and isoflavone such as pseudobaptigenin, tectorigenin and chalcone derivatives were in agreement with the published results in some of the *Salvia* species [9, 14, 24, 25, 31, 33, 34, 38, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57]. Some of the flavonoid compounds in this research were first reported for Iranian *Salvia* species such as sakuranin (flavanones) and tectorigenin-7-*o*-glucoside (isoflavones) (Table 4) and it needs further studies. According to the reports of *Lu* and *Foo* (2002) and *Kharazian* (2013) iso sakuranetin (flavanones) and iso sakuranetin-7-*o*-rhamnoglucoside were encountered in *S. nicolsoniana* and *S. limbata* [9, 24]. Based on our findings and other published results, there is a relationship between the habitat where the plant grows and production of these compounds [25]. Moreover, it seems that the studied parts such as leaf, flower and root will produce the different flavonoid compounds.

The flavonoid constituents of seven *Salvia* species were first reported for Iran, especially *S. sharifii* and *S. reuterana* which are the endemic species for this country (Table 4). According to Wollenweber et al. (1992), Matloubi Moghaddam et al. (2008) and Gohari et al.'s (2011) reports, some of the flavones, flavone glycosides and flavonoid aglycones were extracted from the aerial part (flower) of *S. macrosiphon* [11, 13, 32]. They reported six flavonoid compounds such as apigenin-7,4'-dimethylether, *ß*-sitosterol, salvigenin, apigenin-7-*o*-glucoside, luteolin-7-*o*-glucoside and eupatorin [11, 13, 32]. Moreover, Wollenweber et al. (1992) reported the flavonoid aglycones methylated and flavonoid glycosides in *S. macrosiphon* [32] which are based on our results. Abdalla (1984) identified the flavonoid glycosides in *S. spinosa* [37] which does not agree with our results. Despite the high morphological similarity between *S. spinisa*, *S. macrosiphon* and especially *S. reuterana*, these are very different using flavonoid constituents (Table 4). As mentioned above, *S. reuterana* has more flavonoid compounds (28 compounds) than the two other species. Moreover, due to high morphological characters between *S. macrosiphon* and *S. sharifii*, and between *S. nemorosa* and *S. virgata* these are certainly different in their flavonoid compounds (Table 4). Earlier surveys suggested that the flavones such as apigenin were exhibited in *S. nemorosa* which is supported by our results [58] (Table 4). The flavonoid compounds in *S. syriaca* were different from the results of Hatam et al. (1992) in *S. syriaca* from Iraq [59]. *S. syriaca* is very different species in terms of flavonoid compounds which is related to the presence of 50 spots in chromatogram. Moreover, the flavonoid compounds in *S. mirzayanii* and *S. virgata* were not supported by the results of Ulubelen and Ayanoglu (1975) and Wollenweber et al. (1992) [32, 60]. It can be concluded that the flavonoid constituents are appropriate markers in chemotaxonomic studies.

Finally, flavonoid compounds in the studied species show extreme variety in Iran. Compound segregation might be related to the geographical and ecological situations [13, 61]. The ecological role of the externally accumulated flavonoids has been noted [25, 62]. Moreover, the ecological adaptation of plants applies to the results of chemotaxonomy [25, 63]. Our research showed that flavonoid variation patterns may be considered to be specific to the *Salvia* species.

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