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Identification of flavonoids in leaves of seven wild growing *Salvia* L. (Lamiaceae) species from Iran

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Abstract

This study documents the flavonoid constituents of seven *Salvia* species in Iran namely *S. atropatan*a Bunge, *S. limbata* C. A. Mey, *S. sclarea* L., *S. ceratophylla* L., *S. multicaulis* Vahl., *S. hydrangea* Dc. ex Benth., and *S. eremophila* Boiss. The studied species were collected from their natural habitats in Iran and were analyzed for their flavonoid constituents using two-dimensional thin layer chromatography with silica gel 60F 254 as solid phase. The purification of flavonoid compounds of each species was carried out using column chromatography with sephadex LH20. Based on the results, 53 flavonoid compounds were identified. The most frequent flavonoid subclasses among seven *Salvia* species were flavones (35.7%) and the least of these were dihydroflavonoles (5.3%). The most important structural variation observed in flavonoid was related to hydroxylation patterns. Among the identification of flavonoid, eight of them were reported for the first time in *Salvia* species of Iran. The highest numbers of flavonoid compounds were identified in *S. multicaulis* and *S. hydrangea*. It can be concluded that the flavonoid constituents seem to be a suitable indicator in chemotaxonomic studies in *Salvia* genus.

Key Words: identification, Iran, Lamiaceae, leaves, flavonoid, *Salvia* .

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Introduction

Salvia L. genus with about 1000 species worldwide and 55 species in Iran, is the largest genera of Lamiaceae. It is distributed all around the world, in temperate, subtropical, arctic and sub- arctic areas as well as the tropical regions of Iran (1, 2). Some of these species are perennial, herbaceous, suffruticose, fruticose and sub shrubby (1, 3). *Salvia* genus displays a remarkable range of variation and represents an enormous and cosmopolitan distribution (2). The main speciation centers of this taxon are considered to the east of Mediterranean regions, south- west, western, eastern and central Asia, South Africa and central and South America (2, 4, 5).

In recent years, studies on chemical compounds of plant species were generally constrained to the phenols and essential oils (6). *Salvia* genus is a rich source of phenolic compounds, essential oils and polysaccharides (6, 7, 8, 9, 10, 11). The flavonoid constituents have been generally identified in some of *Salvia* species (12, 13, 14, 15, 16, 17, 18, 19, 20, 21). Lu and Foo (6) revealed that the flavonoid constituents in *Salvia* genus were mostly present as flavones, flavonols and their glycosides. Furthermore, B-ring and A-ring substitutions, oxygenation on the A-ring in *c*6 and/or *c*6 plus *c*8 positions were detected in *Salvia* genus, and mono-substituted (4') and di-substituted (3',4') B-ring are frequent (22). Investigation of the chemical compounds of *Salvia* extracts can probably help to better understand the biological potential and the taxonomic relationships among the investigated species (23). It is known that Salvia species are used in traditional medicines and natural activity all around the world such as antiviral, antitumor, antioxidant, etc. (24).

As Iran is one of the centers of diversity for *Salvia* species and the flavonoid compounds of this genus have not been identified, there is a need for elucidating this genetic resource in this country. Accordingly, this study aims to identify the flavonoid constituents from seven *Salvia* species such as *S. atropatana* Bunge, *S. sclarea* L., *S. ceratophylla* L., *S. limbata* C. A. Mey., *S. multicaulis* Vahl., *S. hydrangea* Dc. ex Benth. and *S. eremophila* Boiss. and reveal the chemotaxonomic value of these compounds.

Materials and methods

Plant materials

Seven *Salvia* species were collected from their natural habitats in Iran (Table 1). The voucher specimens were deposited in the Herbarium of Shahrekord University (HSU).

Extraction and identification methods

Extraction of flavonoids was based on the protocol suggested by Markham (25). The flavonoids were extracted from air-dried leaf sample (10.5 g) of seven *Salvia* species using 85% MeOH at 60°C. The extracts were concentrated using a rotary evaporator at 70°C for total solvent removal. The separation of chlorophyll was initiated with H_2O at 60°C, filtered by Whatman paper and carotenoid pigments were removed from the flavonoid extracts using n-BuOH. The flavonoid extracts were separated from n-BuOH using a rotary evaporator at 85°C and solved in 100% MeOH. Subsequently, the crude extract was analyzed by two-dimensional thin layer chromatography

(TLC; 3 µm, 20× 20 cm) on silica gel 60F 254 (15 mg silica gel, 67.5 ml H_2O). Silica gel plates with the following solvent systems were used: 1) BuOH-$C_2H_4O_2$-H_2O (BAW 3:1:1V/V/V) representing an organic system and 2) H2O- $C_2H_4O_2$ (WA 85:15V/V) representing an aqueous system. Spots' detection with natural product identifiers (H_2SO_4/ MeOH solution) was performed under UV-366 nm (26). 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 thin layer chromatography on silica gel plates (3 µm). 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.

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

Species	Locality	Height (m)
S. hydrangea (114)	Fars- Abadeh; 31°08'N (latitude), 52°40'E (longitude); July 2010	1800
S. multicaulis (148)	Isfahan- Semirom, Vanak; 31°32'N (latitude), 51°25'E (longitude); July 2010	1950
S. ceratophylla (144)	Chaharmahal va Bakhtiari- Bostanshir; 32°05'N (latitude), 50°55'E (longitude); July 2010	2120
S. sclarea (139)	Isfahan-Daran, Damane; 33°01'N (latitude), 50°29'E (longitude); August 2010	1856
S. atropatana (142)	Kordestan- Marivan; 35°30'N (latitude), 46°25'E (longitude); August 2010	1820
S. limbata (125)	Chaharmahal va Bakhtiari- Saman, Horeh; 32°32'N (latitude), 50°45'E (longitude); July 2010	2070
S. eremophila (25)	Isfahan- Kolah Ghazi; 32°39'N (latitude), 51°43'E (longitude); August 2010	1670

Results

The two-dimensional thin layer chromatography of flavonoid patterns from each *Salvia* species showed coloured spots on chromatography plates. Total numbers of spots obtained for each species are as follows: 1) *S. hydrangea* 57 spots, 2) *S. multicaulis* 53 spots, 3) *S. ceratophylla* 24 spots, 4) S. *sclarea* 40 spots, 5) *S. atropatana* 34 spots, 6) *S. limbata* 46 spots and 7) *S. eremophila* 17 spots (Figure 1 and 2).

The yellow, blue and violet spots were common in *Salvia* species (Table 2). Orange, brown, black, dark yellow, white-blue, yellow fluorescent, blue fluorescent, pale orange, dark brown, yellow-orange and yellow-blue spots were found in some species (Table 2). In some of the studied species, colour variations and new colour spots were observed after detection with natural product identifiers which were yellow, blue, violet, brown, orange, yellow fluorescent, dark yellow, yellow-blue and pale orange (Table 2). These colour spots were first reported from *Salvia* species for Iran. The Rf

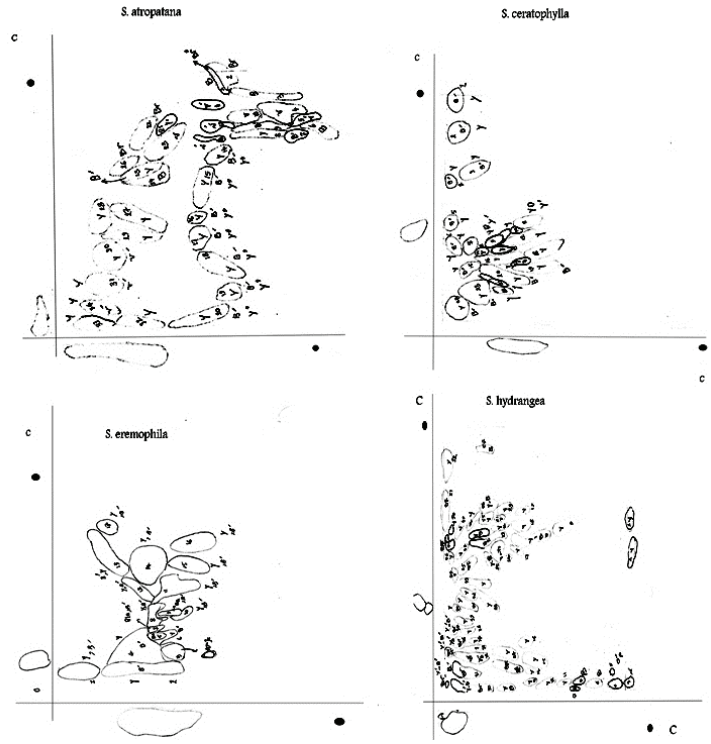

Figure 1. TLC plates in four *Salvia* species.

C: control, y: yellow, yd: dark yellow, b: blue, o: orange, op: pale orange, bf: blue fluorescent, br: brown, v: violet.

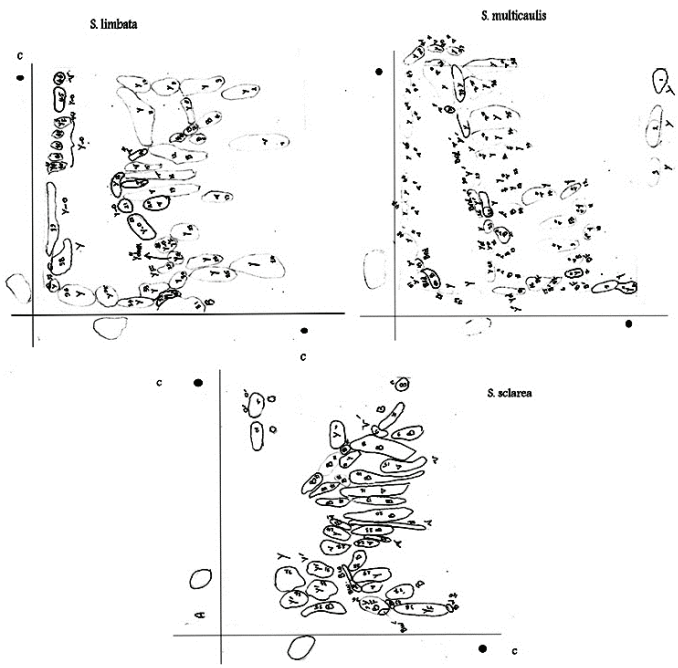

Figure 2. TLC plates in three *Salvia* species.

C: control, y: yellow, yd: dark yellow, yf: yellow fluorescent, b: blue, bw: blue- white, bf: blue fluorescent, v: violet, o: orange, y-o: yellow-orange, brd: dark brown.

Table 2. the colour spots in Salvia species before and after detection with natural product identifiers

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. hydrangea	+,	+	+,+a	+	+		+a		+		+a			+,+a
S. multicaulis	+,+a	+a	+,+a				+		+			+		
S. ceratophylla	+,+a		+a				+							
S. sclarea	+,+a	+,+a	+	+a				+	+	+				
S. atropatana	+,+a	+,+a	+,+a		+,+a									
S. limbata	+	+,+a	+,+a				+a	+,+a					+	
S. ceratophylla	+		+a			+								

1: yellow, 2: violet, 3: blue, 4: orange, 5: brown, 6: black, 7: dark yellow, 8: yellow fluorescent, 9: blue fluorescent, 10: white-blue, 11: pale orange, 12: dark brown, 13: yellow-orange, 14: yellow-blue, a: the spots after detection with natural product identifiers.

value for each species was estimated before and after detection with natural product identifiers (Table 3). The highest Rf was observed in *S. ceratophylla* (Rf= 1.54) and the lowest was in *S. atropatana* (Rf= 0.01).

Based on these results, the variations of *falvonoid* patterns in *Salvia* species displayed more diversity which is as follows:

A-ring*ortho*-dihydroxylation was observed in *S. hydrangea*, *S. multicaulis*, *S. sclarea*, *S. atropatana* and *S. limbata* (Table 4). B-ring*ortho*-dihydroxylation was observed in *S. ceratophylla*, *S. sclarea*, *S. limbata*, *S. eremophila*. The frequency of each variation was 35.48% hydroxylation, 19.3% glucosylation, 16.1% methoxylation, 6.4% methylenedioxidation, and 3.2% rhamnoglucosylation, glucuronosylation, galactosylation and ß-glucopyranosylation (Table 4). In this research, the most frequent flavonoid compounds in seven *Salvia* species were flavones (20 derivatives) and the least of these were dihydroflavonols (3 derivatives) (Table 5). Moreover, 37.5% flavone, 22.2% flavonol, 12.5% chalcone and isoflavone, 8.9% flavanone and 5.3% dihydroflavonols were observed. Consequently, the flavonoid subclasses in seven *Salvia* species are flavones, isoflavones, flavanones, flavonols, dihydroflavonols and chalcones. In this research, we found 53 flavonoid compounds from seven *Salvia* species leaves in Iran (Table 5). The amounts of flavonoid compounds in *S. multicaulis* and *S. hydrangea* were significantly higher than the other species: 22 compounds in *S. multicaulis*, 21 in *S. hydrangea*, 18 in S. limbata, 15 in *S. sclarea*, 14 in *S. ceratophylla* and *S. atropatana*, and one compound in S. eremophylla which ranged from 100%-4.5% (Table 5). The bathochromic shift of Band I with shift reagents as $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3Bo_3 was studied for each flavonoid compound. The highest bathochromic shift was observed in flavones, flavonols, isoflavones, dihydroflavonols and chalcones (Table 6).

Discussion

According to the colour of each spot, it appears that the variation of flavonoid type is incompletely

Table 3. the Rf value of each spot in seven *Salvia* species before and after detection with identifiers

species	Rf
S. sclerea	Before: 1:1.34, 2: 1.31, 3: 0.61, 4: 0.96, 5: 0.73, 6: 0.94, 7: 0.9, 8: 0.84, 9: 0.64, 10: 0.9, 11: 0.99, 12: 1.06, 13: 0.91, 14: 0.84, 15: 0.69, 16: 0.88, 17: 1.04, 18: 1, 19: 0.82, 20: 0.86, 21: 0.92, 22: 0.83, 23: 0.82, 24: 0.95, 25: 0.76, 26: 0.8, 27: 0.96, 28: 0.82, 29: 0.8, 30: 0.86, 31: 1.03, 32: 1.18, 33 1.03, 34: 1.15, 35: 1.04, 36: 0.76, 37: 0.63, 38: 0.59, 39: 0.64, 40: 0.33
	After: 1: 1.34, 2: 1.31, 3: 0.73, 4: 1.03, 5: 1.03, 6: 1.15
S. atropatana	Before: 1: 0.37, 2: 0.37, 3: 0.28, 4: 0.18, 5: 0.03, 6: 0.01, 7: 0.07, 8: 0.29, 9: 0.28, 10, 0.3, 11: 0.44, 12: 0.44, 13: 0.44, 14: 0.4, 15: 0.44, 16: 0.48, 17: 0.48, 18: 0.44, 19: 0.37, 20: 0.56, 21: 0.58, 22: 0.69, 23: 0.64, 24: 0.72, 25: 0.74, 26: 0.78, 27: 0.79, 28: 0.86, 29: 0.82, 30: 0.86, 31: 0.89, 32: 0.77, 33: 0.94, 34: 0.93
	After: 1: 0.37, 2:0.03, 3: 0.07, 4: 0.44, 5: 0.44, 6: 0.44, 7: 0.4, 8: 0.44, 9: 0.48, 10: 0.48, 11: 0.44, 12: 0.37, 13: 0.56, 14: 0.58, 15: 0.74, 16: 0.86, 17: 0.89, 18: 0.93
S. limbata	Before: 1: 0.33, 2: 0.35, 3: 0.53, 4: 0.46, 5: 0.53, 6: 0.49, 7: 0.52, 8: 0.58, 9: 0.65, 10: 0.8, 11: 0.8, 12: 0.67, 13: 0.44, 14: 0.75, 15: 0.78, 16: 0.78, 17: 0.79, 18: 0.83, 19: 0.78, 20: 0.69, 21: 0.8, 22: 0.75, 23: 0.57, 24: 0.31, 25: 0.49, 26: 0.51, 27: 0.58, 28: 0.64, 29: 0.63, 30: 0.58, 31: 0.63, 32: 0.63, 33: 0.7, 34: 0.8, 35: 0.9, 36: 1.09, 37: 1.1, 38: 1.08, 39: 1.31, 40: 1.1, 41: 1.05, 42: 1.09, 43: 1.08, 44: 1.07, 45: 1.09, 46: 1.07
	After: 1: 0.75, 2: 0.64, 3: 0.79, 4: 0.63, 5: 1.1, 6: 1.07
S. hydrangea	Before: 1: 0.53, 2: 0.74, 3: 1.36, 4: 1.38, 5: 1.3, 6: 1.37, 7: 1.38, 8: 1.38, 9: 0.57, 10: 0.62, 11: 1.36, 12: 1.37, 13: 1.31, 14: 1.3, 15: 1.25, 16: 1.16, 17: 0.66, 18: 0.54, 19: 0.46, 20: 0.46, 21, 0.48, 22: 0.54, 23: 0.63, 24: 0.53, 25: 0.74, 26: 0.98, 27: 1.2, 28: 1.29, 29: 1.38, 30: 1.39, 31: 1.39, 32, 1.24, 33: 1.17, 34: 1.15, 35: 1.2, 36: 1.1, 37: 1.1, 38: 1.1, 39: 1.04, 40: 0.97, 41: 0.9, 42: 0.9, 43: 0.84, 44: 0.81, 45: 0.78, 46: 0.71, 47: 0.69, 48: 0.64, 49: 0.58, 50: 0.56, 51: 0.53, 52: 0.29, 53: 0.58, 54: 0.53, 55: 0.52, 56: 0.45, 57: 0.13
	After: 1: 1.3, 2: 1.39, 3: 1.39, 4: 1.24, 5: 1.2, 6: 1.1, 7: 1.1, 8: 1.1
S. multicaulis	Before: 1: 0, 2: 0, 3: 0, 4: 0.13, 5: 0.27, 6: 0.47, 7: 0.43, 8: 0.48, 9: 0.55, 10: 0.33, 11: 0.4, 12: 0.47, 13: 0.6, 14: 0.7, 15: 0.54, 16: 0.66, 17: 0.87, 18: 0.86, 19: 0.85, 20: 0.9, 21: 0.86, 22: 0.91, 23: 0.93, 24: 0.84, 25: 0.93, 26: 1, 27: 0.84, 28: 0.93, 29: 1, 30: 1.12, 31: 1.18, 32: 0.99, 33: 0.81, 34: 1.01, 35: 1.03, 36: 1.06, 37: 1.12, 38: 1.06, 39: 1.18, 40: 1.28, 41: 1.42, 42: 1.31, 43: 1.48, 44: 1.43, 45: 1.43, 46: 1.45, 47: 0.1, 48: 1.42, 49: 1.44, 50: 1.26, 51: 1.34, 52: 1.19, 53: 1.08
	After: 1: 0.27, 2: 0.47, 3: 0.43, 4: 0.48, 5: 0.55, 6: 0.33, 7: 0.4, 8: 0.47, 9: 0.47, 10: 0.6, 11: 0.7, 12: 0.54, 13: 0.87, 14: 0.86, 15: 0.85, 16: 0.86, 17: 0.91, 18: 0.84, 19: 1, 20: 1, 21: 0.93, 22: 0.99, 23: 0.81, 24: 1.01, 25: 1.03, 26: 1.06, 27: 1.06, 28: 1.18, 29: 1.31, 30: 1.18, 31: 1.28, 32: 1.42, 33: 1.42, 34: 1.31, 35: 1.48, 36: 1.43, 37: 1.43, 38: 1.45, 39: 0.1, 40: 1.42, 41: 1.44, 42: 1.34
S. ceratophylla	Before: 1: 0.11, 2: 0.33, 3: 0.58, 4: 0.64, 5: 0.94, 6: 1.1, 7: 1.09, 8: 1.1, 9: 1, 10: 0.95, 11: 0.91, 12: 1.03, 13: 1.08, 14: 1.1, 15: 1.26, 16: 1.17, 17: 1.16, 18: 1.11, 19: 1.22, 20: 1.29, 21: 1.31, 22: 1.36, 23: 1.44, 24: 1.54
	After: 1: 0.11, 2: 0.33, 3: 0.58, 4: 0.64, 5: 0.94, 6: 1.1, 7: 1, 8: 0.91, 9: 1.26, 10: 1.16, 11: 1.44, 12: 1.54
S. eremophila	Before: 1: 1.05, 2: 1.01, 3: 0.95, 4: 0.96, 5: 0.84, 6: 0.84, 7: 0.8, 8: 0.77, 9: 0.73, 10: 0.73, 11: 0.62, 12: 0.64, 13: 0.51, 14: 0.55, 15: 0.48, 16: 0.38, 17: 0.29
	After: 1: 1.05, 2: 1.01, 3: 0.96, 4: 0.84, 5: 0.8, 6: 0.77, 7: 0.73, 8: 0.73, 9: 0.64, 10: 0.62, 11: 0.51, 12: 0.48, 13: 0.38, 14: 0.55, 15: 0.29

detected in previous researches. Lu and Foo (6), Nikolova *et al*. (20) and Tomas-Barberan and Wolenweber (22) have reported that all of the Salvia species represent flavones, isoflavones and flavonols. In our research, it appears that these compounds were flavone, flavones-7–*o*-rahmnoglucoside, flavonol, 5-hydroxylflavonol, isoflavone, flavanone, 5-hydroxylflavanone and dihydroflavonol (Table 2). Nevertheless, the flavonoid determination needs to be identified with column chromatography.

Based on the results, flavonoid spots displayed variation. The colour spots in some of *Salvia* species are partially accorded with the Nakiboglu (26) results. The presence of yellow fluorescent, blue and violet spots is based on the chemotaxonomy results of Nakiboglu (26).

Table 4. The variation of flavonoid patterns (oxidation) in seven *Salvia* species

Variation patterns/ species	hydr.	mult.	cera.	scl.	atro.	limb.	eremo.
A-ring*ortho*-dihydroxyltion	+	+	-	+	+	+	-
B-ring*ortho*-dihydroxylation	-	-	+	+	-	+	+
2-hydroxylation	-	-	-	-	-	+	-
3-hydroxylation	+	+	+	+	+	+	+
4-hydroxylation	-	+	-	+	+	-	-
5-hydroxylation	+	+	+	+	+	+	+
6-hydroxylation	+	+	-	+	-	+	-
7-hydroxylation	+	+	+	+	+	+	+
8-hydroxylation	+	+	-	-	-	+	-
2'-hydroxylation	+	+	+	+	+	+	-
3'-hydroxylation	+	+	+	+	+	+	-
4'-hydroxylation	+	+	+	+	+	+	+
5'-hydroxylation	+	-	-	-	-	-	-
6-methoxylation	+	+	+	+	+	+	-
7-methoxylation	-	+	+	-	-	+	-
8-methoxylation	+	+	+	+	+	+	-
3'-methoxylation	+	+	+	-	+	+	-
4'-methoxylation	+	+	+	+	+	+	-
7-*o*-rhamnoglucosylation	-	+	-	-	-	+	-
3-methylenedioxidation	-	-	+	+	-	+	-
3'-methylenedioxidation	+	+	+	-	+	+	-
5-*o*-glucosylation	-	-	-	-	-	+	-
8-*c*-glucosylation	-	-	+	-	-	-	-
6-*c*-glucosylation	-	-	+	-	-	-	-
3-*o*-glucosylation	-	+	-	+	+	-	-
7-o-glucosylation	-	-	-	-	+	+	-
7-*o*-glucuronosylation	+	+	-	+	+	+	-
3-*o*-galactosylation	-	+	-	-	-	-	-
3-*o*- ß-glucopyranosylation	-	-	+	-	-	+	-

From the findings on the final fraction and the UV absorption spectra, a tendency towards 3-hydroxylation, 5-hydroxylation, 7-hydroxylation and 4'-hydroxylation was present in all of the seven *Salvia* species. Whereas, 2-hydroxylation, 4-hydroxylation, 6-hydroxylation,

Table 5. the flavonoid constituents (frequency %) in seven *Salvia* species

Compounds/ Species	mult.	hydr.	cera.	scl.	atro.	lim.	eremo.
3,4',7-trihidroxyflavone-7-*o*-rhamnoglucoside (flavones)	9.1	-	-	-	-	-	-
7-hydroxyflavone (flavones)	22.7	-	-	-	-	-	-
5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin) (flavones)	4.5	4.5	-	-	-	-	-
3',4'-dihydroxyflavone (flavones)	4.5	-	-	6.6	-	-	-
3,4',7-trihydroxyflavone (flavones)	4.5	9.1	7.1	-	23.1	5.5	-
5,7-dihydroxyflavone (chrysin) (flavones)	-	4.5	-	-	-	-	-
Hymenoxin (flavones)	4.5	9.1	7.1	-	7.6	11.1	-
Saponarin (flavones)	-	4.5	-	-	-	-	-
6-hydroxy luteolin-7,3',4'-trimethylether (flavones)	4.5	-	-	-	-	-	-
5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin) (flavones)	9.1	-	-	6.6	-	5.5	-
5,7,8-trihydroxyflavone (norwogonin) (flavones)	4.5	63.6	21.4	-	-	22.2	-
Norwogonon-7-*o*-glucuronide (flavones)	-	-	-	-	30.7	-	-
5-hydroxy-6,7,4'-trimethoxyflavone (salvigenin) (flavones)	-	-	7.1	-	-	-	-
5,7,4'-tribydroxyflavone (apigenin) (flavones)	-	-	21.4	-	-	-	-
Violanthin (flavones)	-	-	7.1	-	-	-	-
5,7-dihydroxy-6,8,4'-trimethoxyflavone (nevadensin) (flavones)	-	-	-	13.3	-	-	-
3,3',4'-trihydroxyflavone (flavones)	-	-	-	-	-	5.5	-
5,7,8-trihydroxyflavone-7-*o*-glucoside (flavones)	-	-	-	-	-	5.5	-
5,7,3' ,4'-tetrahydroxyflavone (luteolin) (flavones)	-	4.5	-	13.3	-	5.5	100
5,6,7-trihydroxyflavone-7-*o*-glucuronide (baicalin) (flavones)	4.5	9.1	-	6.6	-	5.5	-
Isosakuranetin-7-*o*-rhamnoglucoside (flavanones)	-	-	-	-	-	5.5	-
Pinocembrin (flavanones)	4.5	-	14.2	-	-	5.5	-
Pomiferin (flavanones)	-	9.1	-	-	-	5.5	-
Eriodictyole (flavanone)	27.2	18.1	14.2	-	30.7	16.6	-
Naringenin (flavanones)	-	-	-	13.3	-	5.5	-
3-hydroxy-4-'methoxyflavone (flavonols)	9.1	4.5	-	-	-	5.5	-
Isorhamnetin-3-*o*-galactoside (flavonols)	4.5	-	-	-	-	-	-
Fisetin (flavonols)	-	-	7.1	-	15.3	-	-
Fisetin-3-*o*-glucoside (flavonols)	4.5	-	14.2	26.6	-	-	-

Table 5. (continue)

Compounds/ Species	mult.	hydr.	cera.	scl.	atro.	lim.	eremo.
Quercetin 3-methylether (flavonols)	4.5	-	-	6.6	-	-	-
Kaempferol (flavonols)	4.5	-	14.28	-	7.6	-	-
Kaempferol-3-*o*- ß-glucopyranoside (flavonols)	-	-	7.1	-	-	5.5	-
Kaempferol-3-*o*-glucoside (flavonols)	-	-	-	6.6	7.6	-	-
5,7-dihydroxy -3',4'-dimethoxyflavone (ermanin) (flavonols)	-	9.1	-	-	7.6	-	-
Galangin (flavonols)	-	4.5	-	-	-	-	-
Galangin-3-methylether (flavonols)	-	4.5	-	-	-	-	-
Pseudobaptigenin (isoflavones)	31.8	18.1	35.7	-	3.07	16.6	-
3',4',7-trihydroxyisoflavone (isoflavones)	-	4.5	-	-	-	-	-
5,7-dihydroxyisoflavone (isoflavones)	-	13.6	21.4	-	-	-	-
Tectorigenin (isoflavones)	-	4.5	-	6.6	7.6	11.1	-
Irigenin (isoflavones)	-	4.5	-	-	-	-	-
Biochanin A (isoflavones)	-	-	-	-	23.1	-	-
Lanceolarin (isoflavones)	-	-	-	-	7.6	-	-
Dihydrorobinetin (dihydroflavonols)	4.5	-	-	-	-	-	-
Taxifolin (dihydroflavonols)	-	4.5	-	26.6	7.6	-	-
Dihydrokaempferol (dihydroflavonols)	-	4.5	-	-	-	-	-
3,4-dihydroxychalcone	4.5	-	-	-	7.6	-	-
2,2'-dihydroxychalcone	-	-	-	-	-	16.6	-
3,4'-dihydroxychalcone	-	-	-	6.6	-	-	-
2',3',4'-trihydroxychalcone	9.1	-	-	-	-	-	-
2',3,4,4'-tetrahydroxychalcone	-	-	-	6.6	-	-	-
4'-hydroxychalcone	-	-	-	6.6	-	-	-
3',4'-dihydroxychalcone	-	-	-	6.6	-	-	-

8-hydroxylation, 2'-hydroxylation, 3'-hydroxylation and 5'-hydroxylation were present in some of *Salvia* species (Table 4). As mentioned above, the highest flavonoid variation belongs to hydroxylation (35.48%). These variation patterns are based on the findings of *Salvia* species in previous reports (6, 12, 15, 18, 20, 22, 23, 27, 28, 29, 30, 31, 32). Other flavonoid variations such as 6-methoxylation, 7-methoxylation, 8-methoxylation, 3'-methoxylation and 4'-methoxylation were present in some of these *Salvia* species. A substantial degree of methoxylation was observed. These results were based on the reports of Lu and Foo (7), Nikolova et al. (20), Tomas-Barberan and Wollenweber (22) and Ullubelen et al. (28).

Table 6. the effect of shift reagent on flavonoid compounds (bathochromic shift of Band I) in *Salvia* species

Compounds/ Species	AlC_{l3}	AlC_{l3}/HCl	NaOAc	NaOAc/H_3BO_3
3,4',7-trihidroxyflavone-7-*o*-rhamnoglucoside (flavones)	39	0	60	24
7-hydroxyflavone (flavones)	0	65	51	2
5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin) (flavones)	46	39	23	4
3',4'-dihydroxyflavone (flavones)	38	2	60	25
3,4',7-trihydroxyflavone (flavones)	63	62	22	1
5,7-dihydroxyflavone (chrysin) (flavones)	67	68	46	2
Hymenoxin (flavones)	29	21	40	7
Saponarin (flavones)	45	42	56	5
5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin) (flavones)	25	23	37	3
5,7,8-trihydroxyflavone (norwogonin) (flavones)	34	21	7	6
Norwogonon-7-*o*-glucuronide (flavones)	5	3	-	-
5-hydroxy-6,7,4'-trimethoxyflavone (salvigenin) (flavones)	30	20	46	1
5,7,4'-tribydroxyflavone (apigenin) (flavones)	48	45	40	2
Violanthin (flavones)	52	48	53	13
5,7-dihydroxy-6,8,4'-trimethoxyflavone (nevadensin) (flavones)	27	22	53	43
3,3',4'-trihydroxyflavone (flavones)	100	61	64	22
5,7,3' ,4'-tetrahydroxyflavone (luteolin) (flavones)	77	36	35	21
5,6,7-trihydroxyflavone-7-*o*-glucuronide (baicalin) (flavones)	28	23	38	32
Isosakuranetin-7-*o*-rhamnoglucoside (flavanones)	48	28	10	0
Pinocembrin (flavanones)	86	84	34	2
Pomiferin (flavanones)	10	11	0	2
Eriodictyole (flavanone)	89	84	36	0
Naringenin (flavanones)	86	82	34	1
3-hydroxy-4-'methoxyflavone (flavonols)	61	62	2	0
Fisetin (flavonols)	96	61	16	19
Fisetin-3-*o*-glucoside (flavonols)	41	80	29	25

Other substitutions such as 5-*o*-glucosylation, 3-*o*-galactosylation, 3-*o*-glucosylation, 7-*o*-glucosylation, 7-*o*-glucuronosylation, 6-*c*-glucosylation, 8-*c*-glucosylation, 3 and 3'-methylenedioxidation and 3-*o*-ß-glucopyranosylation were found in our results (Table 4). Some of the variations coincide with the literature reports on flavonoids of some *Salvia* species (6, 11, 13, 31, 32, 33, 34, 35). In addition, ß-glucopynanosylation variation was

Table 6. (continue)

Compounds/ Species	AlC_{13}	AlC_{13}/HCl	NaOAc	NaOAc/H_3BO_3
Quercetin 3-methylether (flavonols)	85	44	25	20
Kaempferol (flavonols)	57	57	20	5
Kaempferol-3-*o*- ß-glucopyranoside (flavonols)	0	46	-	-
Kaempferol-3-*o*-glucoside (flavonols)	50	48	55	10
Galangin (flavonols)	54	53	29	2
Galangin-3-methylether (flavonols)	127	125	98	1
Pseudobaptigenin (isoflavones)	1	0	38	1
3',4',7-trihydroxyisoflavone (isoflavones)	3	1	38	4
5,7-dihydroxyisoflavone (isoflavones)	108	108	68	1
Tectorigenin (isoflavones)	111	99	72	1
Irigenin (isoflavones)	103	106	70	0
Biochanin A (isoflavones)	114	112	66	1
Lanceolarin (isoflavones)	120	18	1	1
Dihydrorobinetin (dihydroflavonols)	1	1	25	3
Taxifolin (dihydroflavonols)	85	85	37	2
Dihydrokaempferol (dihydroflavonols)	91	87	36	5
3,4-dihydroxychalcone	48	0	12	36
2,2'-dihydroxychalcone	71	64	88	4
2',3',4'-trihydroxychalcone	61	39	49	10
2',3,4,4'-tetrahydroxychalcone	111	48	18	36
4'-hydroxychalcone	0	0	57	2

supported by the reports of Wang *et al*. (35) in *S*. *officinalis* L. This substitution is first recorded for Iran. In our results, rhamnoglucosylation, glucuronosylation, ß-glucopynanosylation and galactosylation were exhibited in the lowest quantities. In addition, Flavone *c*-glycosides are extensive in nature and those present in *Salvia* are mostly those of vitexin (6).

Based on the variation of flavonoid patterns, Tomas-Barberan and Wollenweber (22) reported that the substituted B-ring and A-ring are characteristic of Salvia species. Also, 5,7-dihydroxy-6-methoxyflavone with a substituted B-ring was partially observed which is an aspect of this genus. In our results, the substituted B-ring and A-ring, 5,7-dihydroxyflavone, 5,7-dihydroxy-6,8,4'-trimethoxyflavone and 5,7-dihydroxy-3',4'-dimethoxyflavone were found which is partially in agreement with their results (Table 5). Mono-substituted (4'-) or di-substituted (3', 4'-) B-rings are frequent (Table 4) which is

based on Thomas-Barberan and Wollenweber (22) results.

In *Salvia* species, the majority of flavonoids are flavones of apigenin (5,7,4'-trihydroxyflavone) and luteolin (5,7,3',4'-tetrahydroxyflavone) and their corresponding 6-hydroxylated derivatives (6). In our results, the apigenin and luteolin compounds (flavone glycoside) were observed in *Salvia* species which is accorded with the results of Lu and Foo (6, 7). In addition, 6-methylated derivatives of apigenin and 6-hydroxylated derivatives of luteolin have been found in *Salvia* species (12, 34). In this research, apigenin, luteolin and 6-hydroxylated luteolin were encountered which is in accordance with the reports of Ullubelen et al. (36) in *S. tomentosa* L., Miski *et* al. (33), Ullubelen *et al*. (13) in *S. sclarea* and S. palaestina Benth., Dordevic *et al*. (37) in *S. officinalis*, Lu and Foo (6), Nikolova *et al*. (20) and Ciesla *et al*. (23) (Table 5). The 6-hydroxyflavones are the flavonoids that illustrate species of *Salvia*. They include the variation of 6- hydroxylated apigenin and luteolin derivatives, with 6-hydroxyapigenin-6,7-dimethyl ether (cirsimaritin) and 6,7,4'-trimethyl ether (salvigenin) being the most common (6). Liu *et al*. (31) reported that flavone-o-glucoside and flavones-c-glucoside are apparently numerous in Salvia genus which is based on our results. Moreover, the 7-*o*-rhamnoglucosyle position and trihydroxyflavones with 7-*o*-glucuronsyle position were observed in *Salvia* species which is in accordance with Abdalla *et al*. (12) in *S. triloba* L., Miski et al. (33) in *S. palaestina*, El-Sayaed *et al*. (38), Lu and Foo (6) in *S. officinalis*, and Liu *et al*. (31). In flavonol derivatives, galactosyl substitution was found which was reported by Kamel *et al*. (39) in *S. farinacea* Benth.

In some of *Salvia* species Kaempferol derivatives (flavonols compounds) such as 3-robinoside were reported by Tomas-Barberan and Wollenweber (22), and Zhao *et al*. (40), whereas in our results kaempferol-3-*o*-glucoside and Kaempferol-3-o-ß-glucopyranoside were observed (Table 5) which is based on the results of Nigel et al. (41), Ishikawa *et al*. (42) and Suzuki *et al*. (43). In addition, the flavonoid compounds such as quercetin 3-methylether were in accordance with the findings of previous reports in *S. glutinosa* L., *S. triloba* L. and some genera of Lamiaceae (22, 29, 44, 45) (Table 5). According to Lu and Foo (6), Flavonols are typically those of kaempferol and quercetin methyl ethers. These compounds, together with other flavones were mainly identified in Salvia *species* (6). The chemodiversity among flavonols is less different, and remarkably flavonols derivatives are known for this genus (6).

In flavanone derivatives, the hydroxylation in the 5, 7 and 4' positions were extensive which agrees the reports of *Salvia sapinae* Epling and *S. dorii* (Kellogg) Abram (29, 46). Other hydroxylation positions such as 8 and 3' were in accordance with the results of Cuvelier et al. (47). In quercetin, the etherified positions were reported in the 3, 7, 3', and 4' positions in *Salvia* species (20, 48). Quercetin substitution was found in the 3 position. Based on our findings and other published results, there is a correlation between the habitat of plant and production of flavonoid compounds (22). Moreover, it appears that the leaf, flower and root will produce the different flavonoid compounds.

C-6-substitution and *c*-8-substitution include the flavonoid derivatives in Salvia genus (22). In our results, the flavonoid variations such as 6-*c*-glucosyl and

8-c-glucosyl were mainly recorded (Table 3).

The flavone derivatives such as 6, 7, 8, 3' hydroxyflavones were confirmed by the results of Gonzalez *et al*. (16) in *S. texana* (Scheele) Torr. and Cuvelier et al. (48) in *S. officinalis*. Other flavone, flavonol, flavanone, isoflavone, dihydrofalvonol and chalcone derivatives as 5,7-dihydroxyflavone (chrysin), 5,6,7-trihydroxyflavone 7-*o*-glucuronide (Baicalin), norwogonin, nevadensin, hymenoxin, apigenin, saponarin, luteolin, 6-hydroxyluteolin, diosmetin, salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone),cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone), 5,7,8-trihydroxy-7-*o*-glucoside, ermanin, galangin, galangin-3-methylether, isorhamnetin, pomiferin, naringenin, pinocembrin, eriodictyole, isosakuranetin-7-*o*-rhamnoglucoside, biochanian A, lanceolarin and taxifolin were in agreement with the published results in some of the *Salvia* species (6, 18, 20, 22, 29, 32, 34, 37, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58). Valant-Vetschera *et al*. (18) reported that there is a strong tendency toward accumulation of 6-hydroxyflavone and their methyl ethers. Moreover, chemo-diversity in *Salvia* species is somewhat increased and the flavones composition largely aggregated which is in accordance with our results. Some of the flavonoid compounds in this research were first reported for Iranian Salvia species such as fisetin, fisetin-3-*o*-glucoside (flavonols), pseudobaptigenin (isoflavones), tectorigenin (isoflavones), irigenin (isoflavone), violantine (flavone), dihydrorobinetin, dihydrokaempferol (dihydroflavonol) (Table 5) and there is need for further studies.

The flavonoid constituents of three *Salvia* species studied as *S. multicaulis*, *S. hydrangea* and especially *S. eremophila* (endemic species) were first reported for Iran, (Table 5). Based on the previous studies, the flavonoid compounds of four species as S. limbata, S. sclarea, *S. atropatana* and *S. ceratophylla* were incompletely reported. According to Shamsudinov' *et al*. (59) results, four flavonoid compounds were identified for *S. limbata* such as apigenin, apigenin-7-*o*-glucoside, luteolin and luteolin-7-glucoside which is nearly based on our results (Table 5). In this research, 17 flavonoid compounds were found in S. limbata as flavones, flavanones, flavonols and isoflavones (Table 5). Ullubelen et al. (13) and Adzet *et al*. (14) reported apigenin, luteolin, salvigenin and 5-hydroxyflavones for *S. sclarea* which is partially in accordance with our results (Table 5). It seems that these flavonoid differentiations were due to polymorphism, hybridization between species and geographical distribution (60). Ozdemir and Senel (61) also showed the morphological properties of *S. sclarea* in Turkey, which has some similarities and differences compared to other findings in taxonomic literature. In our results, the flavonoid diversifications were due to the environmental or ecological conditions. Consequently, flavones, flavanones, flavonols, isoflavones, dihydroflavones and chalcones were observed in this species (Table 5). Goren *et al*. (62) reported one flavone for *S. ceratophylla*.

In our research flavones, flavanones, flavonols and isoflavones were recognized (Table 5). Habibi *et al*. (63) were identified 5-hydroxy-7,4'-dimethoxyflavone and salvigenin for *S. atropatana* which is not supported by our results. The flavones, flavanones, flavonols, isoflavones, dihydroflavones and chalcones derivatives were observed for this species (Table 5). Hedge (60) and Kharazian (64) stated that this species displays variability in vegetative and reproductive features. It can be concluded that the flavonoid constituents frequently change in incident environment.

Chemotaxonomically, *S. atropatana*

and *S. limbata* were similar in flavones, flavanones and isoflavones. The flavanols derivatives were observed in *S. sclarea* and S. ceratophylla. In *S. hydrangea*, *S. multicaulis* and *S. ceratophylla* were encountered flavones, flavanones and isoflavones. Furthermore, these compounds seem to be appropriate markers in chemotaxonomic studies especially in infra-specific levels. According to the previous researches, the flavonoid derivatives have been reported to be of particularly taxonomic significance to this genus (22).

In conclusion, flavonoid constituents in the *Salvia* species studied show excessive diversity in Iran, and they are often differentially distributed. As mentioned above, chemical differentiation might be correlated to the geographical and ecological conditions under which they grow and the large variability of structures (11, 65). The ecological correlations in the adaptation of plants to habitats apply to the results of chemotaxonomy (22). Our research showed that hydroxylation, methoxylation and glycosylation patterns may be considered to be specific to the *Salvia* species. Their presence could be significant in taxonomy of this genus.

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