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Fatty Acid Composition Analysis of Aerial Parts of Selected *Salvia* Species Growing in Iran and Chemotaxonomic Approach by Shoot Fatty Acid Composition

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In this study, fatty acid (FA) composition of aerial parts of selected *Salvia* species from Iran was analyzed by gas chromatography. The amount of FAs was quantified for leaf and shoot of species as mg per kg (mg kg^{-1}) of dry weight. The results showed that FA contents of aerial parts for the studied plants vary significantly between 73.05 and 739.50 mg kg^{-1} of dried weight. Caprylic (C8:0, 1.00-380.49 mg kg^{-1}), elaididc (C18:1n9t, 0.73-97.29 mg kg^{-1}), stearic (C18:0, 1.1-62.97 mg kg^{-1}), palmitic (C16:0, 1.19-36.48 mg kg^{-1}), and α-linoleic (C18:3n3, 1.34-19.36 mg kg^{-1}) acid were main FAs identified. The numerical analysis was performed on FA composition of shoot and leaf of specimens and the shoot FA composition was selected to identify the systematic position of studied species. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram showed that the species are classified into two clusters. Caprylic acid (C8:0), behenic acid (C22:0), and lignoceric acid (C24:0) were chief characters in the infrageneric grouping the species in the genus. *S. chloroleuca* and *S. atropatana* were placed in cluster I and separated from other species based on shoot FA composition. The discrimination of *Salvia* species based on their botanical classification was supported by results. The results confirmed that FA composition of shoot are distinguishable and can be used as chemotaxonomic markers.

Keywords: *Salvia*, Fatty acid composition, Chemotaxonomy, Gas chromatography

INTRODUCTION

Salvia L. is one of the largest genera in the family of Lamiaceae (subfamily Nepetoideae) and represented by 1000 species [1]. The genus of *Salvia* is found in different parts of Iran by 17 endemic species [2]. Many of *Salvia* species have been used in traditional medicine and food flavoring agents [3]. Many secondary metabolites have been reported from *Salvia* species including terpene, flavonoid, essential oil, fatty acid and phenolic acid [3-6]. The diversity of the reported compounds introduces the genus for using in the medicinal, food, flavor and cosmetic industries [7,8].

Fatty acids (FAs) play an important pharmaceutical role in human health. The essential FAs including α-Linolenic acid (ALA, C18:3n3, omega-3) and Linoleic acid (LA, C18:2n6, omga-6) must be ingested into the body [9-11]. Many of diseases could be observed due to the lack of FAs in human diet [12,13]. The previous studies showed that the seed oils of *Salvia* species are the source of essential FAs. For examples, ALA was reported from *S. nemorosa* by 53.6% of total seed oils and LA from *S. officinalis* reported by 60.64% [14]. In spite of essential FAs, the major FAs from *Salvia* species have been reported including oleic acid (OA, C18:1n9), stearic acid (SA, C18:0) and palmitic acid (PA, C16:0) [15-18].

The ratio of omega-6 to omega-3 (LA/ALA) essential

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FAs in mankind diets is very important. The high ratio of LA/ALA (excessive amount of LA) causes many diseases including cancer, cardiovascular, inflammatory and autoimmune diseases. The previous studies have shown that the w-6/w-3 ratio lower than 5 could be effective on suppressing of related diseases [9,19-21]. For example, rheumatoid arthritis inflammation was suppressed by a ratio of (3-4), [22] and in the other study a ratio of 4 was reported to reduce 70% of total mortality of cardiovascular disease [23].

The secondary metabolite analysis was empolyed for chemotaxonomic studies [24-28]. FA composition of seeds and aerial parts of plants has been the subject of many chemotaxonomic studies. For example, FA composition of seed oils was used as a chemotaxonomic marker for evaluation of species of *Salvia* from Turkey [29,30]. In another study, FA composition of photosynthetic tissues was applied for chemotaxonomic studies of Rubiaceae family [26,31]. FA composition analysis has been widely used for solving the problems in plant systematic studies [32].

Many of the previous studies have been focused on analysis of FA composition of seed oils for phytochemical or chemotaxonomy purposes [16,18,25,29,30]. Few studies have been focused on areal parts of the FA composition [26,31,33,34]. Most of the previous studies have used seeds' FA composition of *Salvia* as chemotaxonomic markers [35]. However, the accessibility of seeds is often impossible. So, chemotaxonomic study based on the areal parts analysis could be valuable for identifying the species. In this study, FA composition of the areal parts of a selected *Salvia* from Iran is investigated and their FA fingerprints are obtained for leaf and shoot separately. In addition, the areal parts of FA composition is applied for chemotaxonomic study of the *Salvia* species from Iran.

EXPERIMENTAL

Plant Materials

The plant materials were collected during spring and summer of 2015. Locality, altitude and herbarium number of the species of *Salvia* are shown in Table 1. The voucher specimens were deposited in the Herbarium mentioned in Table 1.

Chemicals and Reagents

Methanol, heptane, potassium hydroxide, and diethyl ether were obtained from Merck (Darmstadt, Germany). Supelco 37 component fatty acid methyl esters (FAMEs) with analytical standard mixture (10 mg ml^{-1}) was purchased from Sigma-Aldrich (USA).

Sample Extraction and Derivatization

Shoot and leaf materials of species were separated from the rest parts of the plants. The amount of 1 g dried and powdered samples were carefully weighed. Lipids content of the samples was extracted via maceration using 20 ml of diethyl ether for 24 h. At the end of the extraction process, the extract was separated from plant material and concentrated by rotary evaporator to remove extraction solvent. The trans-esterification of lipids to obtain FAMEs was carried on by five ml of methanolic KOH (2 M) at 70 °C for 30 min. The FAMEs were extracted using 0.5 ml of heptane and stored at 4 °C until GC analysis [36].

GC Analysis

A Young-Lin 6000-GC model capillary gas chromatograph system was applied for separation and quantification of the FAMEs. Autochrom 3000 software was used for acquiring the data. The FAME mixtures were separated on a capillary column (14% Cyanopropyphenyl-86% dimethyl polysiloxane, bonded and cross-linked phase, 60 m × 0.25 mm, 0.2 µm film thickness, Teknokroma, Spain) according to the following instrumental conditions. The split ratio was set at 1:50. The initial column temperature was set at 40 ºC for 5 min, then raised at 10 ºC min^{-1} to 250 ºC and remained for 20 min. Injector and detector temperature were 250 and 280 ºC, respectively. The flow rate for N_2 as carrier and make-up gas were 1 and 20 ml min^{-1}, respectively.

Cluster Analysis

To evaluate the taxonomic position of species, we performed a cluster analysis based on Euclidean Distances with unweighted pair-group method and arithmetical mean

Table 1. The Locality of Salvia Species in Natural Habitat of Iran

Species	Locality	Altitude	Herbarium No.
S. virgata	Farooj, Khorasan	1850 m	38127 (FUMH)[a]
S. aethiopis	Ferdows, Abegarm, Khorasan	1560 m	38356 (FUMH)
S. chorassanica	Pivejan, Khorasan	2100 m	40341 (FUMH)
S. nemorosa	Ghoochan, Oghaze Kohne, Khorasan	1780 m	36605 (FUMH)
S. choloroleuca	Pivejan, Khorasan	2090 m	36528 (FUMH)
S. sclarea	Ferdows, Abegarm, Khorasan	1500 m	22520 (FUMH)
S. leriifolia	Bajestan, Ghonabad, Khorasan	1336 m	26879 (FUMH)
S. spinosa	Ferdows, Abegarm, Khorasan	1500 m	32280 (FUMH)
S. atropatana	Einali, Tabriz	1700 m	A1-2227[b]
S. sahendica	Kandovan, Tabriz	2350 m	MPH-848[c]

[a]Ferdowsi University of Mashhad Herbarium. [b]Herbarium of Research Institute of Forests and Rangelands, Iran. [c] Medicinal Plants and Drug Research Institute Herbariuom, Shahid Beheshti University.

(UPGMA) method by using the program Past [35].

RESULTS AND DISCUSSIONS

A high level of chemical diversity of FAs was found in *Salvia* species areal parts. A total of 30 FAs were identified and quantified from aerial parts of *Salvia*. The amount of each FA was assigned as mg per kg of dried plant material (mg kg^{-1}). In addition, the GC fingerprints of studied plants were obtained for shoot and leaf (Fig. 1 and Figs. S1-S8). The obtained data are shown in Tables 1 and 2. The FA contents were reported between 0.01 and 369.20 mg/kg. The main FAs in the aerial parts of specimens were distinguished as caprylic acid (C8:0; 041-369.20 mg kg^{-1}), palmitic acid (C16:0; 0.29-22.53 mg kg^{-1}), stearic acid (C18:0; 0.41-30.15 mg kg^{-1}), oleic acid (C18:1n9t; 0.80-94.98 mg kg^{-1}) and erucic acid (C22:1n9c; 0.28-23.48 mg/kg). *S. chorassanica* and *S. sahendica* had the highest and lowest amount of total FA composition, respectively. The total saturated FA contents of the species were determined between 23.46 and 446.76 mg kg^{-1}, while unsaturated FA contents of the species were determined between 23.10 and 188.55 mg kg^{-1} (Table 3).

The amounts of w-6 and w-3 for the studied species are shown in Table 4. *S. sahendica* shoot FA composition showed the highest value of w-3 by 13.65 mg kg^{-1} and the lowest value of w-6/w-3 ratio as 0.31. So, the use of *S. sahendica* shoot as culinary plant could be helpful to the health. Also, *S. sahendica* has been used as seasoning, flavoring and preserving agent in North West of Iran [37-39].

S. leriifolia showed considerable w-6/w-3 ratios of 1.02 and 2.90 for shoot and leaf, respectively. So, the aerial parts of this plant could be important as a source of essential FAs by total w-3 as 9.75 mg kg^{-1}. *S. spinosa* showed a close range of w-6/w-3 ratios for shoot and leaf by 2.76 and 2.99, respectively. The highest content of w-3, 16.77 mg kg^{-1}, was identified for leaf and 19.36 mg kg^{-1} for sum of leaf and shoot of *S. aethiopis*. This plant also showed close w-6/w-3 ratio for shoot and leaf by 4.97 and 4.37,

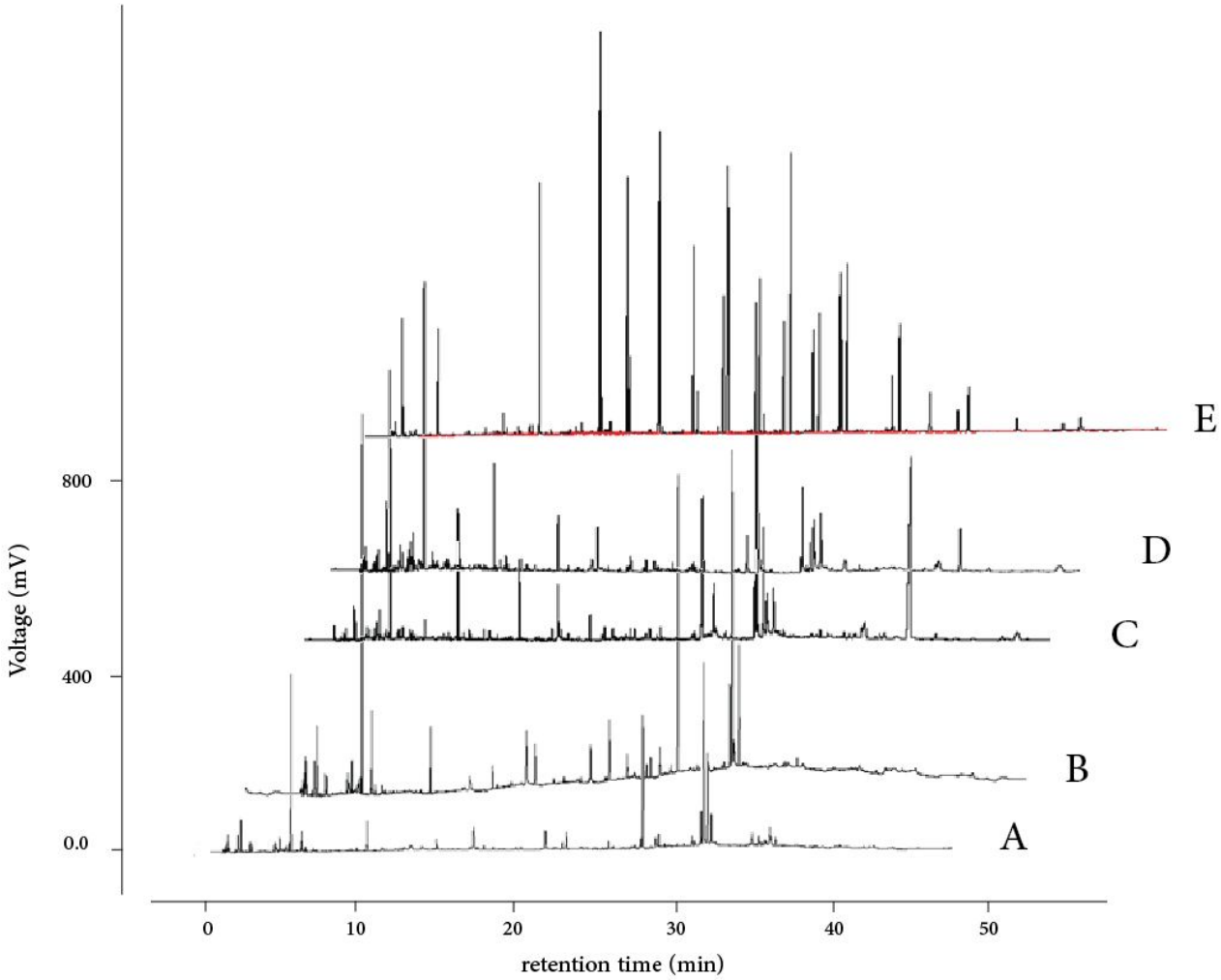

Fig. 1. GC chromatograms for fatty acids of A) *S. nemorosa* leaf, B) *S. nemorosa* shoot, C) *S. chorassanica* shoot, D) *S. chorassanica* leaf, and E) FAMEs mixture standard

respectively. *S. nemorosa* also has shown the same results similar to *S. aethiopis* of w-6/w-3 ratio of 3.48 for shoot and 4.0 for leaf. The obtained results (Table 4) showed the importance of mentioned plants based on their w-6/w-3 ratio of aerial parts FA composition.

The systematic position of the *Salvia* species was chemotaxonomically studied according to the amount of FA composition. Cluster analysis showed that the species are placed in two clusters. *S. chorassanica*, *S. sclarea*, *S. spinosa*, *S. nemorosa*, *S. sahendica*, *S. virgate*, *S. leriifolia* and *S. aethiopis* are placed in cluster I, while *S. atropatana* and *S. chloroleuca* are placed in cluster II (Fig. 2). Principal component analysis (PCA) showed that caprylic acid (C8:0), behenic acid (C22:0), and lignoceric acid (C24:0) were chief characters in grouping the species (Fig. 3).

There are 70 species of *Salvia* recorded in the Flora Iranica (Rechinger).They are divided into five Grex. *S. spinosa*, *S. leriifolia*, *S. virgate*, and *S. nemorosa* are placed in Grex D, and *S. chorassanica*, *S. sclarea*, *S. sahendica*, *S. aethiopis*, *S. atropatana* and *S. chloroleuca* are placed in Grex E. As demonstrated in Table 2, C20:5n3, C20:3n3, C22:1n9 and C18:3n3 have obvious taxonomic significance

Table 2. Shoot Fatty Acid Composition (mg kg^{-1}) of *Salvia* Species from Iran

	Grex D					Grex E				
	S. spinosa	*S. leriifolia*	*S. virgate*	*S. nemorosa*	*S. chorassanica*	*S. sclarea*	*S. sahendica*	*S. aethiopis*	*S. atropatana*	*S. chloroleuca*
C6:0	0.34	N.D	N.D	0.74	1.12	0.58	0.74	0.41	1.25	1.60
C8:0	12.75	0.64	0.39	12.64	22.65	8.80	0.41	0.71	183.6	369.2
C10:0	2.62	1.39	3.71	2.27	11.52	4.76	0.73	1.25	0.61	0.60
C11:0	0.98	0.49	1.45	3.18	7.46	3.59	0.09	2.39	0.42	0.40
C12:0	5.13	1.13	2.79	1.09	10.01	8.17	4.39	3.64	0.14	16.46
C13:0	1.79	0.41	4.42	4.15	1.75	1.00	0.23	1.94	1.19	13.59
C14:0	4.35	3.19	1.48	5.61	2.64	1.64	1.69	12.07	2.08	4.08
C14:1	0.49	0.78	2.00	3.13	2.23	0.72	0.42	6.85	0.62	4.33
C15:0	0.37	0.39	1.41	4.10	2.67	1.13	0.75	3.74	0.14	5.41
C15:1	0.80	0.38	0.26	5.73	1.99	0.55	0.22	3.50	0.62	8.07
C16:0	2.84	0.29	0.93	22.53	19.64	17.40	4.65	3.66	3.55	10.01
C16:1	0.83	1.01	2.47	9.43	14.05	10.08	0.07	7.38	1.64	4.55
C17:0	1.41	0.94	0.77	4.11	2.26	0.36	0.05	1.44	0.60	5.93
C17:1	1.11	0.66	0.86	4.33	2.00	0.78	0.04	2.69	0.40	4.84
C18:0	0.74	5.20	5.98	19.94	8.90	30.15	0.41	4.08	14.30	3.93
C18:1n9t	0.97	1.30	3.89	10.44	17.23	1.16	0.42	36.35	0.80	94.98
C18:2n6t,C18:1n9c	1.38	0.79	3.52	4.70	11.10	N.D	3.03	2.13	2.36	4.88
C18:2n6c	2.87	0.66	2.94	3.13	3.63	6.52	0.01	2.19	0.70	3.86
C20:0	N.D	1.34	5.06	9.92	13.72	3.87	0.07	4.83	0.28	3.02
C18:3n6	2.22	3.77	5.50	4.52	2.13	0.75	0.20	3.90	1.54	7.33
C20:1	0.71	1.41	5.24	4.05	1.85	0.89	0.09	3.03	0.67	2.12
C18:3n3	3.26	8.45	1.98	4.63	3.01	1.70	13.65	2.59	1.52	1.01
C21:0	N.D	2.59	3.45	6.35	3.84	2.40	0.06	3.29	0.19	0.85
C20:2, C22:0	6.02	2.09	2.44	13.60	2.11	2.91	0.30	7.39	0.13	2.62
C20:3n6	0.39	2.48	4.89	3.93	2.37	1.03	0.42	3.74	1.01	2.86
C22:1n9	1.96	1.13	3.16	5.23	1.10	1.59	0.28	1.84	6.88	23.48
C20:3n3	1.61	1.55	N.D	2.23	9.31	2.29	1.26	1.28	3.46	7.90
C20:4n6	2.54	3.40	2.07	3.78	3.58	8.72	1.08	4.71	2.40	6.33
C23:0	3.78	3.54	1.20	5.69	4.75	3.18	8.82	3.67	3.83	8.93
C22:2	1.89	2.42	1.60	6.02	4.42	2.07	4.32	4.71	3.64	11.94
Unsaturated FA	23.10	30.24	40.46	37.84	80.94	49.57	25.56	86.97	28.33	188.55
Saturated FA	43.18	25.21	35.57	161.16	200.7	128.11	23.46	55.23	212.40	446.76
Total FA	66.28	55.45	76.03	199	281.64	177.68	49.02	142.2	240.73	635.31

The highlighted values are the greatest content of fatty acid among studied salvia species. N.D : not-detected

Table 3. Leaf Fatty Acid Composition (mg kg^{-1}) of *Salvia* Species from Iran

	Grex D					Grex E				
	S. spinosa	*S. leriifolia*	*S. virgate*	*S. nemorosa*	*S. chorassanica*	*S. sclarea*	*S. sahendica*	*S. aethiopis*	*S. atropatana*	*S. chloroleuca*
C6:0	0.72	1.26	N.D	0.22	1.18	0.62	2.06	N.D	0.94	1.55
C8:0	15.19	0.36	0.90	11.24	18.70	11.19	1.67	3.48	7.57	11.29
C10:0	2.68	2.18	1.06	2.60	6.55	3.73	0.21	8.11	1.67	4.68
C11:0	1.39	1.80	0.51	2.24	3.97	1.90	0.09	19.33	0.97	3.89
C12:0	4.51	4.40	1.16	1.90	5.89	1.65	4.10	16.96	2.61	4.51
C13:0	0.84	2.31	0.79	1.24	1.16	0.64	0.92	10.26	0.73	1.37
C14:0	3.60	1.65	0.98	4.58	2.18	0.84	3.72	10.22	0.74	1.37
C14:1	1.05	1.65	1.10	2.37	1.20	0.47	0.71	10.28	0.18	1.21
C15:0	1.45	2.00	0.75	1.64	1.39	0.67	1.30	18.29	0.90	1.44
C15:1	1.23	2.33	0.58	1.09	1.40	1.01	0.34	12.75	0.36	2.29
C16:0	1.08	0.90	0.51	13.95	3.56	1.85	1.13	29.89	4.10	5.42
C16:1	0.42	1.05	1.33	3.19	8.58	5.90	0.15	39.71	17.72	7.37
C17:0	0.60	0.16	0.20	2.44	0.43	0.34	0.01	27.74	0.19	0.43
C17:1	0.90	0.43	0.48	1.99	0.33	0.18	0.01	44.59	0.52	0.33
C18:0	1.06	2.09	2.25	21.85	7.16	2.27	0.69	58.89	5.09	5.78
C18:1n9t	1.14	1.12	1.28	6.13	2.49	0.94	0.31	38.81	1.15	2.31
C18:2n6t,C18:1n9c	0.74	0.39	0.97	2.98	4.86	2.86	1.29	17.24	3.32	4.70
C18:2n6c	0.63	0.51	1.53	1.69	0.68	0.55	0.01	21.60	56.22	2.25
C20:0	0.30	0.46	1.44	2.19	6.98	5.09	0.05	32.80	14.34	6.03
C18:3n6	0.41	2.17	2.48	1.30	0.84	1.50	0.20	28.14	0.60	6.75
C20:1	0.93	N.D	1.38	1.91	0.27	0.36	0.03	26.57	0.67	N.D
C18:3n3	0.90	1.30	3.70	1.91	0.89	0.97	0.30	16.77	1.68	0.33
C21:0	0.67	1.30	0.69	2.18	0.58	0.94	0.06	17.29	0.36	0.21
C20:2, C22:0	0.50	0.37	3.08	2.45	0.32	0.47	0.22	23.18	0.50	0.39
C20:3n6	0.93	1.52	0.62	0.58	3.21	1.87	1.69	15.90	0.22	N.D
C22:1n9	0.94	0.69	0.70	1.04	1.45	0.37	0.43	3.75	0.44	0.41
C20:3n3	1.31	1.52	1.43	1.04	0.41	0.21	0.15	2.11	0.26	N.D
C20:4n6	0.91	0.71	0.79	1.66	0.73	0.73	1.08	6.39	0.52	1.35
C23:0	0.73	1.73	2.11	0.38	4.09	3.00	0.88	23.90	0.28	0.78
C22:2	1.28	1.67	3.67	1.98	7.75	1.38	0.09	12.44	1.55	2.86
Unsaturated FA	13.78	17.13	22.10	30.95	34.79	19.38	6.85	297.12	85.49	32.23
Saturated FA	35.39	23.04	16.52	71.16	64.21	35.27	17.18	300.39	41.05	49.20
Total FA	49.17	40.17	38.62	102.11	99.00	54.65	24.03	597.51	126.54	81.43

The highlighted values are the greatest content of fatty acid among studied salvia species. N.D: not-detected.

Table 4. Omega-3 and Omega-6 Content (mg kg^{-1}) of *Salvia* Species

Species	Leaf		Shoot		w-6/w-3		Total		
	w-6	w-3	w-6	w-3	Leaf	Shoot	w-6	w-3	Species
S. chorassanica	6.76	0.89	20.44	3.01	7.57	6.78	27.2	3.9	31.10
S. chloroleuca	15.06	0.33	22.41	1.01	44.30	22.05	37.47	1.34	38.81
S. atropatana	60.68	0.68	7.02	1.52	36.08	4.61	6.77	2.2	8.97
S. sclarea	5.66	0.97	16.00	1.70	5.81	9.39	21.66	2.67	24.33
S. spinosa	2.69	0.90	9.03	3.26	2.99	2.76	11.72	4.16	15.88
S. nemorosa	7.65	1.91	16.15	4.63	4.00	3.48	23.8	6.54	30.34
S. sahendica	2.59	0.30	4.32	13.65	8.56	0.31	6.91	13.95	20.86
S. virgata	5.78	3.70	14.05	1.98	1.56	7.07	19.83	5.68	25.51
S. leriifolia	2.79	1.30	8.62	8.45	2.90	1.02	11.41	9.75	21.16
S. aethiopis	73.39	16.77	12.94	2.59	4.37	4.97	86.33	19.36	105.69

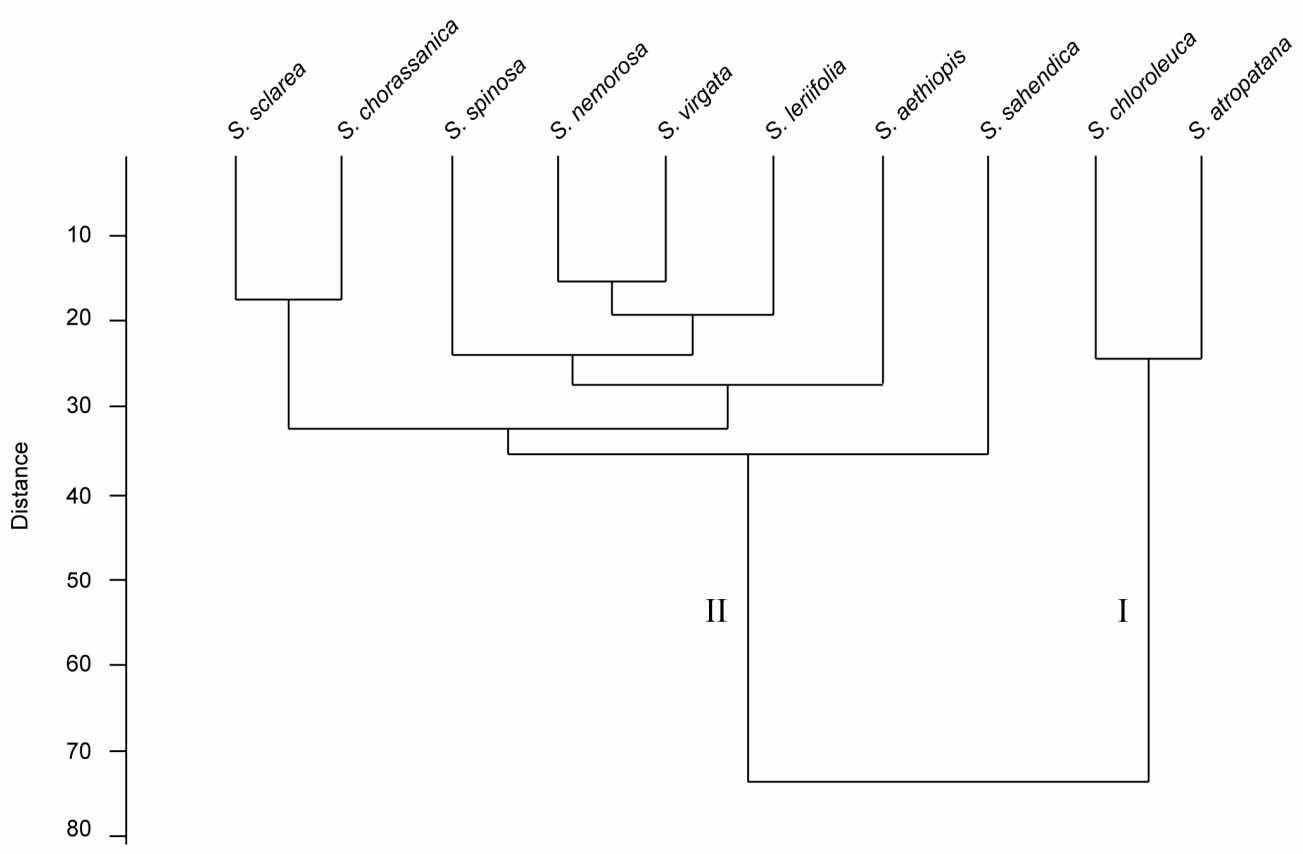

Fig. 2. UPGMA dendrogram showing relationship among 10 species of *Salvia* based on shoot fatty acid composition.

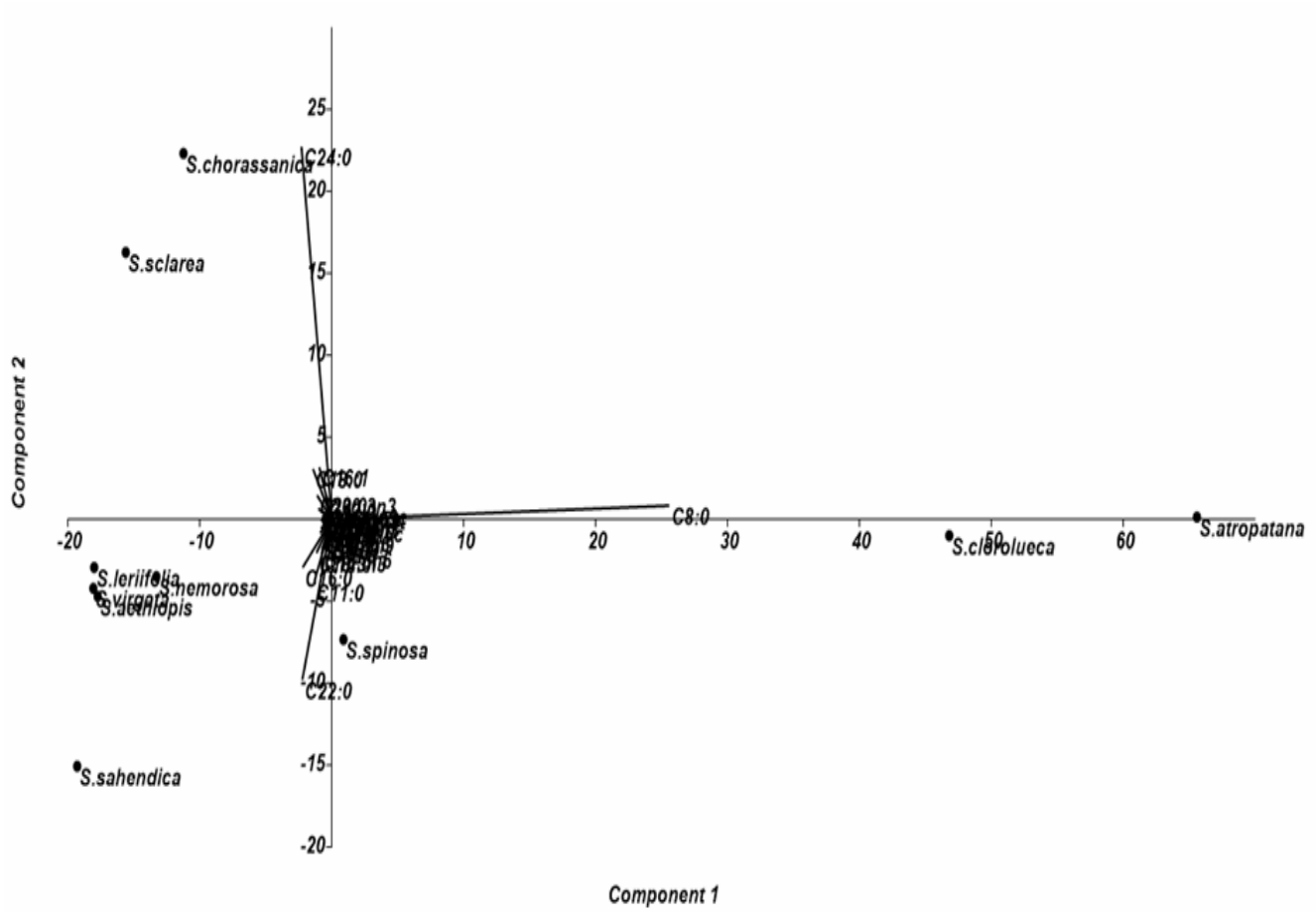

Fig. 3. Principal component analysis (PCA) of 10 species of *Salvia* based on shoot fatty acid composition, Cophe.Corr: 0.94.

in Grex D. A good agreement was found between classical taxonomy and chemical composition data. Morphologically, *S. spinosa*, *S. leriifolia*, *S. virgate*, and *S. nemorosa* are closely related. Species belong to Grex D have shown similar FA composition especially in the main components, so obtained results support the morphological classification (Fig. 2). In cluster analysis, *S. nemorosa* was clearly separated from *S. virgate*, which is based on Sajjadi *et al*. [40] and kharazian [41] reports. Notably, these two species have a high morphological similarity, which makes it difficult to distinguish them.

In Grex E, morphological diagnostic of *S. sclarea* and *S. aethiopis* is difficult, because the leaf characters, hair types and nutlet shape appear more similar. Also, these two species display the stamina character of total fusion of the posterior thecae into what Claβen-Bockhoff *et al*. [42] described as "stamen type v". In the present study, the FAs profiles were found to be appropriate markers to distinguish two species. The high content of C18:3n3 was found in *S. aethiopis,* while a high content of C8, C16, C18 and C24 were found in *S. sclarea*. In addition, FA composition of *S. aethiopis* was similar to species belonging to Grex D.

CONCLUSIONS

In this study, FA composition was leading to separating of *S. atropatana* and *S. chloroleuca* from the other studied species of Grex E. Notably, the two species presented

similar profiles of FAs. As demonstrated in Fig. 3, caprylic acid (C8:0) has obvious taxonomic significance for separation of *S. atropatana* and *S. chloroleuca*, because, caprylic acid was found in species of linkage I in low amounts. Previous studies had shown that *S. atropatena* was different from other taxa. According to Kharazian [43], *S. atropatana* displayed considerable variation in morphological characters. Anatomical observation on nutlets of some *Salvia* species showed that *S. atropatana* could be differentiated from *S. aethiopis* and *S. sclarea* based on thickness of pericarp layer and parenchymatous layers of color [44]. In similar study, Salimpour *et al*. [45] showed that *S. atropatana* is separated from *S. nemorosa* and *S. virgate* based on anatomical characters such as trichomes, stomata and cross section of stem and petiole. Based on essential oil composition, *S. atropatana* could be separated from *S. aethiopis* and *S. sclarea* [46]. We observed FAs variations in all of the *Salvia* species belonging to Grex E. It seems that these taxonomic differentiations are due to polymorphism in the morphological characters, hybridization among species and geographical distribution.

In this study, we also performed cluster analysis based on leaf, shoot and total FA profiles. Accordingly, we suggest that the FA composition of shoot is appropriate as chemotaxonomic marker. The results showed that the FA composition is varied among species, and they are appropriate compounds for taxonomic studies.

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