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Chemical Analysis of Some *Salvia* species Native to West Azarbaijan (Iran)

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Abstract: Present survey was performed on nine species of *Salvia* (Lamiaceae) growing wild in different regions of West Azarbaijan in Iran (*S. limbata*, *S. vertisillata*, *S. macrochlamys*, *S. nemorosa*, *S. ceratophylla*, *S. candidissima*, *S. syriaca*, *S. multicaulis*, *S. sclarea*). In this research, three of the most important biochemical characters such as total protein content, total oil content and fatty acids combination in seeds of these species were considered. Analysis of species average comparison variance conducted with ANOVA and Duncan's test ($p<0.05$) revealed that there is a significant difference about protein content, oil content and fatty acid composition among species.

Key words: *Salvia*, seed, oil content, protein content, fatty acid, Iran

INTRODUCTION

The genus *Salvia* is one of the largest members of the Lamiaceae family. It comprises more than 900 species, which are widespread all over the world. The Mediterranean, Central Asia, America and South Africa are the main centers of diversity of this genus (Malenčić *et al*., 2003). *Salvia* species are an important group of useful plants. Some of this are shrubby or subshrubby and perennial (Nakıboğlu, 1993).

Salvia (Lamiaceae) and their essential oil are used in food flavoring, pharmaceuticals and in perfumery. *Salvia* species have been reportedly used in folk medicine for wound healing and in alleviating stomach, liver and rheumatism pains and for treating the common cold in the form of infusion and Detection (Sezik and Yesilada, 1999). Antioxidant activities of the seed oils of *Salvia* have been reported (Azcan *et al*., 2004). Fatty acid components of the seed oils of these plants are palmitic, palmitoleic, stearic, oleic, linolenic, linolenic and arachidic acids. These fatty acids are responsible for the antioxidant activity (Castoro *et al*., 1986).

Chia seeds contain 21% protein, a level markedly greater than other nutritional grains such as wheat (14%), corn (14%), rice (8.5%) and oat (15.3%). Chia seeds have an oil content of approximately one third of their weight, about 60% of which is α-linolenic acid, making this ingredient a rich source of ω3 fatty acids (Craig and Sons, 2005). Chia seeds are also a rich source of vitamins B, calcium, phosphorus, potassium, zinc and copper (Malenčić *et al*., 2003).

The most popular species of the genus *Salvia*, sage (*Salvia officinalis* L.) is a well-known medicinal plant. The sage acts as an antiphlogistic, stomachic, antiseptic, antiasthematic, astringent drug and used as spice (Chiej, 1988). However, the majority of wild-growing *Salvia* species have not been fully evaluated from their phytochemical point.

The aim of this study was determined the protein content, oil content and fatty acid composition of selected *Salvia* species, in order to evaluate their nutritive value. There are almost no reports on the protein content, oil content and fatty acid composition of the species found in the world.

MATERIALS AND METHODS

We initiated the biochemical studies at the Laboratory of Biochemistry and Artemia Researches Center of Urmia University in West Azarbaijan in Iran, using living materials which were collected from their natural habitats during mid May and end of June 2006.

The sampling localities and *Salvia* species used in this study are given in Table 2.

Nine taxa of *Salvia* were used in this experiment: *S. limbata* C.A.MEY, *S. vertisillata* L., *S. macrochlamys* Boiss, *S. nemorosa* L., *S. ceratophylla* L., *S. candidissima* Vahi, *S. syriaca* L., *S. multicaulis* Vahi, *S. sclarea* L. For this purpose, dried and crushed seeds were used.

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The Lowry *et al.* (1951) method was used for determination of the total protein content. For measurement of total oil content, ether method was used (Leiboritz *et al.*, 1987).

Fatty acids were determined by Gass Chromatography after the preparation of their methyl esters. The analysis of fatty acid methyl esters were performed by Gass Chromatograph (model: GC-1000) on a EC-1000 column (30×0.33 mm i.d., 0.25 μm film thickness) using hydrogen, equipped with a FID (220°C) (Siavah *et al.*, 2005). Identification of the methyl esters were made by comparison of retention times of standard fatty acid methyl esters (Saglik *et al.*, 2002). For statistical analysis, ANOVA and Duncans' test were used ($p<0.05$).

RESULTS AND DISCUSSION

The fatty acid composition of the seed oil was determined by gas chromatography and the results are shown in Table 1 and Fig. 1-9.

Previous reports on seed oil fatty acids composition indicated that the 18:3/18:2 ratio could be used as a taxonomic marker in some subfamilies of the Lamiaceae (Azcan *et al.*, 2004). The main fatty acid components of the seed oils of *Salvia* species were reported as palmitic, stearic, oleic linolenic and linolenic acid (Azcan *et al.*, 2004).

There were 17 fatty acids identified by comparison with the fatty acid methyl ester standards. In the investigated *Salvia* species, the lowest oil yield was obtained from *S. syriaca* (29.86%) and *S. vertisillata* seeds gave the highest oil yield (50.42%) (Table 2).

Palmitic acid was the main constituent in seed oils of the *S. limbata* (2.83%), *S. vertisillata* (3.2%), *S. macrochlamys* (5.51%), *S. nemorosa* (3.23%), *S. ceratophylla* (3.15%), *S. candidissima* (4.9%) and *S. multicaulis* (5.1%). In the seed oils of *S. limbata* (2.7%), *S. vertisillata* (4.28%), *S. macrochlamys* (3%), *S. nemorosa* (2.55%), *S. ceratophylla* (2.77%), *S. candidissima* (2.68%) and *S. syriaca* (1.65%), palmitoleic acid was the main component.

Stearic acid was the main constituent in the seed oils of *S. limbata* (1.39%), *S. ceratophylla* (1.35%), *S. candidissima* (1.19%), *S. multicaulis* (1.88%) and *S. sclarea* (1.97%). Oleic acid was the main constituent in seed oils of the *S. limbata* (13.93%), *S. vertisillata* (8.13%), *S. macrochlamys* (8.86%), *S. nemorosa* (11.05%), *S. ceratophylla* (13.33%), *S. candidissima* (16.3%), *S. multicaulis* (13.93%) and *S. sclarea* (2.47%). In the seed oil of *S. macrochlamys*, Cis-vaccenic acid (7.37%) was the main component.

Linolenic acid was the main constituent in seed oils of *S. limbata* (13.78%), *S. vertisillata* (10.88%), *S. macrochlamys* (40.17%), *S. nemorosa* (14.88%), *S. ceratophylla* (16.76%), *S. candidissima*, (11.88%) and *S. sclarea* (21.53%). In the seed oils of Linolenic acid *S. limbata* (18.23%), *S. vertisillata* (19.47%), *S. nemorosa* (25.66%), *S. ceratophylla* (16.29%) and *S. sclarea* (10.77%). Arachidic acid was the main component in the seed oils of *S. limbata* (17.61%), *S. vertisillata* (25.1%), *S. ceratophylla* (14.82%) and *S. sclarea* (4.79%). Gadoleic acid was the main component of *S. candidissima* (16.9%) and *S. sclarea* (27.78%). Eicosatrienoic acid was the main component in seed oil of *S. sclarea* (13.7%).

Table 1: Mean amounts of fatty acid compositions of 9 Salvia species (%)

Fatty acids	1	2	3	4	5	6	7	8	9
14:0	0.054	0.041	0.14	0.022	0.032	0.38	0.42	0.24	0.134
14:1	0.04	0.02	0	0	0	0	0	0	0
16:0	3.1	4.9	6.39	3.21	2.83	5.51	3.23	5.1	0
16:1	2.7	2.686	1.65	4.28	2.72	3.006	2.55	3.69	4.73
18:0	1.356	1.19	1.86	0.41	1.39	1.38	1.34	1.88	1.9
18:1n7	0	0	0	0	0	7.37	0	0.03	0
18:1n9	13.33	16.36	24.37	8.132	11.36	8.86	11.05	13.93	2.4
18:2	16.76	11.88	35.74	10.83	13.78	40.17	14.88	0.44	21.5
18:3	16.29	6.54	34.32	19.47	18.23	0.61	25.66	24.26	10.7
20:0	14.82	26.91	8.55	25.1	17	0.38	0	0.05	4.7
20:1	0.031	16.91	0.05	0	0	0.077	0.19	0.42	27.8
20:2	0.046	0.06	0.06	0.05	0.059	0.035	0.07	0.06	0.05
20:3	0	0.025	0	0.02	0	0	0	0	13.7
20:4	0	0.06	0	0	0	0	0	0.085	0.05
20:5	0	0.057	0	0	0.049	0	0	0	0.08
22:0	0	0	0.198	0	0	0.26	0.09	0.29	0
22:1	0	0	0.05	0	0	0.057	0	0.09	0
ΣSaturated	21.83	28.78	7.91	5.08	19.7	33.03	17.54	7.27	6.89
ΣUnsaturated	46.16	42.75	60.16	54.4	19.37	54.5	96.19	43.15	81.27
U/S	2.11	1.48	7.6	59.4	2.54	1.63	5.64	5.93	11.97
18:3/18:2	1.32	1.79	0.01	1.72	0.97	0.55	0.96	55.13	0.5

(Numbers 1, 2, 3, …and 9 represent species of *Ceratophylla*, *Candidissima*, *Syriaca*, *Vertisillata*, *Limbata*, *Macrochlamys*, *Nemorosa* and *Multicaulis*, *Sclarea*, respectively)

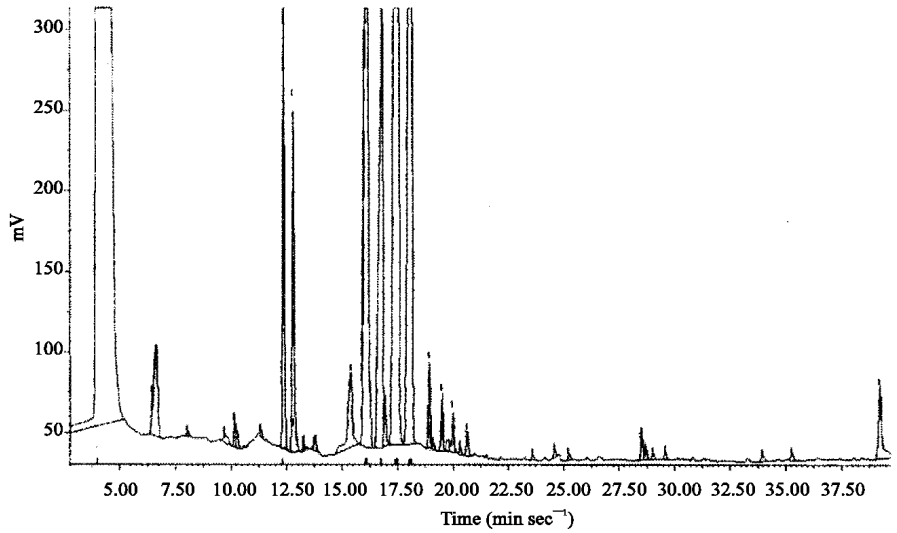

Fig. 1: Chromatographical of *S. multicaulis*

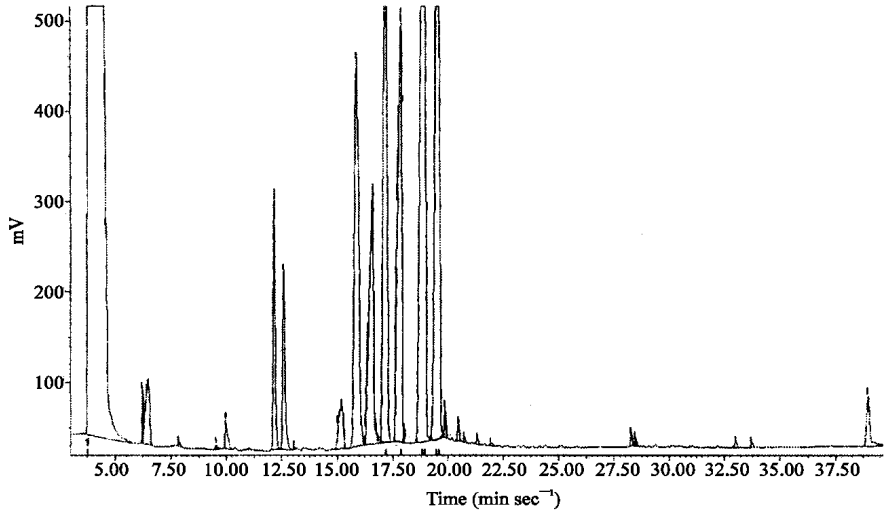

Fig. 2: Chromatograph of *S. nemorosa*

S. limbata: In the seed oil of *S. limbata*, 10 fatty acids identified by comparison with the fatty acid methyl ester standards. The majority fatty acids were α-linolenic acid (18.23%), arachidic acid (17.6%) linolenic acid (13.78%) and oleic acid (11.37%), followed by palmitic acid (2.83%), Stearic acid (1.39%) and palmitoleic acid (2.7%). On the other hand, 3 fatty acids were detected in small quantities (0.03-0.05%).

There were 4 saturated fatty acids in the sample, of which, the total percentage of them was 21.83%. In the unsaturated fatty acids, α-linolenic acid (18.23%) was the predominant fatty acid in them and the total percentage was 46.16%. The ratio of unsaturated fatty acids to the saturated (U/S) was 2.11% and the 18:3/18:2 ratio was 1.32%.

S. vertisillata: In the seed oil of *S. vertisillata*, 10 fatty acids identified. The major fatty acids were arachidic acid (25.1%), α-linolenic acid (19.47%), linolenic acid (10.88%) and oleic acid (8.13%), followed by palmitoleic acid (4.28%) and palmitic acid (3.2%). Also, 4 fatty acids were detected in small quantities (0.02-0.05%) except 18:2 (0.41%). There were 4 saturated fatty acids in the sample of which, the total percentage of them was 28.78%. In the

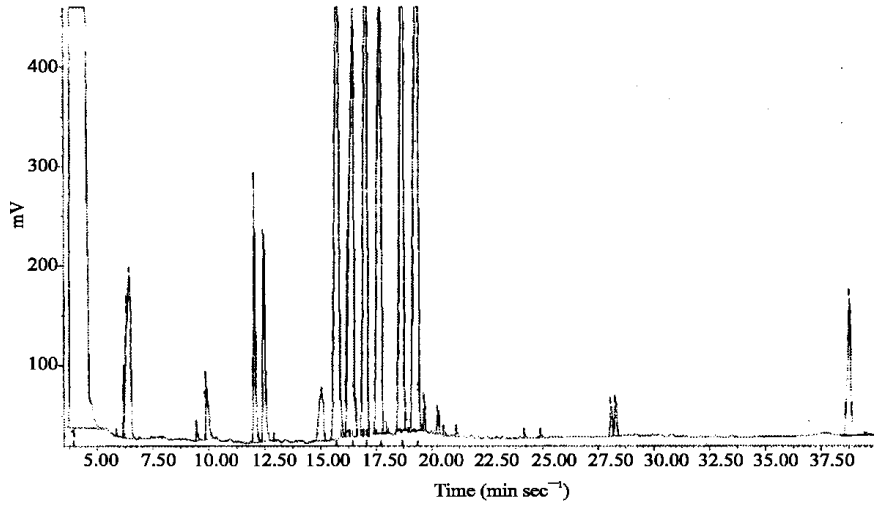

Fig. 3: Chromatographical of *S. candidissima*

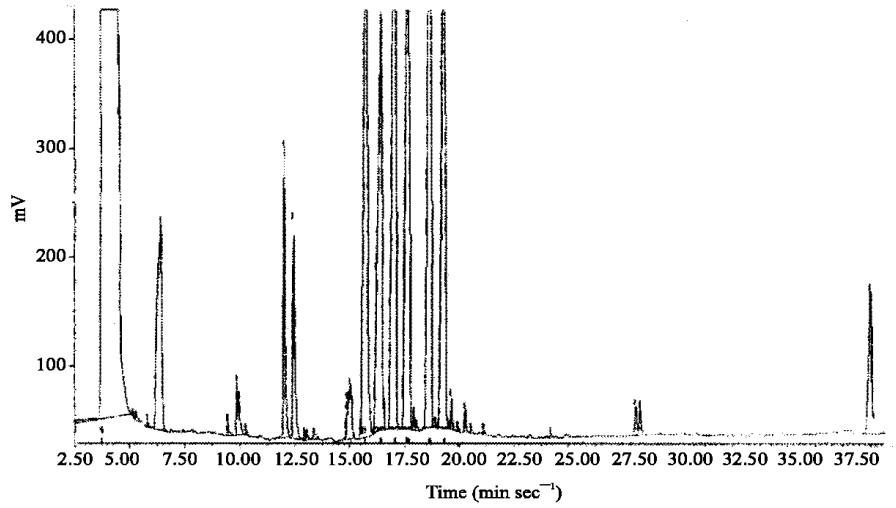

Fig. 4: Chromatographical of *S. ceratophylla*

unsaturated fatty acids, α-linolenic acid (19.47%) was the predominant fatty acid in them and the total percentage was 42.75%. The ratio of unsaturated fatty acids to the saturated (U/S) was 1.48% and the 18:3/18:2 ratio was 1.79%.

S. macrochlamys: In the seed oil of *S. macrochlamys*, 13 fatty acids identified. The major fatty acids were linolenic acid (40.17%), oleic acid (8.86%), Cis-vaccenic acid (7.37%), palmitic acid (5.51%) and stearic acid (1.38%), Also, 8 fatty acids were detected in small quantities (0.03-0.38%). There were 5 saturated fatty acids in the sample of which, the total percentage of them was 7.91%. In the unsaturated fatty acids, linolenic acid (40.17%) was the predominant fatty acid in them and the total percentage was 60.16%. The ratio of U/S was 7.6% and the 18:3/18:2 ratio was 0.01%.

S. nemorosa: In the seed oil of *S. nemorosa*, 10 fatty acids identified. The major fatty acids were α-Linolenic acid (25.66%), linolenic acid (14.88%), oleic acid (11.5%), palmitic acid (3.23%) followed by palmitoleic acid (2.55%) and stearic acid (1.34%). Also, 4 fatty acids were detected in small quantities (0.07-0.09%) except 20:1

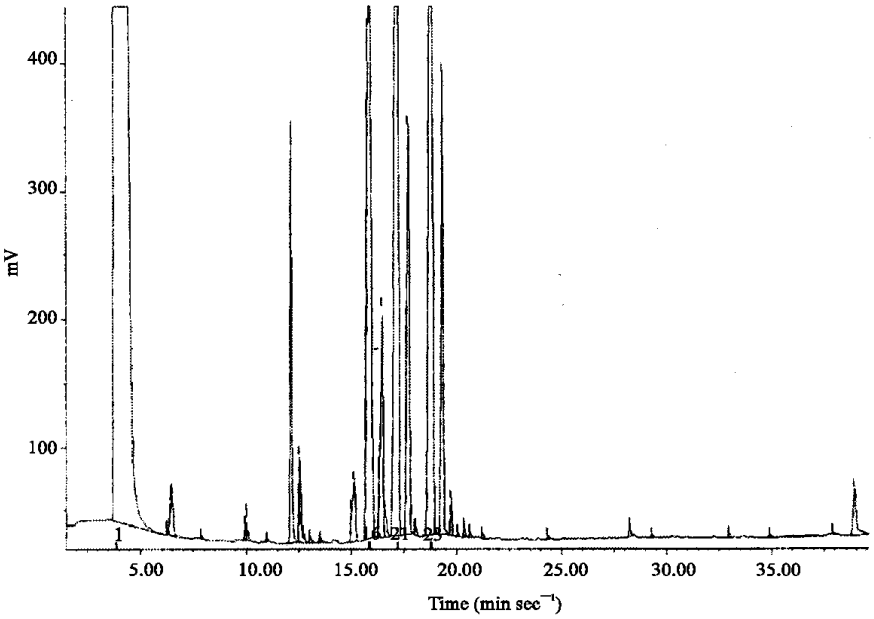

Fig. 5: Chromatographical of *S. syriaca*

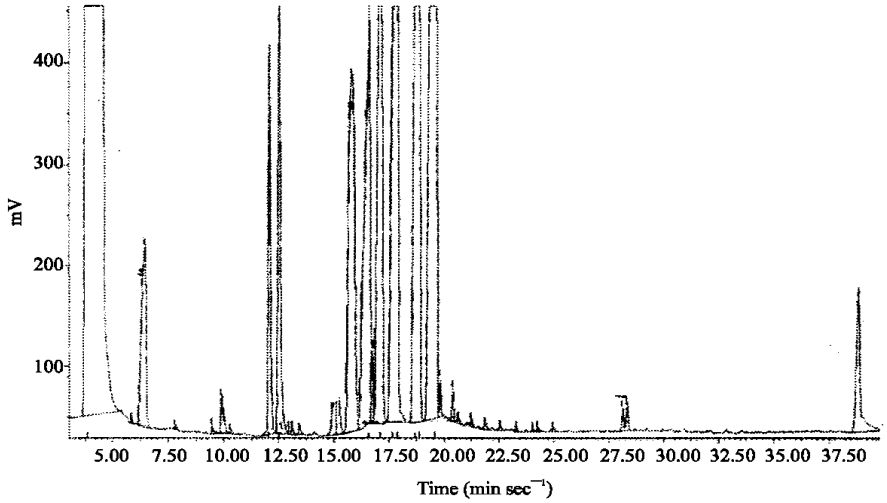

Fig. 6: Chromatographical of *S. vertisillata*

(0.19%) and 14:0 (0.42%). There were 4 saturated fatty acids in the sample of which, the total percentage of them was 5.08%. In the unsaturated fatty acids, α-linolenic acid (25.66%) was the predominant fatty acid in them and the total percentage was 54.4%. The ratio of U/S was 59.4% and the 18:3/18:2 ratio was 1.72%.

S. ceratophylla: In the seed oil of *S. ceratophylla*, 11 fatty acids identified. The major fatty acids were linolenic acid (16.76%), α-linolenic acid (16.29%), arachidic acid (14.82%), oleic acid (13.33%) and palmitic acid (3.15%) followed by palmitoleic acid (2.77%) and stearic acid (1.35%). Also, 4 fatty acids were detected in small quantities (0.03-0.05%). There were 4 saturated fatty acids in the sample of which, the total percentage of them was 19.37%. In the unsaturated fatty acids, linolenic acid (16.76%) was the predominant fatty acid in them and the total percentage was 19.37%. The ratio of U/S was 2.54% and the 18:3/18:2 ratio was 0.97%.

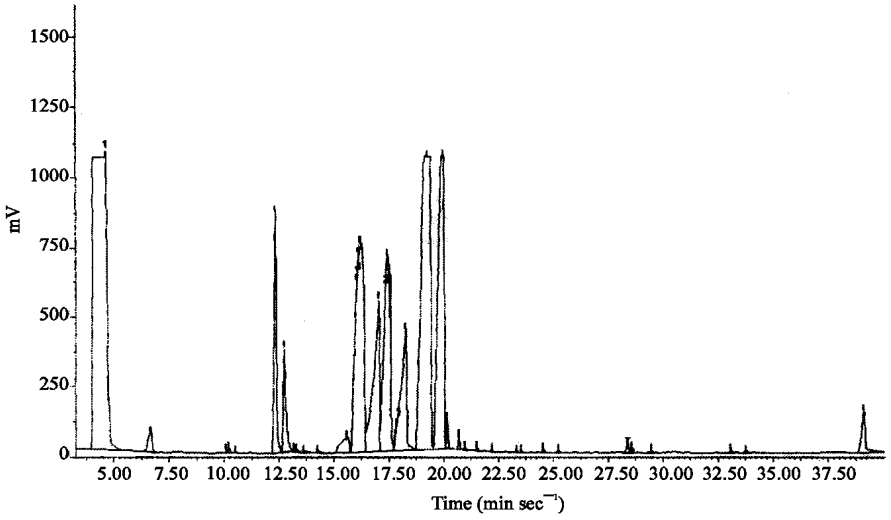

Fig. 7: Chromatographical of *S. limbata*

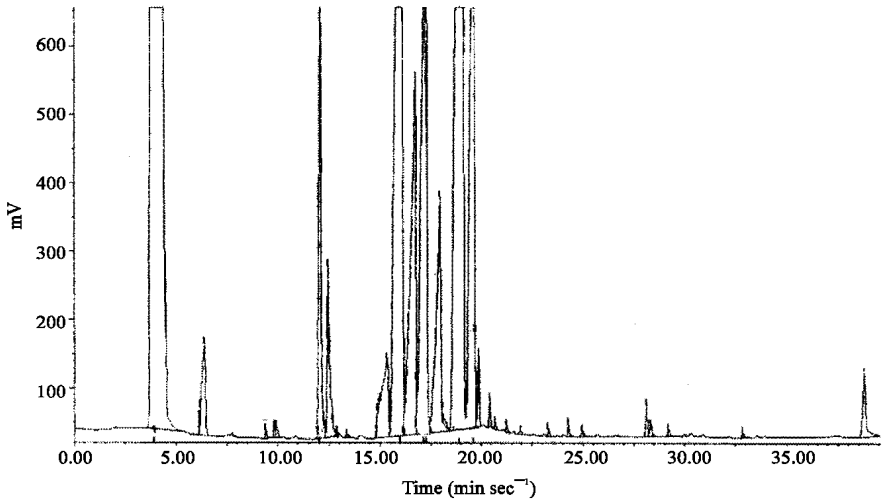

Fig. 8: Chromatographical of *S. sclarea*

S. candidissima: In the seed oil of *S. candidissima*, 14 fatty acids identified. The major fatty acids were arachidic acid (26.9%), gadoleic acid (16.9%), oleic acid (16.3%), linolenic acid (11.88%) and α-linolenic acid (6.5%) and palmitic acid (4.95), followed by palmitoleic acid (2.68) and stearic acid (1.195). Also, 6 fatty acids were detected in small quantities (0.02-0.06%). There were 4 saturated fatty acids in the sample of which, the total percentage of them was 33.3%. In the unsaturated fatty acids, gadoleic acid (16.9%) was the predominant fatty acid in them and the total percentage was 54.5%. The ratio of U/S was 1.63% and the 18:3/18:2 ratio was 0.55%.

S. syriaca: In the seed oil of 12 fatty acids identified. The major fatty acids were linolenic acid (35.74%), α-linolenic acid (34.32%), oleic acid (24.37%), arachidic acid (8.55%) and palmitic acid (6.39%), followed by stearic acid (1.86%), palmitoleic acid (2.68) and palmitoleic acid (1.65%). Also, 5 fatty acids were detected in small quantities (0.05-0.06%) except 22:0 (0.19%) and 14:0 (0.14). There were 5 saturated fatty acids in the sample of which, the total percentage of them was 17.54%. In the unsaturated fatty acids, linolenic acid (35.74%) was the predominant fatty acid in them and the total percentage was 96.19%. The ratio of U/S was 5.64% and the 18:3/18:2 ratio was 0.96%.

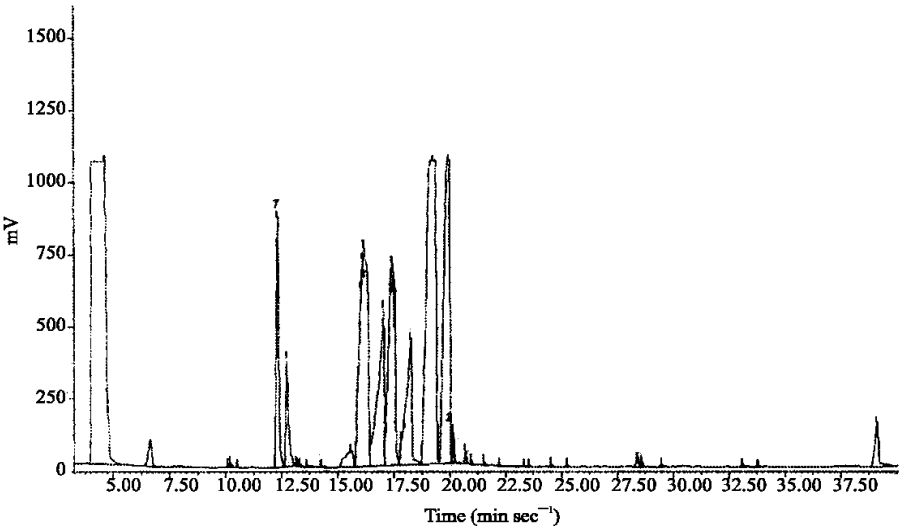

Fig. 9: Chromatographical of *S. macrochlamys*

Table 2: seed oil yields and collection sites of *Salvia* species

Species	Collection area	Oil yield (%)
S. limbata C.A.MEY	Urmia: Ghooshchi Mountains	40.60
	Urmia: Marmisho	
	Urmieh: Gharebagh Village	
S. vertisillata L.	Urmia: Ghooshchi Mountains	50.42
	Urmia: Marmisho	
	Maku: Bazargan	
S. macrochlamys Boiss	Urmia: Marmisho	33.30
S. nemorosa L.	Urmia: Shohada Vallay	48.70
	Chaldran	
	Salmas: Between Koozehrash and Sarichichak Village	
	Maku: Siahcheshmeh	
S. ceratophylla L.	Urmieh: Gharebagh Village	41.37
	Urmieh: between Gharebagh and Agziarat Village	
S. candidissima Vahi	Urmia: Marmisho	33.16
S. syriaca L.	Urmia: Ghooshchi Mountains	29.86
	Oshnavieh: Mir Abad Jungle	
S. multicaulis Vahi	Naghadeh: Soltan Yaaghub Vallay	39.54
	Oshnavieh: Barden Zard	
	Urmia: Shohada Vallay, Khan Vallay	
	Oshnavieh road	
	Urmia: Nooshinshahr, Keshish Mountain	
S. sclarea L.	Maku: Siahcheshmeh	43.20
	Urmia: Nazloo Village	
	Urmia: Shamlakan Village	
	Khoy: 25 km of road of Ghareziaaddin	
	Sardasht: 25 km of Piranshahre	

S. multicaulis: In the seed oil of *S. multicaulis*, 12 fatty acids identified. The major fatty acids were α-Linolenic acid (24.26%), oleic acid (13.93%), palmitic acid (5.1%), palmitoleic acid (3.69%) and stearic acid (1.88%). Also, 7 fatty acids were detected in small quantities (0.03-0.09%) except 18:2 (0.44%). There were 3 saturated fatty acids in the sample of which, the total percentage of them was 7.27%. In the unsaturated fatty acids, α-linolenic acid (24.26%) was the predominant fatty acid in them and the total percentage was 43.15%. The ratio of U/S was 5.93% and the 18:3/18:2 ratio was 55.13%.

S. sclarea: In the seed oil of *S. sclarea*, 12 fatty acids identified. The major fatty acids were gadoleic acid (27.78%), linolenic acid (21.53%), Eicosatrienoic acid (13.7%), arachidic acid (4.79%) and palmitoleic acid (4.74%), followed by oleic acid (2.47%) and stearic acid (1.97%). Also, 4 fatty acids were detected in small

Table 3: Analysis of variance for parameters in *Salvia* species

Source of variation	df	Mean squares												
		Oil	Pr	1	2	3	4	5	6	7	8	9	10	11
Between Groups	8	146.6	31.8	0.06	0.001	10.45	2.65	0.614	17.89	110.26	463.31	325.04	343.09	298.8
Within Groups	18	0.104	3.62	0.003	0	0.027	0.04	0.012	0.001	0.01	0.025	0.05	0.185	3.7
		12	13	14	15	16	17							
Between groups	8	0.013	62.72	0.003	0.003	0.036	0.004							
Within groups	18	0.004	0.001	0	0	0	0							

Pr: Protein, Numbers 1, 2, 3, …and 17 represent fatty acids, 14:0, 14:1, 16:0, 16:1, 18:0, 18:1n7, 18:1n9, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 20:5 and 22:1, respectively

quantities (0.05-0.13%). There were 3 saturated fatty acids in the sample of which, the total percentage of them was 6.89%. In the unsaturated fatty acids, gadoleic acid (27.78%) was the predominant fatty acid in them and the total percentage was 81.27%. The ratio of U/S was 11.79% and the 18:3/18:2 ratio was 0.5%. The oil of *S. ceratophylla* seeds contained the lowest content of total unsaturated fatty acids (19.37%) while they were the main components in the seed oil of *S. sclarea*.

As to the saturated fatty acid contents in the oils examined, the lowest yield was obtained in the seeds of *S. nemorosa* (5.08%) and highest content was encountered in the seeds of *S. candidissima* (33.03%). The ratio of unsaturated fatty acids to the saturated ranged between 1.63 to 59.4%. Present results showed that protein content was high, ranging from 8.4 to 16.77%. The highest amounts were found in the seeds of *S. vertisillata* (16.77%) followed by *S. syriaca* (15.16%), *S. candidissima* (15.15%), *S. nemorosa* (15.2%), *S. macrochlamys* (10.67%), *S. limbata* (10.23%), *S. ceratophylla* (9.83%), *S. sclarea* (8.84%) and *S. multicaulis* (8.4%).

In all the taxa studied, this is the first study of the seed proteins of *Salvia* species, therefore, a comparison with the previously published data has not been possible. However present results agree with those of previously published studies on other members of the genus *Salvia* (Craig, 2005). Analysis of species average comparison variance conducted with ANOVA and Duncan's test ($p<0.05$) revealed that there is a significant difference about protein content, oil content and fatty acid composition among species (Table 3). In all the taxa studied, the ratio of unsaturated fatty acids is greater than that of saturated fatty acids. This is a typical feature of the seed oils of the family Lamiaceae (Azcan *et al*., 2004).

This is the first study on fatty acids composition, 14:0, 20:2, 20:4, 20:5 and 22:1 and high amounts of Cis-Vaccenic acid, arachidic acid and gadoleic acid. Cis-Vaccenic acid was determined in some different Umbelliferae seed oils (Reiter *et al*., 1998) and also pulp lipids of commonly available fruits (Shibahara *et al*., 1987). In seed lipids it is generally found in lower concentrations (0.5-2%), although it's higher amounts in some species (Spitzer, 1996). In this study it was found as 7.23% (*S. macochlamys*) and this value is high comparing the general literature findings (Soukup and Holman, 1987), also, 0.03% (*S. multicaulis*). In all the taxa studied, this is the first study of the seed oils of *Salvia* species apart from *S. sclarea*, *S. candidissima*. Seed oil of *S. sclarea*, was reported to be rich in Linolenic (36.6%), as the main fatty acid constituent followed by oleic (19.4%), linolenic acid (18.1%) (Azcan *et al*., 2004). In this taxa, oil yield as 4%, was reported. Seed oil of *S. candidissima*, was reported to be rich in oleic acid (21.1%), palmitic acid (21%), Linolenic acid (20.9%) and linolenic acid (19.2%) (Azcan *et al*., 2004). In this taxa oil yield as 5.6%, was reported. From the aspect of fatty acids percentage and oil yield, our results don't agree with those of previous studies conducted on other members of the genus *Salvia* (Azcan *et al*., 2004). The investigation of the fatty acid composition of the *Salvia* species showed that the plants except *S. macrochlamys* were well supplied with Omega 3 fatty acid that this fatty acid is necessary for human body.

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