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Chemotaxonomic Evaluation of Species of Turkish Salvia: Fatty Acid Composition of Seed Oils. II.

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Abstract: Fatty acids composition of seed oil of *Salvia viridis, S. hydrangea, S. blepharochleana, S. chianantha, S. staminea, S. hypergeia,, S. cilicica, S. caespitosa, S. sclarea, S. cadmica, S. microstegia, S. pachystachys* and *S. verticillata* were analyzed by GC/MS. The main compound were found to be as linoleic acid (18:2; 12.8 % to 52.2 %), linolenic acid (18:3; 3.2 % to 47.7 %), oleic acid (18:1; 11.3 % to 25.6 %), palmitic acid (16:0; 0.7 % to 16.8 %) and stearic acid (18:0; 1.8 % to 4.8 %). A phylogenetic tree of species of *Salvia* were reported and compared to 18:3/18:2 ratio of the seed oils. Fatty acid composition of *Salvia* seed oils could be used as a chemotaxonomical marker.

Key words: *Salvia*; Fatty acid; Linoleic acid; Linolenic acid; chemotaxonomy

1. Introduction

There are 89 species and 93 taxa of *Salvia* recorded in the Flora of Turkey [1-4] The ratio of endemism of species of *Salvia* in Turkey is 48 % and Anatolia is a major centre for the genus in Asia [1]

Fatty acid compositions of the *Salvia* species were reported in the literature. The genus *Salvia* belongs to the Nepetoideae subfamily of the Lamiaceae of angiosperms. The family has been characterized by the occurence of linolenic, linoleic and oleic acids in their seeds oil [5,6]. Fatty acid compositions of the seed oils of *Salvia hispanica* L, [7-11] *S. scleara* [12] and 25 Turkish *Salvia* species were reported in the literature and the main constituents of them were similar to other Nepetoideae subfamily members. The main fatty acid composition of reported 25 *Salvia* species were palmitic acid, oleic acid, linoleic acid and linolenic acid [6,13-14].

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As part of our continuing studies of fatty acid composition of seed oils and chemotaxonomy of species of *Salvia* [5,14], we have now investigated fatty acid composition of 13 species of *Salvia: Salvia viridis, S. hydrangea, S. blephorachleana, S.chianantha, S. staminae, S. hyperegia, S. cilicica, S.caespitosa, S. sclarea, S. cadmica, S. microstegia, S. pachstachys* and *S. verticillata,* and continued the chemotaxonomic evaluation of 35 species of *Salvia* were reported.

2. Materials and Methods

2.1. Plant Material

Salvia species were collected during seed period. Locality, altitude, collection time and Herbarium number of Thirteen species of *Salvia* are folllows: *Salvia viridis* (Osmaniye, 1600 m, June, 2004, ISTE 83384), *S. hydrangea* (Iğdır, 1200 m, June, 2004, ISTE 83385), *S. blepharochleana* (Kayseri, 1700 m, June 2004, ISTE 83386), *S. chianantha* (Burdur, 1150 m, July 2004, ISTE 83387), *S. staminea* (Erzincan, 2200 m, July 2004, ISTE 83388) *S. hypergeia* (Adana, 900 m, June 2004, ISTE 83389), *S. cilicica* (K.Maraş, 1300 m, July 2004, ISTE 83390), *S. caespitosa* (Kayseri, 2400 m, June 2004, ISTE 83391), *S. sclarea* (Osmaniye, 900 m, June 2004, ISTE 83392), *S. cadmica* (Denizli, 1800 m, July 2004, ISTE 83393), *S. microstegia* (Kayseri, 1600 m, July 2004, ISTE 83394), *S. pachystachys* (Erzincan, 2250 m, July 2004, TD. ISTE 83395), *S. veritcillata* (Sivas, 1200 m, August 2004, ISTE 83396). The voucher specimens were deposited in the Herbarium of ISTE and Department of Biology, Faculty of Arts and Science, Balıkesir University.

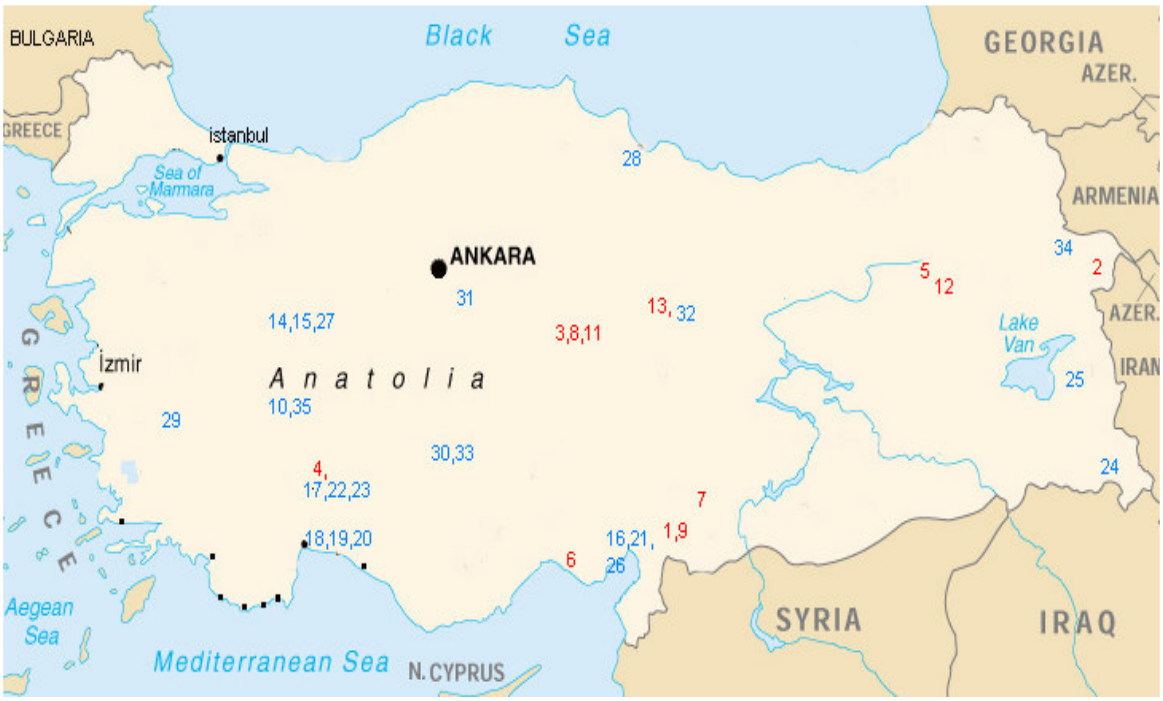
2.2. Sample Extraction and Derivatization

Seeds of species were separated from the rest of the aerial parts of the plants. The seeds of *Salvia viridis* (10.1 g), *S. hydrangea* (10.3 g), *S. blepharochleana* (8.2 g), *S. chianantha* (10.4 g), *S. staminea* (7.8 g), *S. hypergeia* (17.0 g), *S. cilicica* (9.8 g), *S. caespitosa* (8.6 g), *S. sclarea* (12.0 g), *S. cadmica* (10.0 g), *S. microstegia* (15.1 g), *S. pachystachys* (6.2 g) and *S. verticillata* (15.1 g) were extracted with hexane by using the Soxhlet extraction and the oil yields were 150 mg (1.5 %), 200 mg (1.9 %), 160 mg (1.9 %), 140 mg (1.3 %), 225 mg (2.9 %), 187 mg (1.1 %), 143 mg (1.5 %), 140 mg (1.6 %), 180 mg (1.5 %), 600 mg (6 %), 170 mg (1.1 %), 160 mg (2.6 %), 200 mg (1.3 %) respectively.

For the derivatization of free fatty acids: The seed oil (30 mg) was dissolved in toluene (1 mL) in a test tube and H_2SO_4 in methanol (2ml, 1%) was then added. The mixtures was left overnight at 50 ^{0}C, then sodium chloride solution (5ml, 5%) was added and the required esters were extracted with hexane (2x 5 mL), then the organic layer was separated using Pasteur pipettes for both samples. The hexane layers were washed with potassium bicarbonate solution (4 ml, 2%) and dried over anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure on a rotary evaporator to give fatty acid methyl esters [5,15].

For the derivatization of total fatty acids: 100 mg of the seed oil was refluxed in 0.1 M KOH solution in ethanol (2 mL) for 1 hour. The solution was cooled and 5 mL water was added. The aqueous mixture was neutralized with 0.5 mL HCl solution and extracted with hexane:diethyl ether (1:1; 3 x 5 mL). The organic layer was separated and washed with water (10 ml), dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure on a rotary evaporator to give fatty acid methyl esters [5-7]. Derivatization procedure was described above.

Figure 1. Locality of reported species



1. *Salvia viridis* L., *(Osmaniye) ;* **2.** *S. hydrangea* DC ex Benth., *(Iğdır);***3.** *S. blepharochlaeana* Hedge & Hub.-Mor.,*(Kayseri);* **4.** *S. chianantha* Boiss.,*(Burdur) ;* **5.** *S. staminea* Montbret & Aucher ex Benth.,*(Erzincan);* **6**. *S. hypargeia* Fisch. & Mey., (Adana); **7.** *S. cilicia* Boiss. & Kotschy,*(K.Maraş);* **8.** *S. caespitosa* Montbret & Aucher ex Benth,*(Kayseri)*; **9.** *S. sclarea* L., *(Osmaniye);* **10.** *S. cadmica* Boiss., *(Denizli);***11.** *S. microstegia* Boiss.&Ball.,*(Kayseri) ;***12.** *S. pachystachys* Trautv., *(Erzincan);* **13.** *S. vetricillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm.,*(Sivas);* **14.** *S. bracteata* Banks & Sol., *(Afyon);* **15.** *S. aethiopis* L., *(Afyon);* **16.** *S. candidissima* Vahl ssp *candidissima, (İçel) ;***17.** *S. syriaca* L., *(Burdur) ;***18.** *S. potentillifolia* Boiss. & Heldr. ex Benth.,*(Antalya);***19.** *S. candidissima* L. ssp. *occidentalis* Hedge, (Antalya); **20.** *S. tomentosa* Miller,*(Antalya);* **21.** *S. recognita* Fisch. & Mey., (İçel);**22.** *S. virgata* Jacq., (Burdur);**23.** *S. ceratophylla* L., (Burdur) ;**24.** *S. macrochlamys* Boiss. & Kotschy,*(Hakkari)*; **25.** *S. poculata* Nab., *(Van) ;***26.** *S. albimaculata* Hedge & Hub.-Mor.,(İçel); **27.** *S. cryptantha* Montbret & Aucher ex Benth., (Eskişehir); **28.** *S. forskahlei* L., (Samsun);**29.** *S. fruticosa* Miller, (Strict of Aegean); **30**. *S. halophila* Hedge, (Konya); **31**. *S. tchihatcheffii* (Fisch. & Mey.) Boiss.(Ankara); **32**. *S. euphratica* Montbret & Aucher ex Benth., *var. eupharitica*, (Sivas); **33**. *S. aucheri* Benth var. *canascens* Boisss & Heldr., (Konya);**34**. *S. limbata* C.A. Meyer, (Ağrı) ; **35**. *S. cedronella* Boiss.(Denizli)

2.3. GC/MS conditions

The fatty acid methyl esters were analyzed using Trace 2000 GC series gas chromatography and Thermo mass spectrometer. SGE BPx70 column (60 m x 0.25mm,

0.25 μm film thicknes) was used. The carrier gas was helium at a rate of 1mL/min. GC oven temperature was kept at 100 ^{0}C for 5 min and programmed to 240 ^{0}C at a rate of 4 ^{0}C/min and kept constant at 240 ^{0}C for 5 min. The injection temperature and source temperature were 250 ^{0}C and 220 ^{0}C, respectively. MS interface temperature was 240 ^{0}C. The injection volume was 0.5 μL with a split ratio of 1:30. EI/MS were taken at 70 eV ionization energy. Mass range was from m/z 50 to 650 amu. Scan time 0.5 sec. with 0.1 interscan delay. The library search carried out using NIST and Wiley GC-MS library and TÜBİTAK-UME library. Supelco^{TM} 37 components FAME mixture (Catalog no:47885-U) were used for the comparison of the GC chromatograms. The relative percentage of separated compounds were calculated from Total Ion Chromatography by the computerized integrator.

3. Results and discussion

The main free fatty acids in the seeds of *Salvia* were determined as linoleic acid (18:2; 12.8 % to 52.2 %), linolenic acid (18:3; 2.9 % to 47.7 %), oleic acid (18:1; 11.3 % to 32.4 %), palmitic acid (16:0; 0.7 % to 16.8 %) and stearic acid (18:0; 1.8 % to 6.5 %) and, the main total acid composition of the reported species were determined as linoleic acid (18:2; 14.3 % to 52.2 %), linolenic acid (18:3; 3.2 % to 35.9 %), oleic acid (18:1; 13.7 % to 32.1 %), palmitic acid (16:0; 7.1 % to 14.5 %) and stearic acid (18:0; 2.5 % to 5.5 %) (Table.1)

In the Flora of Turkey, the Turkish species of *Salvia* were classified into seven subgroups. Using the shapes of leaves, calyx texture, the length and colour of corolla as criteria [1-2] The reported species were classified as follows; **2**, **3**, **8** and **12** (Group A), **10** (Group B), **4, 9** and **11** (Group D), **6** and **10** (Grup E), **1,7** and **13** (Group F), **5, 7 and 11** (Group G) [2]. In our previous study, 18:3/18:2 ratio of seed oil of species of *Salvia* has been used as a taxonomic marker [6]. Linoleic acid, linolenic acid, oleic acid, palmitic acid and stearic acid were the main fatty acids in the majority of species of *Salvia* (Table 1). The ratio of linolenic acid to linoleic acid showed good agreement in each subgroup (Table 2). In this study, 18:3/18:2 (linolenic acid/linoleic acid) ratio for the species of Turkish *Salvia* were found in the ranges of 0.36-0.4 for species of Group A, 0.01-0.06 for species of Group B, 0.01-0.11 for Group C, 0.99 -2.17 for species of Group D, 001-0.58 for species Grup E, 0.82-1.31 for species of Group F, and 0.82-2.64 for species of Group G except, *S aethiopis*.

Table 1 Fatty Acid Composition of *Salvia* species*

C	1[a]	1[b]	2[a]	2[b]	3[a]	3[b]	4[a]	4[b]	5[a]	5[b]	6[a]	6[b]	7[a]	7[b]	8[a]	8[b]	9[a]	9[b]	10[a]	10[b]	11[a]	11[b]	12[a]	12[b]	13[a]	13[b]
6 :0	t	t	t	t	-	-	t	t	t	t	-	-	t	t	t	t	t	-	0.2	0.4	-	-	t	t	t	t
8 :0	t	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	-	t	t	t	t
8 :1 (2Z)	-	-	-	0.2	t	0.3	-	-	-	-	1..3	1..3	0.6	t	0.4	t	0.1	t	0.3	t	-	-	t	t	t	t
10 :0	-	-	-	-	-	-	-	-	0.5	0.3	2.5	1.7	t	t	-	-	-	-	t	t	t	t	t	t	-	-
10 :1 (2Z)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	t	t	0.3	0.2	-	-
12 :0	t	t	t	t	t	t	-	-	-	0.5	6.4	5.1	t	t	0.4	t	t	t	t	0.4	t	t	t	t	t	t
14 :0	t	t	0.1	0.2	0.2	0.3	t	-	0.4	0.5	4.2	3.4	t	0.6	0.2	0.5	0.1	0.1	0.2	0.4	0.2	0.2	0.3	0.4	t	0.1
15 :0	t	t	-	-	-	-	0.3	t	-	-	-	-	t	0.3	t	0.2	t	0.1	t	t	t	t	0.2	0.3	t	t
15 :1Z (9Z)	t	t	-	-	0.2	0.3	0.6	t	0.7	1.4	-	-	t	t	11.1	t	-	-	t	t	t	t	0.5	0.8	t	t
16 :0	**10.1**	**9.6**	**9.1**	**9.4**	**6.4**	**7.5**	**9.8**	**10.8**	**6.4**	**7.1**	**16.8**	**14.5**	**9.4**	**11.2**	**0.7**	**11.6**	**8.2**	**9.7**	**7.4**	**9.6**	**8.6**	**9.6**	**7.9**	**8.5**	**8.1**	**10.2**
16 :1 (9Z)	t	t	t	t	t	t	t	t	0.2	0.5	t	t	0.9	0.9	t	1.0	t	0.4	0.2	0.8	t	0.8	0.7	0.8	t	t
16 :1 (11Z)	0.2	0.2	0.3	0.3	0.5	0.6	t	0.5	0.1	t	1.0	2.3	t	t	t	t	0.1	t	0.2	t	0.2	t	t	t	0.2	0.4
17:0	0.1	0.1	0.1	0.1	0.3	t	t	0.2	0.2	0.2	t	t	t	0.1	0.51	0.4	0.1	0.1	0.2	1.4	0.1	0.2	0.2	0.3	0.2	0.2
17 :0	0.1	0.1	0.2	0.3	t	t	t	0.1	0.1	t	t	t	t	t	t	0.3	0.1	0.2	0.3	t	0.1	0.2	0.2	0.2	t	0.1
18 :0	**3.6**	**3.4**	**2.5**	**2.5**	**1.8**	**2.5**	**4.1**	**4.8**	**2.9**	**3.5**	**6.5**	**5.5**	**3.4**	**3.6**	**2.19**	**3.1**	**3.3**	**4.1**	**2.8**	**3.3**	**3.7**	**4.1**	**2.1**	**2.7**	**2.8**	**3.8**
18:1 (9Z)	**21.8**	**20.7**	**25.1**	**25.6**	**16.8**	**18.8**	**28.6**	**31.9**	**14.1**	**15.4**	**15.5**	**13.7**	**11.3**	**14.8**	**21.3**	**23.7**	**17.1**	**20.6**	**24.2**	**27.8**	**32.4**	**32.1**	**17.3**	**18.9**	**20.6**	**25.0**
18:1 (11Z)	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
18 :2 (9Z ,12Z)	**26.5**	**26.5**	**51.2**	**52.2**	**52.2**	**52.1**	**14.2**	**14.3**	**18.1**	**18.5**	**12.8**	**12.8**	**17.1**	**21.8**	**50.1**	**44.8**	**20.8**	**22.8**	**47.3**	**34.7**	**21.2**	**19.1**	**47.3**	**45.4**	**27.1**	**25.7**
18 :3 (9Z, 12Z,15Z)	**34.8**	**35.8**	**5.2**	**3.2**	**10.3**	**5.9**	**20.4**	**24.4**	**47.7**	**42.4**	**7.4**	**7.4**	**16.2**	**20.9**	**5.6**	**5.4**	**45.2**	**35.9**	**2.9**	**4.1**	**30.6**	**25.8**	**8.9**	**10.8**	**38.1**	**29.7**
20 :0	t	t	t	t	t	t	t	t	t	t	2.2	2.2	t	t	0.9	0.6	t	t	t	t	t	t	t	t	t	t
20 :1 (11 Z)	0.7	0.7	1.2	1.3	0.9	1.1	1.6	0.8	0.8	0.8	t	t	0.7	0.9	0.1	1.2	1.3	1.2	0.7	1.1	0.7	0.6	t	1.2	0.5	0.5
20 :2 (10 Z,13 Z)	0.1	0.2	0.2	0.1	0.2	0.2	t	0.2	0.3	t	t	t	t	t	0.3	0.2	t	t	t	t	t	t	t	t	t	t
22 :0	0.2	0.2	0.2	0.2	1.1	1.5	0.3	0.3	0.8	1.5	2.6	2.6	t	t	0.2	0.7	0.2	0.2	0.2	0.7	0.1	0.3	0.3	1.8	0.2	0.2
22 :1 (13 Z)	0.2	0.2	0.3	0.2	0.7	0.4	0.1	0.3	0.3	0.2	1.1	1.1	1.6	0.8	0.3	0.3	0.2	0.2	0.1	0.2	0.1	0.2	0.3	t	0.1	t
24:0	0.2	0.2	0.3	0.2	0.5	0.3	0.2	0.2	0.7	0.4	2.4	2.4	0.9	1.2	0.4	0.5	0.2	0.2	0.3	0.3	0.1	0.2	0.6	0.4	0.1	0.1
25 :0	0.2	0.3	0.5	0.5	0.8	0.6	0.1	0.4	0.2	0.2	t	t	3.1	1.3	0.3	0.7	0.2	0.3	0.5	0.3	0.2	0.2	0.6	0.5	0.1	0.2
26:0	t	0.1	0.1	0.1	0.8	0.4	t	0.1	-	0.2	2.2	2.2	2.6	0.6	0.6	0.3	0.1	0.1	t	0.4	t	0.1	t	t	t	t
27 :0	t	0.3	0.2			0.3	0.1		-		1.3	1.3	3.1	0.6	0.7	0.6	t	t	-		t	t	1.1	0.6		
28 :0	0.1	0.2	0.4	0.3	0.3	0.9	0.2	0.3	t	0.6	2.6	2.6	2.6	1.8	0.9	0.8	0.3	0.3	0.9	0.9	0.2	0.2	1.2	0.8	0.3	0.3
Σ Saturated FA	14.8	14.7	14.0	14.1	12.8	14.9	15.1	17.7	12.3	15.0	50.7	45.8	25.1	21.2	8.0	20.3	12.9	15.3	13.2	18.1	13.5	15.3	14.7	16.5	12.0	15.6
Σ Unsaturated FA	84.1	84.1	83.2	82.8	81.3	79.1	65.5	71.9	82.2	79.2	38.1	36.3	48.4	60.2	89.2	76.6	84.7	81.2	75.7	68.7	85.0	78.6	75.3	78.1	86.4	80.9
18:3/18:2	1.31	1.35	0.10	0.06	0.20	0.11	1.44	1.71	2.64	2.29	0.58	0.58	0.95	0.96	0.11	0.12	2.17	1.57	0.06	0.12	1.44	1.35	0.19	0.24	1.41	1.16
Total FA	98.8	98.7	97.1	96.9	94.0	94.1	80.6	89.9	94.5	94.2	88.8	82.1	74.0	81.4	97.2	96.9	97.6	96.5	88.9	86.8	98.5	93.9	90.0	94.6	98.4	96.5
Oil yield	1.5	-	2.0	-	2.0	-	1.4	-	3.2	-	1.1	-	1.5	-	1.7	-	1.5	-	6.0	-	1.2	-	2.6	-	1.4	-

[a] Free Fatty acid composition of species [b]Total fatty acid composition of species (Supelco™ 37 components FAME mixture (Catalog no:47885-U) were used for the comparison of the GC chromatograms) *The species**:** **1**: *Salvia viridis,* **2**: *S. hydrangea,* **3**:*S. blepharochleana,* **4**: *S. chianantha*, **5**: *S. staminea*, **6**: *S. hypergeia*, **7**: *S. cilicica*, **8**: *S. caespitosa* **9**: *S. sclarea*, **10**: *S. cadmica* **11**: *S. microstegia*, **12**: *S. pachystachys* **13**: *S. verticillata.*

Table. 2. Classification of the species of *Salvia* by 18:3/18:2 ratio of seed oil of studied Turkish Species of *Salvia* [a]*[Ψ]

Group A	Group B	Group C	Group D	Group E	Group F	Group G
2 (0.10)	**10** (0.06)	**10** (0.06)	**4** (1.44)	**6a** (0.58)	**1** (1.31)	**5** (2.64)
3 (0.20)	**14a** (0.05)	**27** (0.02)	**9a** (2.17)	**6b** (0.47)	**7a** (0.95)	**7a** (0.95)
8 (0.10)	**14b** (0.04)	**30** (0.01)	**9c** (2.13)	**6c** (0.44)	**7b** (0.82)	**7b** (0.82)
12 (0.20)	**21** (0.05)	**32** (0.11)	**11** (1.44)	**10** (0.06)	**13** (1.41)	**11** (1.44)
18 (0.40)	**29** (0.01)		**16** (0.99)	**24** (0.30)	**25** (0.90)	**15** (0.04)
26 (0.05)			**19** (1.40)	**20** (0.05)	**22** (1.05)	**17** (0.90)
31 (0.03)			**34** (1.45)	**28** (0.04)	**30** (0.97)	**22** (1.05)
35 (0.04)				**29** (0.01)		**23** (1.20)
				33 (0.02)		**25** (0.90)

[a]The bold number indicate the number of species of *Salvia* given in figure 1.
*The 18:3/18:2 ratios of seed oil of species are given in parenthesis and the values were taken from the literature for species **14-35** [5-6,12-13].
[Ψ]Groups A-G of species of *Salvia* were described in references [1-2]

In conclusion, the results of this study show that linolenic/linoleic acid mixtures with the ratios identified above from the seed oils of species of *Salvia* can be used as chemotaxonomical marker as described previously [14]

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