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Fatty Acid Composition of Seed Oils of Eight *Salvia* Species Growing in Iran

Zahra Sepehry Javan[1]*, Fatemeh Rahmani[2], Leilasadat Asadyar[3]

[1]Department of Biology, Faculty of Sciences, Urmia University, Iran,
[2]Department of Biology and Institute of Biotechnology, Urmia University, Iran
[3]Master's degree, Department of Biology, Faculty of Sciences, Urmia University, Iran.

***Corresponding Author**

Abstract: *Pharmacological and taxonomic studies have been mainly driven by investigations about distribution and composition of fatty acids of seed oils. Seed oils of eight saliva species (S. hydrangea, S. xanthocheila, S. limbata, S. aethiopis, S. macrochlamys, S. nemorosa, S. sclarea and S. verticillata) collected from Ardebil province in Iran were obtained by Soxhlet apparatus using hexane. The oil yields were found to be between 2.2% and 25%. Fatty acids in the oils were converted to methyl esters and determined by GC/MS in methyl ester form. The main fatty acid components were linoleic acid, linolenic acid, oleic acid and palmitic acid. The broad sense heritability was assessed between 0.992 and 1. Moreover, there were noticeable qualitative and quantitative differences among Salvia species based on fatty acid composition and oil yield in seeds. Hence, these traits could be used as selection criteria to improve seed yield and quality.*

Keywords: *Fatty Acids, Lamiaceae, Salvia Seed Oils.*

INTRODUCTION

Salvia is the largest genus of *Lamiaceae* family with over 900 species throughout the world. This genus has 58 species in Iran which 17 of them are endemic (Saeidnia *et al.,* 2012). *Salvia* has a strong environmental adaptability and widely distributed throughout the world (Delamare *et al.,* 2007), especially in subtropical and temperate regions (Okada, 2007). Many *Salvia* species have been used in traditional medicine as a tonic, anti rheumatoid, antimicrobial, and carminative and also as a flavored spice (Li *et al.,* 2013). Moreover, *Salvia* seeds are used for nutrition and medicinal purposes such as for the treatment of eye diseases. Potential therapeutic usages of *Salvia* species are due to their essential oils which contain antioxidant, antimicrobial, antifungal, and aromatic properties (Ben Taarit *et al.,* 2014; Farhat *et al.,* 2015).

Fatty acid is a carboxylic acid with long aliphatic tail which is divided into saturated and unsaturated acid, depending on the presence of unsaturated double bond in the fatty acid chain. *Lamiaceae* family has been characterized by linolenic, linoleic and oleic acids in their seed oils (Azcan *et al.,* 2004). It is worth mentioning that linoleic and α-linolenic acids are essential for normal growth, health promotion, and disease resistance. In addition, the oils which contain high proportions of oleic acid can reduce cardiovascular diseases (Farhat *et al.,* 2015).

The oil concentration and fatty acid composition of seeds are completely varied due to the genetic and environmental differences (Farhat *et al.,* 2015). As *Salvia* species predominantly propagate sexually by seeds (Song *et al.,* 2010) chemical composition of essential oils can reveal the differences among *Salvia* species. *Salvia* species also show great intraspecific essential oil variations according to their geographical origin. For

example, Pitarokili et al. (2006) analyzed essential oil composition of *Salvia verticillata*, *S. verbenaca*, *S. glutinosa* and *S. candidissima* growing wild in Greece. They reported that *β*-phellandrene and (*E*)-caryophyllene were the main essential oil components in studied species while sabinene, cadinene, terpinen-4-ol, and pinene were typical compounds of *S. verbenaca* essential oil originated from Saudi Arabia (Al-Howiriny, 2002).

To the best of our knowledge, chemical composition of *Salvia* species grown in Ardabil region of Iran has not been investigated before. In addition, there are numerous studies which are focused on aerial parts of *Salvia* species, while works interested in seeds are scanty. Hence, the results of the present study can be seen as a starting point for future studies to improve seed yield and quality. For this purpose, we analyzed and compared the seed fatty acid composition of eight *Salvia* species to determine their potential usages for human or animal consumptions.

Materials and Methods

Plant materials

The sample collection for the present investigation was carried out during August of year 2010 in Randomized Design with three replicates. The seeds were collected from randomly selected plants/ replications. Voucher specimens were deposited at the Herbarium laboratory of Ardabil Agriculture Research Center. Locality, climate data, collection time and Voucher number of the *Salvia* species were indicated in Table 1. The geographical distribution of *Salvia* species has been represented in Figure 1.

Sample extraction

Seeds of eight species of *Salvia* were separated from the rest of the aerial parts of the plants. Seeds of species were extracted with hexane by using the Soxhlet extraction. The solvent was evaporated and residue was refluxed with 0.5 N sodium hydroxide solutions in 5 ml methanol for 10 min. Subsequently, 5 ml of 14% BF_3-MeOH solution was added by a pipette through condenser and boiled for 2 min. Then 5 ml of heptane was added through condenser and boiled for 1 min. The solutions were cooled. Then, 5 ml saturated NaCl solution was added and the fatty acid methyl esters were extracted with heptane (2 × 5 ml), the organic layer was dried over anhydrous Na_2SO_4. The fatty acid methyl esters were recovered after solvent evaporation under vacuum (Gören *et al.,* 2003). Data were reported as mean for three replicates (Table 2).

GC/MS analysis conditions

The fatty acid methyl esters were analyzed using Trace 2000 GC series gas chromatography and thermo mass spectrometer. SGE BPx70 column (60 m × 0.25 mm, 0.25 µm film thickness) was used. The carrier gas was helium at a rate of 1 ml/min. GC oven temperature was kept at 100 °C for 5 min and programmed to 240 °C at a rate of 4 °C / min and kept constant at 240 °C for 5 min. The injection temperature and source temperature were 250 °C and 220 °C, respectively. MS interface temperature was 240 °C. The injection volume was 0.5 ml with a split ratio of 1:30. Mass range was from *m/z* 50 to 650 amu, scan time was 0.5 s with 0.1 inter scan delay. The relative percentage of separated compounds was calculated from Total Ion Chromatography by the computerized integrator.

Broad sense heritability estimates

The heritability estimates facilitate the evaluation of genetic and environmental effects, which aid in selection. Thus, the plant breeder can anticipate improvement. In order to assess the heritable portion of total variability, phenotypic variance (δ^2p) was partitioned into genotypic (δ^2g) and error variance (δ^2e). Heritability, estimates were calculated by the formulae used by Khan et al. (1992), $\delta^2p = \delta^2e + \delta^2g$ and $h^2 = \delta^2g / \delta^2p$.Genetic ($\delta^2g$) and environmental ($\delta^2e$) variances were computed from the mean squares in the analysis of variance of each trait (Coakes *et al.,* 2009).

Statistical analysis

Data were analyzed using two-way ANOVA followed by Tukey's post hoc test for multiple comparisons. All statistical analyses were performed using Statistical Analysis System (SAS) 9.1.3 software (SAS Institute Inc, 2005). A significance level of $P \leq 0.05$ was used for all statistical analyses.

Results

Oil yield and fatty acid composition

Among the eigh *Salvia* species, the highest oil yield was obtained from *S. sclarea* seeds (25%), and *S. aethiopis* seeds gave the lowest oil yield (2.2%) (Table 2). Fourteen fatty acids identified by comparison with the fatty acid methyl ester standards. A total of fourteen compounds of the essential oils were identified. The content of major fatty acid components varied according to the *Salvia* species studied as follows: linoleic acid (18:2; 3.44 – 29.6%), linolenic acid (18:3; 2.92 – 25.3%), oleic acid (18:1; 3.9 – 26.03%) and palmitic acid (16:0; 1.67 – 17.5%). The major fatty acid was erucic acid for *S. aethiopis* (57.85%), *S. sclarea* (52.3%), *S. limbata* (37.84%), *S. hydrangea* (28.7%), *S. macrochlamys* (26.93%) and *S. nemorosa* (14.4%) whereas, *S. verticillata* and *S. xanthocheila* completely lacked this fatty acid. Myristic acid, pentadecanoic acid and arachidic acid were only identified in *S. verticillata* and *S. xanthocheila*.

Broad sense heritability (h^2)

To assess the heritable portion of total variability, phenotypic variance (δ^2p) was partitioned into genotypic (δ^2g) and error variance (δ^2e). The broad sense heritability of fatty acid composition for studied species was determined to be between 0.992 and 1. The least heritability (0.992) was observed for pentadecanoic acid (15:0) and palmitoleic acid (16:1) content (Table 3).

Discussion

Our main findings were (1) all studied species were well supplied with Omega-3 and Omega-6 fatty acids and (2) there were noticeable qualitative and quantitative differences among *Salvia* species based on fatty acid composition and oil yield in seeds.

According to our results, the main fatty acid components were linoleic acid, linolenic acid, oleic acid and palmitic acid. The highest levels of linolenic and linoleic acids were belonged to *S. xanthocheila*, and the highest levels of oleic and palmitic acids were determined in *S. macrochlamys*.

The *Salvia* genus belongs to *Lamiaceae* family that has been characterized by the occurrence of linolenic, linoleic, and oleic acids in their seed oils. Our results were in accordance with the previous that indicated palmitic acid, oleic acid, linoleic acid and linolenic acid were the main fatty acid components of *Salvia* species (Azcan *et al.,* 2004; Gören *et al.,* 2006; Farhat et al. 2015).

However, in this study, stearic acid was the main component in *S. verticillata* (78.5 %) seed oil, Habibvash et al., (2007) reported that arachidic acid was the major fatty acid (25/1%) in *S. verticillata* (Habibvash et al 2007). Further, linoleic acid, linolenic acid and oleic acid were also found as the major compounds in studies on S.hispanica L. (Ayerza et al., 2002; Heuer et al., 2002) and S.sclarea (Ferlay et al., 1993). Our study confirms the existence of these fatty acids in all species except stearic acid, which is absent in S. sclarea, S. aethiopis and S. macrochlamys. The reason of these differences could be some environmental factors such as drought and high temperature, which can decrease the amount of seed oils (Farhat *et al.,* 2015).

According to our results, all studied species were well supplied with Omega-3 (2.92- 25.3%) and Omega-6 (3.44- 29.6%) fatty acids, and the highest level of Omega-3 (25.3%) and Omega-6 (29.6%) fatty acids was determined in S. xanthocheila that was collected from the lowest mean annual temperature (7.9 °C) and the highest mean annual rainfall (509.2 mm) region in comparison to other species. Since these fatty acids are essential for human health, they are considered as the most important oil components (Carvalho *et al.,* 2006). However, human diet requires a balanced source of *n*-3 and *n*-6 essential fatty acids to prevent those diseases

caused by an excess of *n*-6 fatty acids including asthma, coronary heart disease, many forms of cancer, autoimmunity and neurodegenerative diseases (Simopoulos, 2002). The most abundant fatty acid in *S. xanthocheila* oil seeds was linoleic acid, followed by linolenic, oleic acids and palmitic. In 1995, Ohlrogge and Browse suggested that Δ^{12}- desaturase is responsible for desaturation of oleic to linoleic acid in oilseed plants, and so this activity can lead to the accumulation of the latter fatty acid, which is the case of *S. xanthocheila* in our study.

In *S. hydrangea*, *S. limbata*, *S. aethiopis*, *S. nemorosa* and *S. sclarea* the accumulation of linolenic acid was over linoleic acid (Table 2) that can be due to the the fact that Δ^{15}-desaturase which is involved in desaturation of linoleic acid into linolenic is more active than the Δ^{12}-desaturase (Bet Taarit et al. 2007).

Based on our results, in studied all species, the ratio of unsaturated fatty acids to saturated fatty acids is high ranging from 2.25 to 5.46 except for *S. verticillata* (0.14) and the level of unsaturated fatty acids was greater than saturated fatty acid which is a typical feature of the oil seeds of the family *Lamiaceae* (Azcan *et al.,* 2004). Previous reports on seed oil fatty acids composition indicated that the C18:3/C18:2 ratio could also be used as a taxonomic marker in some subfamilies of *Lamiaceae* family (Azcan *et al.,* 2004; Goren *et al.,* 2006; Kilic *et al.,* 2007).

Fatty acid composition is an important index for *Salvia* seed oil quality evaluation (Azcan *et al.,* 2004). Genotype, year, various physiological, geographical and ecological factors influence chemical composition of fatty acids (Cheikh-Rouhou *et al.,* 2007). Bon our results; there is a close relationship between *S. sclarea* and *S. aethiopis* using fatty acid composition (Table 2). These two species were also clustered in one group according to our previous study applying RAPD and ISSR molecular markers (Sepehry-Javan *et al.,* 2012). Therefore, studied traits could be used as selection criteria and our present study can be valuable to highlight the potential usages of some *Salvia* seeds in human diets and for oil industrial purposes.

The variability existed among studied species, can be mainly due to the genotypic variance when the error variance is very low (Yadav *et al.,* 2006). Therefore, based on our results the variation among studied species can be largely due to genotypic variance as the values of error variance were determined between 0 and 0.08. As a result, genetic improvement for these traits can easily be achieved by selection of promising plant types and also through crossing the desirable accessions among themselves followed by selection in segregating generations. The knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through selection (Yadav *et al.,* 2006). It is worth mentioning that most of the fatty acids showed the highest heritability (1). In this study, broad sense heritability was high, indicating the major role of genetic factors on fatty acids content in *Salvia* species.

Conculsion

In conclusion, there are qualitative and quantitative differences among *Salvia* species based on fatty acids composition and oil yield in seeds. Our data also implies that seed oil fatty acid compounds could be employed as a reliable biochemical marker for genetic variation studies.

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Table 1: *Salvia* seeds with their locality, voucher specimen, collection time, altitude and climate data.

Plant samples	Locality	Voucher number	Collection time	Altitude (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)
S. hydrangea	Khalkhal, Ardabil, Iran	1127	August, 2010	2000- 2160	9.6	345.5
S. xanthocheila	Sabalan mountain, Shabil, Ardabil, Iran	619	August, 2010	2000- 2500	7.9	509.2
S. limbata	Ahar-Meshginshahr road, Ardabil, Iran	485	August, 2010	2000- 2160	8.9	334.7
S. aethiopis	Sardabeh, Ardabil, Iran	495	August, 2010	2000- 2500	9.2	414.5
S. macrochlamys	Germi, Ardabil, Iran	3298	August, 2010	2000- 2500	17.55	245.0
S. nemorosa	Ahar-Meshginshahr road, Ardabil, Iran	3963	August, 2010	2000- 2160	8.9	334.7
S. sclarea	Khalkhal, Ardabil, Iran	3953	August, 2010	2000- 2500	9.6	345.5
S. verticillata	Sardabeh, Ardabil, Iran	3472	August, 2010	2000- 2500	9.2	414.5

Table 2: Fatty acid composition of *Salvia* species collected from Ardabil province (Iran).

Compounds	1	2	3	4	5	6	7	8
Myristic acid (14:0)	- a	0.4 b	- a	- a	- a	- a	- a	0.1 c
Pentadecanoic acid (15:0)	- a	1.1 b	- a	- a	- a	- a	- a	6.3 c
Palmitic acid (16:0)	12 a	14.3 b	12.2 c	5.0 d	17.1 e	10.3 f	5.6 g	1.7 h
Palmitoleic acid (16:1)	- a	- a	- a	- a	- a	- a	- a	0.12 b
Heptadecanoic acid (17:0)	- a	4.8 b	- a	- a	- a	- a	- a	0.6 c
Stearic acid (18:0)	2.5 b	8.4 c	3.32 d	-a	-a	3.4 d	- a	78.5 e
Oleic acid (18:1)($C_{18}H_{34}O_2$)	20 a	14.4 b	16.1 c	4.8 d	26.0 e	21.4 f	4.3 g	3.5 h
Linoleic acid (18:2)	12.1 a	29.6 b	10.3 c	5.4 d	22.6 e	11.9 a	3.4 g	3.9 h
Linolenic acid (18:3)	16.1 a	25.3 b	20.3 c	10.6 d	19.5 e	15.8 f	10.3 g	2.9 h
Arachidic acid (20:0)	- a	- a	- a	- a	- a	- a	- a	0.1 b
Eicosenoic acid (20:1)	- a	- a	- a	- a	- a	- a	- a	0.8 b
Behenic acid (22:0)	8.7 b	-a	-a	16.4 c	-a	9.93 d	24.1 e	-a
Erucic acid (22:1)	28.7 b	-a	37.9 c	57.9 d	14.4 e	26.9 f	52.3 g	-a
Ricinoleic acid (18:1)($C_{18}H_{34}O_3$)	-a	-a	-a	-a	-a	-a	-a	0.5 b
∑Saturated FA	23.2 a	30.8 b	15.5 c	21.43 d	17.5 e	24.0 f	29.8 g	87.82 h
∑Unsaturated FA	76.8 a	69.3 b	84.5 c	78.61 d	82.5 e	76.0 f	70.2 g	12.2 h
∑Unsaturated/∑saturated	3.3 a	2.3 b	5.46 c	3.7 d	4.7 e	3.2 f	2.4 g	0.1 h
18:3/18:2	1.3 a	0.9 b	2 c	2 d	0.9 e	1.3 a	3 f	0.8 g
Oil yield (W/V)	15 a	5 b	16 c	2.2 d	2.9 e	14 f	25 g	24 h

The species: 1- *S. hydrangea,* 2- *S. xanthocheila,* 3- *S. limbata,* 4- *S. aethiopis,* 5- *S. macrochlamys,* 6- *S. nemorosa,* 7- *S. sclarea* and 8- *S. verticillata.* Data are expressed as mean of 3 replicates. Means within one row having different letters are significantly different according to Tukey's Post hoc at P≤0.05

Table 3: Estimates of variance components and heritability in *Salvia* species.

compounds	$\delta^2 g$	$\delta^2 e$	$\delta^2 p$	h^2	mean	F value*
Myristic acid (14:0)	0.0628	0.000	0.063	1	0.135	8609.1
pentadecanoic acid (15:0)	0.161	0.001	0.162	0.992	0.224	436.41
Palmitic acid (16:1)	24.664	0.003	24.667	1	9.883	29671
Palmitoleic acid (16:1)	0.002	0.000	0.002	0.992	0.015	432

Heptadecanoic acid (17:0)	2.470	0.001	2.470	0.999	0.677	6618.1
Stearic acid (18:0)	637.966	0.011	637.977	1	12.048	193997
Oleic acid (18:1)(C18H34O2)	212.243	0.007	212.25	1	13.869	29352
Linoleic acid (18:2)	75.713	0.012	75.725	1	12.405	20561
Linolenic acid (18:3)	43.081	0.004	43.085	1	15.079	37995
Arachidic acid (20:0)	0.280	0.000	0.280	1	0.211	38426
Eicosenoic acid (20:1)	0.077	0.000	0.077	1	0.105	21168
Behenic acid (22:0)	73.503	0.002	73.505	1	7.395	99806
Erucic acid (22:1)	416.058	0.008	416.066	1	27.253	185861
Ricinoleic acid (18:1)(C18H34O3)	0.033	0.000	0.033	1	0.064	7803
Oil yeild	69.895	0.080	69.976	1	11.045	2966.4

δ^2g: Genotypic variance, δ^2e: Error variance, $\delta 2p$: Phenotypic variance, h^2: heritability, *significant at $p \leq 0.05$

Figure 1: Geographical distribution of *Salvia* species in Ardabil province of Iran. The species: 1- *S. hydrangea,* 2- *S. xanthocheila,* 3- *S. limbata,* 4- *S. aethiopis,* 5- *S. macrochlamys,* 6- *S. nemorosa,* 7- *S. sclarea* and 8- *S. verticillata*