ESSENTIAL AND FIXED OIL CHEMICAL COMPOSITIONS OF THE SEEDS FROM THE ENDEMIC SPECIES *Salvia Sharifii* RECH. F. & ESFAND

*SAMIRA ALIPOUR-GOUGEH[1], JINOUS ASGARPANAH[2,3]**

[1]*Department of Phytochemistry and Essential Oil Technology, Faculty of Pharmaceutical Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran – Iran (IAUPS).*
[2]*Young Reserahcers and Elit Club, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran – Iran.*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran – Iran (IAUPS).*

ABSTRACT

The essential oil content in the seeds of *Salvia sharifii* growing wild in south of Iran was found to be 0.2% (v/w). The essential oil was analyzed by GC and GC–MS. Twenty eight constituents, representing 85.0 % of the seeds essential oil were identified. The major components of the S. sharifii seeds essential oil were identified as sclareol (21.9%) and n-decane (17.3%). The fixed oil content and fatty acid composition of the seeds were also analyzed in order to determine their potential for human or animal consumption. The oil content in these edible seeds was found to be 19.9 %. The oil was analyzed by GC and GC/MS and five fatty acids identified which constituted 88.0 % of the oil. The main fatty acids of the oil were characterized as linoleic acid (39.7%) and linolenic acid (36.7%).

Keywords: *Salvia sharifii,* Lamiaceae, Seeds, Essential oil, Fixed oil

INTRODUCTION

The largest genus of the Lamiaceae family, the genus *Salvia* L. represents an enormous and cosmopolitan assemblage of nearly 1000 species displaying remarkable variation. It has undergone marked species radiations in three regions of the world: Central and South America (500 spp.), Central Asia/ Mediterranean (250 spp.) and Eastern Asia (90 spp.) [1]. Iran, particularly, is one of the centers of origin of the genus *Salvia* with 67 species, here called with the common Persian name of "Maryam-Goli" and about 53% of endemics [2]. Some species of the genus *Salvia* are used as flavorings, food condiments and perfume additives and cultivated for the aromatic characteristics [3]. *Salvia* species have been widely used in folk medicine as anticancer, antiviral, antimicrobial, antioxidant, anti-inflammatory and spasmolytic treatments and further have been used in relief of mental, nervous and gastrointestinal disorders [4].

Abietane, labdane, ictexane, neoclerodane and phenalenone types of diterpenoids [5,6], triterpenoids and sterols [7], phenolic acids, anthocyanins, flavonoids, coumarins and polysaccharides and their derivatives [4] were reported as major constituents of *Salvia* species. Most *Salvia* species are rich in essential oils, and various biologically active monoterpenoid/sesquiterpenoid have been reported in them possessing diverse biological activities such as antioxidant [8,9], anti-inflammatory [9,10], analgesic [11], anticonvulsant, anti-ulcerogenic, tranquillizing activities [12] and antibacterial activities [13]. Furthermore, the *Salvia* species, often pleasantly aromatic plants of potential economic interest, comprise the majority of the essential oil rich genera of the Lamiaceae, and particularly tend to accumulate monoterpenoid-rich essential oils.

Salvia sharifii Rech. f. & Esfand. is an endemic plant which is found just in south of Iran and grows up to a height of about 70-100 cm. This plant is extensively exploited as a medicinal plant and locally called "Maryam-Goli Jonubi". It is used as antiseptic, carminative, digestive and analgesic in Iranian folk medicine. Significant antibacterial, cytotoxic and antioxidant potential of *S. sharifii* has also been identified [14]. Two flavones, ladanein and 6-hydroxy-5,7,4′-trimethoxyflavone and one labdane-type diterpene, ent-13-epi-manoyloxide, were isolated from the aerial parts of this plant [14]. Since there has been no attempt to study the essential and fixed oils of *S. sharifii* seeds up to now, we were prompted to investigate the volatile components and fatty acid composition of this endemic plant species seeds for the first time.

EXPERIMENTAL

Plant material

Seeds of S. sharifii were collected in July 2014 from the mountain areas of Genow protected area in 30 Km west north of Bandar Abbas, Hormozgan Province, Iran: (27° 24' 6.62" N 56° 10' 46.57" E, 1800m). Specimen was identified by R. Asadpour and voucher was deposited in the Herbarium of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran under code number 3016-AUF.

Essential oil extraction

Seeds (100 g) were submitted to hydrodistillation in a Clevenger-type apparatus for 3 hours. At the end of distillation the essential oil was collected, dried with anhydrous Na_2SO_4, measured, and transferred to clean glass vials and kept at a temperature of −18°C for further analyses.

Fixed oil extraction

Fixed oil extraction was performed with a Soxhlet apparatus using n-hexane as the solvent. 100 g of powdered seeds was extracted for 6 h and then the solvent was evaporated by using a rotary evaporator at 30 °C. The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at -18°C until analyzed for fatty acid composition.

Fatty acid methyl esterification

Fatty acid methyl esters of the extracted oil were prepared according to the method previously reported by Metcalfe et al. [15]. 1 g of the oil was weighed into a volumetric flask. Then, 25 ml of 0.5 N methanolic potassium hydroxide was added and placed in the boiling water for 20 min. Then 12 ml boron trifluoride (BF3) was added and boiled again for 3 min. After that, the flask was cooled and 5 ml n-hexane and adequate saturated NaCl solution were added. The flask was shaken vigorously and left to stand for 5 min. the fatty acid methyl esters were prepared and dissolved in n-hexane (the upper layer). 2 ml of upper layer was transferred to a small vial and stored at0 °C until analyzed by GC/MS.

2.5 Analysis of the essential oil and fatty acid methyl esters

Essential oil and fatty acid samples analyses were separately performed on a Hp-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, temperature programmed as follows: 60°−240°C at 4°C/min. The carrier gas was N_2 at a flow of 2.0 ml/min; injector port and detector temperature were 250°C and 300°C, respectively. Samples were separately injected by splitting and the split ratio was 1:10.

GC/MS analysis was performed on a Hewlett-packard 6890 /5972 system with a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Scan mass range was from 40-400 m/z at a sampling rate of 1.0 scan/s. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the samples were identified by their retention time, retention indices, relative to C_9-C_{28} *n*-alkanes, computer matching with the WILEY275.L library and as well as by comparison of their mass spectra with data already available in the literature [16,17]. The percentage of composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively. The result of each oil analysis is the average of three replicates.

RESULTS

The hydrodistillation of *S. sharifii* seeds gave pale yellow essential oil with pleasant odor and yields of 0.2% (v/w). Table 1 shows the list of compounds whose GC/MS concentration is not less than 0.1% of total peak concentration.

e-mail: taxolfa@yahoo.com

According to Table 1, twenty eight components were identified in the seeds essential oil which represented about 85.0% of the total composition. The major components of *S. sharifii* seed essential oil were identified as sclareol (21.9%) and n-decane (17.3%). The studied essential oil comprised eighteen hydrocarbon (51.1%), three monoterpenoids (5.0%), five sesquiterpenoids (6.2%) and two diterpenoids (22.7%).

In this study, the fatty acid composition of *S. sharifii* seed oil was also determined. The oil extracted was viscous and yellow in color with the total oil content of 19.9%.

Table 1: GC-MS analysis of *S. sharifii* seeds essential oil.

Compound[a]	KI[b]	KI[c]	Content (%)
Nonane	898	900	0.8
α-Pinene	938	939	1.1
Octane, 4-ethyl	965	966	0.7
Nonane, 5-methyl	970	968	0.9
Nonane, 3-methyl	972	971	1.4
β-Pinene	979	979	0.5
n-Decane	1001	1000	17.3
Linalool	1099	1097	3.4
Hexyl isobutyrate	1148	1150	1.1
Dodecane	1203	1200	5.4
Hexyl 2-methyl butyrate	1233	1236	3.0
Hexyl isovalerate	1245	1243	5.2
Hexyl hexanoate	1350	1352	1.8
β-Elemene	1389	1391	0.8
Tetradecane	1397	1400	2.1
Germacrene D	1485	1485	1.0
Bicyclogermacrene	1500	1500	0.6
Pentadecane	1505	1502	0.6
Germacrene A	1511	1509	1.0
Spathulenol	1580	1578	2.8
Hexyl octanoate	1583	1581	7.4
Hexadecane	1602	1600	1.6
Heptadecane	1704	1700	0.5
Hexyl decanoate	1760	1763	0.1
Octadecane	1797	1800	0.7
Sclareoloxide	1910	1914	0.8
Eicosane	1995	2000	0.5
Sclareol	2219	2223	21.9
Total			85.0

[a]Compounds listed in order of elution.
[b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the experimental section.
[c]KI, (Kovats index) from literature.

According to the Table 2, seed oil consists mainly of essential saturated and unsaturated fatty acids. Linoleic acid (C18:2; 39.7%) and linolenic acid (C18:3; 36.7%) were found to be in maximum in *S. sharifii* seed oil, followed by palmitic acid (C16:0; 9.4%) while other fatty acids were in minor proportions. The total saturated fatty acid composition was 11.6% while the unsaturated fatty acid composition found to be so high (76.4%).

DISCUSSION

Identification of the compounds was made by comparing their mass spectra retention indices with those given in the literature. As the Table 1, four compounds were represented in the seeds essential oil at greater than 5% namely: sclareol (21.9%), n-decane (17.3%), hexyl octanoate (7.4%) and dodecane (5.2%). Presence of high amounts of sclareol in the seeds essential oil was noticeable. It is a fragrant bicyclic diterpene alcohol found in the essential oil of the most *Salvia* species. Sclareol is used as a fragrance in cosmetics and perfumes and as flavoring in food. Due to significant anti-inflammatory [18] and antitumor [19] activities of sclareol, these biological activities might be expected from the studied essential oil.

Lamiaceae family has been characterized by the occurrence of linoleic and linolenic acids in their seed oils and their importance as chemotaxonomic markers, for the cosmetic, nutritional and medicinal industries has also been demonstrated [20]. According to the Table 2, the oil from *S. sharifii* seeds showed a high potential for use in food and medicine industries due to their fatty acids profile. Higher content of linoleic and linolenic acids in analyzed oil is noteworthy. In such a way, it may offer protection against cardiovascular diseases and conditions throughout the human life [21]. The linoleic acid/ linolenic acid ratio was 1.08, which could contribute to the chemotaxonomic study of this plant. These overall results are in good agreement with those found for other *Salvia* species [21,22].

Table 2: Fatty acid composition of *S. sharifii* seeds oil.

Compound[a]	KI[b]	KI[c]	Content (%)
Palmitic acid (C16:0)	1917	1921	9.4
Linoleic acid (C18:2)	2071	2076	39.7
Linolenic acid (C18:3)	2111	2108	36.7
Stearic acid (C18:0)	2125	2128	2.1
Arashidic acid (C20:0)	2334	2329	0.1
Total			88.0

[a]Compounds listed in order of elution.
[b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the experimental section. Reported KIs were calculated based on the fatty acid methyl esters.
[c]KI, (Kovats index) from literature.

CONCLUSIONS

In conclusion, the current study is a contribution to the chemical compositions of *S. sharifii* essential and fixed oils grown wild in Iran. Due to the presence of sclareol as the main component of the seeds essential oil, future studies on the biological and pharmacological properties of the studied oil are suggested. The present study also revealed that the studied fixed oil could be a new source of unsaturated fatty acid rich edible oil and its full potential should be exploited. Hence *S. sharifii* seeds oil could be a new source of edible vegetable oil after the future toxicological studies.

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