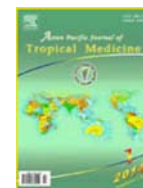


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Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*Reza Sepahvand¹, Bahram Delfan¹, Saeed Ghanbarzadeh², Marzieh Rashidipour³, Gholam Hassan Veiskarami¹, Javad Ghasemian–Yadegari^{4*}¹Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran²Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran³Young Researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran⁴Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran

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ABSTRACT

Objective: To determine the chemical composition, antioxidant activity and antibacterial properties of essential oils of the aerial parts of *Salvia sclareoides* (*S. sclareoides*).**Methods:** The essential oil of the areal parts of *S. sclareoides* was obtained by using hydrodistillation method and the composition of the volatile components analyzed by gas chromatography method coupled with mass spectrometry detector. The antimicrobial capacity of the essential oil of *S. sclareoides* was investigated by microdilution technique. The antioxidant activities were determined employing inhibition of 2,2-diphenyl-1-picrylhydrazyl hydrate radical method.**Results:** Mass spectra were searched against mass spectrometry databases and sixty components were recognized. Non-terpenoids (41.6%) and sesquiterpenes (39.7%) were determined as the main components of the essential oil. The main identified components were, linalool (27.6%), trans-caryophyllene (16.6%), beta.-trans-ocimene (11.831%), germacrene-D (10%), bicyclogermacrene (3.3%) and caryophyllene oxide (2.8%). Two monoterpenes (13.2%) and three oxygenated sesquiterpenes (5.5%) were also obtained from the essential oil of the *S. sclareoides*. In addition, results of our study supported the significant antioxidant activity of essential oil of *S. sclareoides* and potential use as a source of natural antioxidants. The essential oil of *S. sclareoides* aerial parts remarkably inhibited the growth of all tested microorganisms with the exception of *Pseudomonas aeruginosa* and *Candida albicans*.**Conclusions:** Results indicated that essential oil of *S. sclareoides* includes rather higher proportions of non-terpenoid and sesquiterpenes compounds with good antioxidant and antibacterial properties.

1. Introduction

Essential oils could be extracted from leaves, stems, flowers, roots, herbs, brushes, and trees through distillation. They have been used for medicinal and healing purposes for many years in all over the world.

Interest in essential oils has increased in recent decades with the popularity of aromatherapy, which claims that essential oils and other aromatic compounds have beneficial effects. They are widely used in perfumes, cosmetics, soaps, cleaning products and other products and also for flavoring of foods and drinks[1–4].

The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal genera of the Lamiaceae family which contains 900 different species widespread throughout Mediterranean region, South–East Asia and Central America. About 58 species are also found in Iran, where, 17 of these species are endemic in Iran[5–7].

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Some members of this genus are used as food spices to flavor meats and as a flavoring agents in perfumery and cosmetics. Additionally, they have pharmacologically effect and used in folk medicine; they were used to treat colds, wounds, insomnia and skin infections as well as headache, cerebral ischaemia and memory disorders. Sages have been used as antihydrotic, spasmolytic, anticholinesterase, antiseptic, insecticide, antifungal, antioxidant, anti-inflammatory and also in the treatment of mental and nervous conditions. Moreover, cardioactive and antibacterial terpenoids were isolated from the extracts of this genus.

Salvia is a rich source of phytochemicals including phenolic acids, polyphenols, flavonoid glycosides, anthocyanins, sesquiterpenoids, diterpenoids, sesterterpenes and triterpenes[1,2,4,8–12].

Antioxidants have great importance because they can reduce oxidative stress which could cause damage to biological molecules. Antioxidant compounds play a crucial role in the treatment of various diseases related to degenerative disorders, namely, cardiovascular and brain diseases, arthritis, diabetes, cancer and immune system decline, by acting as free radical scavengers, and thus decreasing the extent of oxidative damage. Furthermore, studies about antioxidant substances in foods and medicinal natural sources have attracted increased interest in the recent decades. In addition, the use of plant materials in lipids and lipid-containing foods is important because the plant potentials of decreasing rancidity, delaying the formation of toxic oxidation products, maintaining nutritional quality and increasing the shelf life of food products. Hence, evaluation of radical scavenging properties and antioxidant activity are of commercial interest to the pharmaceutical and food industries as a source of natural antioxidants[7,9,10,12–14].

Microbial actions of essential oils are also one of the most extensively studied features of botanical medicine and various aromatic plant species were being investigated for their pharmacological properties[6,11,15,16].

To the best of our knowledge, there has been no published detailed information about the composition, antioxidant and antibacterial activities of the essential oil of aerial part of *Salvia sclareoides* (*S. sclareoides*). Therefore, the objectives of the present study were to identify chemical composition as well as assess the antioxidant and antibacterial properties of the essential oil of the aerial part of *S. sclareoides* collected from the western of Iran using gas chromatography combined with mass spectrometry (GC–MS) and flame ionization detector.

2. Materials and methods

2.1. Plant material

The aerial parts of *S. sclareoides* were collected during flowering stage on May 2014 from Khorramabad (a city in Lorestan Province in Western Iran) and consequently authenticated by the authors.

2.2. Isolation of the essential oil

Essential oil was obtained from air-dried plant material by hydrodistillation method employing Clevenger-type apparatus. The extraction was carried out for a 4-hours period. To improve their recovery, the essential oil was taken up in xylene (Merck), dried over anhydrous sodium sulphate (Merck) until the last traces of water removing and stored in a dark sealed glass bottle at 4 °C until GC–MS analyses. The essential oil yield was 1.4% (w/w).

2.3. Analysis of the essential oil by gas chromatography

The essential oil analysis was performed using a Agilent 6890N gas chromatograph coupled to Agilent 5973 mass detector and employing a HP–5 column (30 m length, 0.25 mm ID, 0.25 µm stationary phase thickness).

An electron ionization system, with ionization energy of 70 eV was used for GC–MS detection. Helium (99.999%) was the carrier gas, at a flow rate of 0.9 mL/min with linear velocity of 29.6 cm/s and split injection with split ratio of 1:40. The gas chromatography conditions were set as the follows: column temperature, 3 min in 55 °C, 55–200 °C at 5 °C/min and 200–250 °C at 10 °C/min; injector temperature, 250 °C, and 0.5 µL of volume injection of the essential oil. The mass spectrometry operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 230 °C, solvent delay, 5 min; scan speed, 2000 amu/s; scan range, 40–450 amu and EV voltage, 1489 V.

2.4. Identification of the compounds

The components of essential oil were identified based on the comparison of their relative retention times and mass spectra with literature data including, National Institute of Standards and Technology (Nist) 21 as well as Wiley 7n library data (Wiley, New York, NY, USA) mass spectral library of the GC–MS system and quantitative data were obtained electronically from flame ionization detector area percent data[17].

2.5. Antioxidant activity

The antioxidant potential activity of essential oil of *S. sclareoides* was assessed by a spectrophotometric method, named 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging activity assay. Fifty microlitres of various concentrations of the samples (1, 5, 10, 20, 40, 80 and 100 µg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. Tests were carried out with essential oil and reference antioxidant, the synthetic butylated hydroxytoluene (BHT) in concentrations ranging from 1 to 100 µg/mL. The DPPH test is based on the ability of the extracts to donate a radical hydrogen to scavenge the stable DPPH radical. When this radical reacts with the antioxidant compound, it is reduced with the loss of the deep violet colour to light-yellow. The absorbance is measured at 517 nm on a visible light spectrophotometer.

The percent of DPPH radical scavenging activity is calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{essential oil}}}{\text{Abs}_{\text{blank}}} \right] \times 100$$

Where $\text{Abs}_{\text{blank}}$ is the absorbance of the blank sample (time=30 min) and $\text{Abs}_{\text{essential oil}}$ is the absorbance of the essential oil sample (time=30 min)[1,10].

2.6. Antibacterial effect

The fifty microlite of essential oil was tested against a panel of microorganisms, including *Pseudomonas aeruginosa* (ATTC 27853) (*P. aeruginosa*), *Proteus vulgaris* (ATTC 8427) *Klebsiella pneumoniae* (ATTC 500706), as Gram negative bacteria, as well as *Listeria monocytogenes* (ATTC 1298) and *Staphylococcus aureus* (ATCC 25923) as Gram positive bacteria and *Candida albicans* (*C. albicans*) for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. MIC and MBC were calculated based on micro-broth dilution method. All bacteria were obtained from Pastor Institute (Tehran, Iran).

2.7. Statistical analysis

All data were reported as mean±SD of independent replicates. Data were analyzed by an analysis of variance (ANOVA). $P < 0.05$ was considered as significant level.

3. Results

The essential oil was extracted from the aerial parts of *S. sclareoides*. The gas chromatography profile of this oil was displayed in Figure 1.

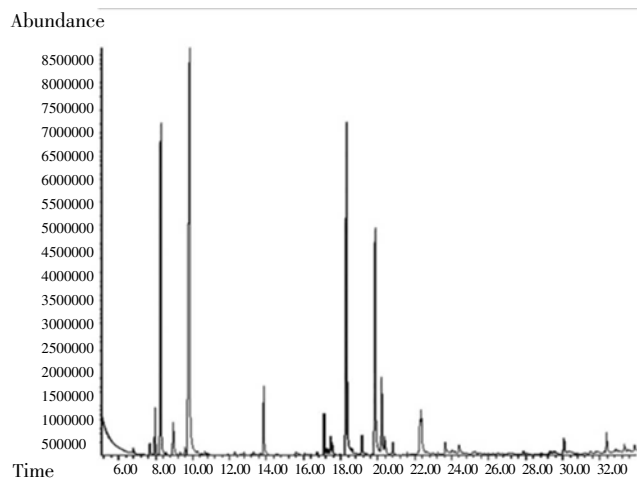


Figure 1. Chromatogram of essential oil composition of *S. sclareoides*.

The GC-MS analysis of the essential oil of the aerial parts of *S. sclareoides* obtained by hydrodistillation resulted in the identification of 60 different components, and these components belong to non-terpenoids (41.6%), monoterpene hydrocarbons (13.2%), sesquiterpene hydrocarbons (39.7%) and oxygenated sesquiterpenes (5.5%). All of the recognized components are arranged in order of their retention time from the HP-5 column and their percentage compositions are summarized in Table 1.

Table 1

Essential oil composition (%) of aerial parts of *S. sclareoides*.

No.	Retention time (min)	Compound	Area (%)
1	6.80	Beta-pinene	0.145
2	7.69	p-Cymene	0.444
3	7.97	cis-Ocimene	1.399
4	8.27	Beta.-trans-ocimene	11.830
5	8.55	Delta. 3 Carene	0.151
6	8.94	1-Octanol	1.978
7	9.32	Alpha.-terpinolene	0.230
8	9.59	Isoamyl-2-methyl butyrate	0.372
9	9.82	Linalool	27.600
10	10.80	Cis-epoxy-ocimene	0.266
11	11.92	Cinereone	0.100
12	12.30	Alpha. terpineol	0.435
13	12.78	Camphene	0.228
14	13.31	cis-3-Hexenyl isovalerate	0.165
15	13.68	d-Carvone	0.112
16	13.85	Linalyl acetate	2.667
17	14.55	Pinen-ol	0.150
18	15.60	Carvacrol	0.447
19	16.74	Neryl acetate	0.189
20	17.11	Alpha.-Copaene	1.504
21	17.25	Geranyl acetate	0.323
22	17.36	Beta. Bourbonene	0.252
23	17.47	Beta-cubebene	0.912
24	17.54	Beta-elemene	0.584
25	18.31	trans-Caryophyllene	16.580
26	18.60	Alpha.-longipinene	0.543
27	18.79	trans-caryophyllene	0.117
28	18.96	Alloaromadendrene	0.099
29	19.15	Alpha.-humulene	1.027
30	19.39	Beta.-Sesquiphellandrene	0.154
31	19.69	Zingiberene	0.085
32	19.85	Germacrene-D	9.989
33	20.20	Bicyclogermacrene	3.312

Table 1 continuedEssential oil composition (%) of aerial parts of *S. sclareoides*.

No.	Retention time (min)	Compound	Area (%)
34	20.37	Alpha-farnesene	1.133
35	20.81	Delta-cadinene	0.721
36	21.59	Beta-bisabolene	0.160
37	22.28	Spathulenol	1.576
38	22.34	Caryophyllene oxide	2.857
39	22.58	Dehydroaromadendrene	0.204
40	22.68	Vulgarol	0.345
41	22.96	Alpha-Bisabolene epoxide	0.261
42	23.24	Isolimonene	0.230
43	23.65	Caryophylla-3,8(13)-dien-5.alpha.-ol	1.043
44	23.93	Aromadendrene	0.164
45	24.03	Alpha-cadinol	0.412
46	24.19	Beta-humulene	0.313
47	24.40	3-Hexadecen-7-yne	0.993
48	24.77	Capnellane-8-One	0.263
49	25.27	Junipene	0.258
50	26.27	Valerenol	0.115
51	26.50	Isoaromadendrene epoxide	0.378
52	27.05	Agathadiol	0.126
53	27.22	Oploenone	0.227
54	29.59	Sesquirosefuran	0.264
55	30.07	O-cymene	1.510
56	32.36	Phytol	0.888
57	32.74	Sclereodiol	0.096
58	32.86	2-p-Tolylpropane	0.379
59	33.32	Geranyl linalool isomer	0.387
60	33.88	Sclareol	0.293
Total			100.000

It was found that the essential oil of *S. sclareoides* was a complex mixture of mainly non-terpenoids, sesquiterpene hydrocarbons, monoterpene hydrocarbons and oxygenated sesquiterpenes. Linalool with 27.6%, and Linalyl acetate with 2.7% were the two abundant constituents presented among the non-terpenoid compounds of the essential oil. Besides, trans-caryophyllene, germacrene-D and bicyclgermacrene with 16.6%, 10% and 3.3%, respectively, were the main sesquiterpene hydrocarbons found in the *S. sclareoides* essential oil. Additionally, caryophyllene oxide (2.9%), spathulenol (1.6%) and caryophylla-3,8(13)-dien-5.alpha.-ol (1%) were the identified oxygenated sesquiterpene hydrocarbons in the essential oil. Furthermore, beta.-trans-ocimene with 11.8% and cis-ocimene with 1.4% were the major monoterpene hydrocarbons.

3.1. Antioxidant activity

Plants with radical scavenging property and antioxidant capacity are useful for medicinal applications and as

food additive. So, in the present study, the antioxidant capacity of *S. sclareoides* was evaluated using DPPH radical scavenging method by comparing with the activity of the BHT as a known antioxidant. Results of the antioxidant capacity of essential oil of *S. sclareoides* and BHT are given in Figure 2.

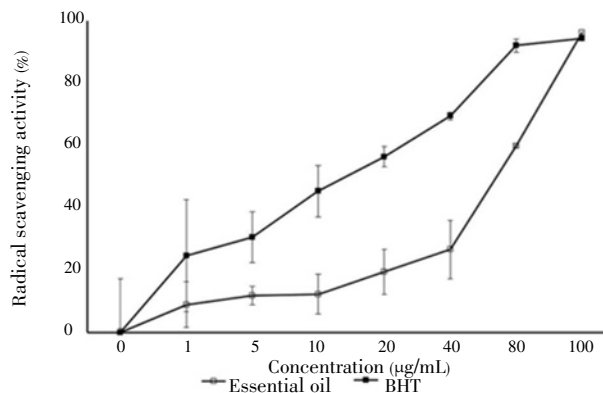


Figure 2. Antioxidant activity of essential oil of *S. sclareoides* and BHT.

The antioxidant capacity of essential oil of *S. sclareoides* was higher than that of the used synthetic antioxidant. Therefore, the antioxidant properties of *S. sclareoides* essential oil could play a beneficial role in the food preservation and also in the prevention of oxidative damage related to the pathophysiology of many diseases, including important and prevalent neurodegenerative diseases such as Alzheimer's disorders[9,12,18,19].

3.2. Antibacterial effect

The essential oil of *S. sclareoides* aerial parts was also screened for its antimicrobial activity. The essential oil remarkably inhibited the growth of tested Gram positive and Gram negative bacteria except for *P. aeruginosa*. It also did not show inhibitory effect on the *Candida albicans* (Table 2).

4. Discussion

Primary studies were conducted in advance especially in practical applications of the essential oils in fragrance and flavor industries, as well as in the chemical and

Table 2MIC and MBC of essential oil of *S. sclareoides*.

Concentration	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>P. aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Candida albicans</i>
MIC (µg/mL)	15.6	15.6	125	15.6	31.25	125
MBC (µg/mL)	15.6	15.6	–	31.25	62.5	125

pharmaceutical industries. In order to distinguish observed the essential oil constituents of *S. sclareoides* with that of other species of genus *Salvia*, additional studies are necessary to determine the intrinsic (such as genetic and growth stage) and extrinsic (such as climatic, seasonal and environmental distillation processes) conditions affecting the biosynthesis pathways of the essential oils[5,17,20,21]. Therefore, further studies are required to evaluate the characteristics of each species which grows in different soil and climatic conditions.

The findings above showed the presence of natural antioxidant property in *S. sclareoides*, which are better than that of BHT, a very efficient synthetic antioxidant agent which is widely used in food technology. Basically, interest has increased noticeably in the research of naturally occurring antioxidants for use in foods or medicinal materials as an alternative to synthetic antioxidants, which are being limited because of their possible toxicity[9,12,13,22]. Therefore, essential oil of *S. sclareoides* with antioxidant properties could be considered as a phytonutrients.

The antibacterial property of the essential oil and extracts have led to the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Furthermore, this property shows an effective way to prevent the development of various off-flavours and undesirable compounds that result from lipid peroxidation in foods[6,8,11,23–25]. This study also demonstrated that the *S. sclareoides* essential oil displayed antimicrobial activity on Gram negative and Gram positive bacteria. These effects were observed regarding the low MIC and MBC values and the high reduction in viability of the tested microorganisms by low concentration of essential oil. The tested microorganisms are pathogens or opportunists for man, animal and plants, and they cause contamination and deterioration in food, water and air. The strong antimicrobial activity of the essential oil against almost all the susceptible microorganisms can be attributed to the presence of high concentration of monoterpenes. This *in vitro* experimental study clearly revealed the efficient antibactericidal action of *S. sclareoides* essential oil and support the freely use of this natural, pleasant and eco-friendly product as a preservative in food and water which are susceptible for generating pleasant odors[6,23,24,26].

The results reported here can be considered as the first information on the antimicrobial and antioxidant properties of aerial part of essential oil of *S. sclareoides*.

In recent times, the essential oils and various extracts

of plants have attracted attention as sources of natural products. They have been studied for their potential uses as alternative remedies for the treatment of many infectious and oxidative diseases as well as the preservation of foods from the toxic effects of bacteria and oxidants. The characterization of *S. sclareoides* has been carried out regarding its component identification, antibacterial and antioxidant properties. The use of gas chromatography coupled with mass spectrometry has been applied to characterize materials based on their chemical compositions. This approach is practical and efficient to identify essential oils obtained from different species or varieties for determining components of the essential oil. In general, aerial parts of *S. sclareoides* showed large amount of different compounds in essential oil. Presence of relatively larger amounts of linalool, a non-terpenoid, trans-caryophyllene and germacrene-D as oxygenated sesquiterpenes as well as beta-trans-ocimene as monoterpene hydrocarbon in the essential oil of the aerial parts *S. sclareoides* is reported for the first time in the present investigation. Essential oil of *S. sclareoides* displayed a rich source of antioxidant and can be used as powerful herbal antioxidant. Antibacterial property of the essential oil of *S. sclareoides* could be considered as an additional health promoting factor. Antibacterial and antioxidant properties indicated that this plant has potential for use in aromatherapy and pharmacy.

Conflict of interest statement

We declare that we have no conflict of interest.

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