Advances in Environmental Biology, 6(1): 221-226, 2012
ISSN 1995-0756

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Essential Oil Composition, Phenolic Content, Antioxidant and Antimicrobial Activity in *Salvia officinalis* L. Cultivated in Iran

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ABSTRACT

The essential oil was obtained by hydro-distillation of the aerial part of *Salvia officinalis* L. cultivated in Iran. The chemical composition of essential oil was determined using Gas Chromatography and Gas Chromatography/Mass Spectrometry. Forty two components were identified in *S. officinalis* oil. That, α-thujone (41.48%), borneol (8.33%), 1,8 cineole (7.94%), β-thujone (6.75%) virdiflorol (5.85%), camphene (3.46%), α-pinene (3.24%), α-humulene (2.64%) and β-pinene (2.25%) determined as the major components. The total phenolic contents and the antioxidant activity of plant extract were determined, by Folin-Ciocalteau and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays respectively. Plant extract of *S. officinalis* had more phenolic content (25.13 mg GAE/g DW) and antioxidant (17.45µg/ml) activity. Antimicrobial activity of the essential oil was evaluated using the disc diffusion method. The oil showed high antimicrobial activity against *Staphylococcus aureus* and *Candida albicans***,** two medically important pathogens compare with standard antibiotics.

Key words: *Salvia officinalis,* Essential oil, phenolic, Antioxidant, Antimicrobial.

Introduction

The essential oils and extracts of many plant species have been shown to exert biological activities, which justified research on traditional medicine focused on the characterization of antioxidant and antimicrobial activities of these plants [26].

The *Salvia* genus belongs to the subfamily Nepetoideae of Mentheae tribe in Lamiaceae family [29]. This genus consisting of about 900 species, of which 58 are distributed in Iranian flora [30]. *Salvia officinalis* grown wild in Mediterranean countries, but in Iran, *S. officinalis* plants are cultivated.

Many *Salvia* species are used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world [9].

Salvia species are generally known for their multiple pharmacological effects including their antibacterial [10,18,25], antiviral [22], antioxidative [19], antimalarial [16], anti-inflammatory [5], antidiabetic [11], cardiovascular, antitumor and anticancer [21], Also, some studies showed that a part of these activities depended on essential oil composition**.**

Recent studies show that some plants from the lamiaceae family are very rich in phenolic compounds, such as flavonoids, phenolic acids and phenolic diterpenes and possess high antioxidant activities [2,23,35]. Phenolic compounds are plant secondary metabolites and naturally present in all plant materials [13,28]. These compounds can delay or inhibit the oxidative damage caused by free radicals [34] and can protect us against major diseases such as coronary heart disease and cancer in human [20].

In this research, we have cultured *S. officinalis* as an importation medicinal plant in Iran. This study focuses on influence of environmental condition in Iran on essential oil composition, phenolic content, antioxidant and antimicrobial activities of *S. officinalis* in an attempt to contribute to use of these as alternative products and natural antioxidant and antimicrobial agent for food and medicinal uses**.**

Materials and Methods

Plant Material:

Seed of *S. officinalis* L. was obtained from institute of Medicinal Plants Research, Esfahan, Iran

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and were grown in Medicinal and Aromatic Plants Garden in Estahban Branch, Islamic Azad University. Plants were harvested in the flowering stage and dried at room temperature for 15 days.

Essential Oil Extraction and Analysis:

Dried aerial part of *S. officinalis* (100 g) was cut and subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4°C.

Gas chromatography analysis was performed on an Agilent technologist model (6990 USA) series II gas chromatograph was equipped with flame ionization detector and capillary column HP (30 m´ 0.25 mm, 0.25 µm film thickness). The chromatographic conditions were as follows: The oven temperature increased from 60° C to 240° C at a rate of 3 C/min. The injector and detector temperatures were 240° C and 250° C, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:50. Quantitative data was obtained from electronic integration of peck areas without the use of correction factors. Essential oil was also analyzed by Hewlett- Packard GC-MS (model 6890 series II)operating at 70e V ionization energy, equipped with a HP-5 capillary column phenyl methyl siloxane (30m´ 0.25 mm, 0.25 µm film thickness) with Helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method [33] using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [1].

Total Phenolic Content and Antioxidant Activity:

The total phenolic content was determined with the Folin-Ciocalteau reagent as described previously [31]. Briefly, 200 µl of plant extract dissolved in methanol (1 mg/ml) were mixed with 2.5 ml of Folin-Ciocalteau reagent (diluted 10 times in distilled water) in glass tubes in triplicate. The samples were incubated at room temperature for 5 min and vortex mixed at least 2 times. Then, 2 ml of Na2CO3 (7.5%) was added and the glass tubes were incubated in the dark for 90 min with continuous shaking. The absorbance of samples was measured at 765 nm using a Spectrophotometer (Perkin-ElmerUV/Vis double beam lambda 1, USA) against a blank of distilled water. Different concentrations of Gallic acid in methanol were tested to obtain a standard curve. Total phenolic content was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dw).

The antioxidant activity was determined byDPPH free radical scavenging assay as described previously with some modifications [7]. Briefly, 4different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH 100 mM in a total volume of 4ml. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Methanol was used as blank and all measurements were carried out in triplicate. Trolox, a water-soluble equivalent of vitamin E, and quercetin were used as reference compounds. All solutions were made daily. The percent inhibition of DPPH free radical was calculated by the formula: Percentage inhibition (%I) = [(A blank -A sample) / A blank] × 100

Where, A blank is the absorbance of the control reaction (DPPH alone), and A sample is the absorbance of DPPH solution in the presence of the test compound.

IC50 values denote the concentrations of the sample, required to scavenge 50% of DPPH free radicals.

Microorganisms:

Standard strain of *Candida albicans* (ATCC No. 10231) and Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) were obtained from Persian Type Culture Collection (PTCC) in Iranian Research Organization for Science and Technology.

Antimicrobial activity:

In vitro antimicrobial activity of the essential oil of *S. officinalis* was evaluated by disc diffusion method, with determination of inhibition zones (IZ), according to the National Committee for Clinical Laboratory Standards [27] using 100 µl of each suspension of the tested microorganisms containing 2.0×10^6 CFU/ml for bacteria and 2.0×10^5 CFU/ml (0.5 McFarland) spore for fungi strain. The bacteria inoculate was prepared by suspending overnight colonies from sabouraud dextrose agar (SDA) media, and the *C. albicans* was prepared by suspending colonies from 48h old potato dextrose agar (PDA) cultures respectively. Fungal or bacterial suspension were seeded into Petri dishes (9cm) containing 20 ml sterile sabouraud dextrose agar (SDA) or potato dextrose agar (PDA) using a sterile cotton swab. The sterile paper discs (6 mm in diameter) were individually impregnated with 20 µl of the oil and then placed on the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37 °C for 24 h and at 30 °C for 48 h for the *C. albicans* strain. After incubation, the mean inhibition zone diameter for each concentration was measured in

millimeters. All the studies were performed in triplicate. Blank discs containing 20µl DMSO were used as negative controls. Tetracycline (30 µg/disk), Oxacillin (1 µg/disk), Ketoconazole (20 µg/disk) and Amoxicillin (10 µg/disk) were used as positive reference standards to determine the sensitivity of the microorganisms. The assessment results shown were resistant (lower than 7mm in diameter), dose-dependent (7-11mm in diameter) and sensitive (larger than 11mm in diameter).

A serial dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) according to the National Committee for Clinical Laboratory Standards [27]. The essential oil was dissolved in dimethylsulfoxide (DMSO), and diluted in a twofold manner to make the concentrations of 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 µl/disc. The sabouraud dextrose agar (SDA) containing varying amounts (logarithmic, serially and 2-fold diluted) of *S. officinalis* oil and the various controls were inoculated with actively dividing microorganism cells. The cultures were incubated for 24 and 48 h at 30°C. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The MBC/MFC is defined as the lowest concentration of the essential oil at which inoculated microorganisms were completely killed. Minimum bactericidal or fungicidal concentration (MBC/MFC) was determined by sub-culturing a 0.01 ml aliquot of the medium drawn from the culture tubes showing no macroscopic growth at the end of 48 h of culture on sabouraud dextrose agar (SDA) plates.

Results and Discussion

GC and GC/MS analysis of the essential oil resulted in the identification of 42 compounds, representing the 98.67% the total oil. Major constituents of the oil were α-Thujone (41.48%), borneol (8.33%), 1, 8 cineol (7.94%), β-Thujone (6.75%) virdiflorol (5.85%), camphene (3.46%), α-pinene (3.24%), α-humulene (2.64%) and β- pinene (2.25%). Other components were present in amounts less than 2 %(Table 1).

According to these result, Bouaziz et al., [6] reported the most abundant components (>4%) of the *S. officinalis* essential oils cultivated in Tunisia were β -thujone (17.76%), 1,8-cineole (16.29%), camphor (14.19%), α-thujone (7.41%), trans-caryophyllene (5.45%), viridiflorol (4.63%), β -pinene (4.41%), α-humulene (4.37%) and camphene (4.07%). Also Delamare et al., [10] reported the main constituents (>2%) of the essential oils of *S. officinalis* cultivated in South Brazil were α-thujone (24.8%), 1,8-cineole(14.8%), camphor (10.9%), β–pinene (9.87%), δ-gurjunene (8.20%), camphen (4.40%), β-thujone (3.97%), α-pinene (3.07) and β–caryophyllene (2.89). For the main constituents in the essential oil of *S. officinalis*, the components observed was similar to that obtained in Italy [12,24] and Yugoslavia [32]. However, the concentrations of some components were different than our report. These differences in the essential oil compositions can be attributed to several environmental factors such as climatic, seasonal and geographical or ontogenesis variations [3].

The total phenolic content and antioxidant activity in *S. officinalis* were measured by Folin Ciocalteau reagent and DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging respectively. According to the Table 2, *S. officinalis* has the high phenolic content (25.13 mg GAE/g DW) and antioxidant activity (17.45µg/ml) and can be used as natural source of phenolics and antioxidants.

The antimicrobial activities of *S. officinalis* essential oil was evaluated against a Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231) as yeast using the microdilution method**.** The results show that *S. officinalis* essential oil has high antimicrobial properties against two microorganisms compared to the positive standard antibiotics (Table 3, 4). The antimicrobial activities of the essential oil of *S. officinalis* on *S. aureus* and *C. albicans* were similar (Table 4). In all treatments, the inhibitory effect of the essential oils increased when the concentration of essential oil increased. Recent studies on the essential oils of many Lamiaceae show that these plants have a broad range of biological activities, notably their antimicrobial potency [4], and this activity is generally correlated to the chemical composition of the oil. Thus, this biological difference can be partly explained by the variation in their chemical composition. The antimicrobial activity of *S. officinalis*, could be attributed to high amount of major components as α–thujone, 1,8-cineole, β-caryophyllene, and other components in essential oil. Several studies have focused on the antimicrobial activity of the essential oils of *Salvia* species**.** Kabouche et al., [15] reported that the essential oil obtained from roots of *Salvia jaminiana* has antibacterial activity. Similarly, the essential oil of *Salvia tomentosa* showed antibacterial activity against eight microorganisms [14,17]. The antimicrobial activity of *Salvia officinalis* was recognized decades ago, and was attributed to the presence of 1,8-cineole, thujone and camphor; in reality, even if antimicrobial activity of an essential oil is often attributed mainly to its major components, today it is known that the synergistic or antagonistic effect of one compound in minor percentage of mixture has to be considered [8].

Antimicrobial properties of the essential oil of *S. officinalis* show that the plant has potential for use in aromatherapy, pharmacy and also in pathogenic systems to prevent the growth of microbes.

Table 1: Essential oil components of *Salvia officinalis* L.

No	Compound	RI[a]	Percentage in Oil
1	Cis-Salvene	852	0.28 ± 0.12
2	Trans-Salvene	859	0.08 ± 0.02
3	Tricyclene	915	0.45 ± 0.19
4	α-Thujene	928	0.05 ± 0.02
5	α-Pinene	937	3.24 ± 0.86
6	Camphene	955	3.46 ± 0.94
7	Sabinene	974	0.09 ± 0.02
8	β-Pinene	978	2.25 ± 0.64
9	1-Octan-3-ol	985	0.14 ± 0.06
10	β -Myrcene	992	0.58 ± 0.23
11	3-Octanol	995	0.44 ± 0.19
12	α -Phellandrene	999	0.09 ± 0.03
13	α - Terpinene	1008	0.37 ± 0.13
14	O-Cymene	1021	1.25 ± 0.36
15	Limonene	1025	1.33 ± 0.43
16	1,8-Cineole	1029	7.94 ± 1.85
17	Cis -Ocimene	1030	0.34 ± 0.17
18	γ - Terpinene	1044	0.21 ± 0.11
19	Cis- Sabinene hydrate	1050	0.17 ± 0.08
20	Linalool oxide	1076	0.17 ± 0.07
21	α- Terpinolene	1083	0.09 ± 0.02
22	Trans- Sabinene hydrate	1093	0.05 ± 0.02
23	Linalool	1101	0.37 ± 0.15
24	α -Thujone	1112	41.48 ± 3.87
25	β -Thujone	1115	6.75 ± 1.43
26	α - Campholene aldehyde	1119	0.05 ± 0.01
27	Camphore	1126	0.12 ± 0.06
28	Borneol	1155	8.33 ± 1.85
29	4- Terpineol	1162	0.56 ± 0.21
30	α - Terpineol	1184	0.19 ± 0.06
31	Trans-Sabinyl acetate	1291	0.75 ± 0.31
32	Carvacrol	1300	0.64 ± 0.27
33	Carvacryl acetate	1352	0.12 ± 0.04
34	*B*- Caryophyllene	1418	1.68 ± 0.43
35	α - Humulene	1454	2.64 ± 0.78
36	Naphthalene	1480	0.09 ± 0.02
37	Ledene	1499	0.05 ± 0.02
38	Caryophyllene oxide	1581	0.63 ± 0.33
39	Viridiflorol	1590	5.85 ± 1.36
40	*B*- Selinene	1604	0.87 ± 0.34
41	Humullene epoxide Π	1606	2.56 ± 0.75
42	Manool	2056	1.87 ± 0.58
	Oil Yield (% w/w)		0.38
	Total		98.67

a RI, retention indices in elution order from HP-5 column.
Each value in the table was obtained by calculating the average of four experiments ±`standard deviation. Data expressed as percentage of total.

Table 2: Total phenolic content and radical scavenging activity of *S. officinalis*

Plant Species	Total phenolic content [a] (mg GAE/g DW)	IC_{50} [b] (µg/ml)
S. officinalis	25.13	17.45

[a] Data expressed as mg of gallic acid equivalents per g dry weight (DW).
[b] IC_{50}. Data expressed as µg per millilitre. Lower IC_{50} values indicated the highest radical scavenging activity.

Table 3: Antimicrobial activitiy of the essential oil of *S. officinalis*

				Inhibition Zone (mm)				
Microorganisms				*S. officinalis* Essential oil (µg/ml)				
	200	100	50	25	12.5	6.25	3.125	1.562
S. aureus	79	68	53	32	18	12	9	0
C. albicans	76	64	48	28	16	9	7	0

Each value in the table was obtained by calculating the average of three experiments.
Diameter of inhibition zone including disc diameter of 6mm.

Table 4. Antimicrobial activity of the standard antibiotics

		Inhibition Zone (mm)		
Microorganism	Oxacillin (1 µg/disk)	Tetracycline (30 µg/disk)	Amoxicilin (10 µg/disk)	Ketoconazol (20 µg/disk)
S. aureus	NA	20	17	NA
C. albicans	30	NA	NA	32

Each value in the table was obtained by calculating the average of three experiments.
Diameter of inhibition zone including disc diameter of 6mm.
NA, not active.

Conclusion:

This study shows that the *S. officinalis*, is the main source for α-thujone, borneol, 1,8 cineole and other valuable components such as β-thujone, virdiflorol, camphene, α-pinene, α-humulene and β-pinene. Also this plant possesses high biological activities and could potentially be used as natural source of phenolics, antioxidants and antimicribials. These results showed that environmental condition of Iran is suitable for sowing Sage.

Acknowledgments

This study was designed and performed in Estahban Branch, Islamic Azad University, Estahban, Iran. The authors sincerely thank the institute for financial support, Grant No. 145183.

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