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Chemical Composition and Antimicrobial Activity of Essential Oil of *Salvia spinosa* L.

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Abstract: The aerial parts of *Salvia spinosa* L. (Labiatae) afford an essential oil on hydro distillation was analyzed by gas chromatography-mass spectroscopy (GC/MS) using direct injection. Out of 19 peaks (representing 99.99% of the oil), 18 Components were identified representing 98.59% of the total oil Composition. The major Components were 1,8-cineol (32.87%), (Z)-β-ocimene (20.03%), Germacrene d (10.66%), 2-Butyl thiophene (9.83%), Trans caryophyllene (5.01%) and 3- Butyl thiophene (3.49%). The oil was also screened for its antimicrobial activity against four bacteria (*Staphylococcus aureus*, *Basillus subtilis*, *Psedomonas aeruginosa* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergilus niger*) using Disc diffusion method and also Minimum Inhibitory Concentration (MIC) values of each active oil concentration were determined. The results showed a significant activity against *Staphylococcus aureus*, *pseudomonas aeruginosa* and *Basillus Subtilis*.

Key words: *Salvia spinosa* L. Labiatae, 1,8-Cineol, antimicrobial activity

INTRODUCTION

Salvia, the largest genus of Labiatae family, includes about 900 species wide spread all over the world. *Salvia* species such as *S. officinalis* and *S. fruticosa* have been credited with a heigh potential medicinal uses such as spasmolytic, antiseptic and astringent and are of economical importance in flavouring, perfumery, Cosmetic, food and pharmaceutical industries (Al-Howiring, 2003). *Salvia spinosa* L. is a perennial plant growing wild in the Mediterranean areas and in Iran, especially in Tehran province (Baher Nik and Mirza, 2005). There is only one report on the essential oil composition of *Salvia spinosa* from Dizin, North Tehran province of Iran (Baher Nik and Mirza, 2005), but no study was found on the microbiological activity of it's essential oil. According to the traditional medicinal uses of *S. spinosa* we aimed to study the composition and antimicrobial activity of *S. spinosa* essential oil, collected from Baraghan.

MATERIALS AND METHODS

Plant material: Aerial parts of the plant were collected during flowering time in July 2005 from Baraghan (15 km North-Western of Karaj in Tehran province) Iran. The voucher specimen is identified and deposited at the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences under number 6649-THE.

Analysis of the essential oil: The air dried aerial parts of plant was powdered (50 g) and Hydro-distilled in a Clevenger apparatus for 3 h to obtain the yellow colored oil (0.1 mL, 0.2% V/W), Which was subjected to analyzed by GC/MS using direct injection in to the split mode under the following conditions:

Ionization voltage: 70 eV; injector temperature: 250°C; DB1 Column and He; was used as carrier gas at a flow rate of 1.5 mL min^{-1}.

Identification of components of the oil were based on retention indices relative to normal alkanes and computer watching with the wiley 275. Library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in literature (Adams, 2004).

Pharmacological screening: Two gram-positive bacteria [*Staphylococcus aureus* (ATCC 6538), *Basillus subtilis* (ATCC 12711)], Two gram-negative bacteria [*Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739)] and two fungi [*Aspergilus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231)] were used as test microorganisms.

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Antimicrobial activity of the essential oil was determined, using the disc diffusion method and Minimum Inhibitory Concentrations (MICs). The bacteria inocula were prepared by suspending over night colonies from Muller-Hinton (MH) agar Media in 0.9% saline. The *Candida albicans* and *Aspergilus niger* inocula were prepared by suspending colonies from 48 and 72 h old Sabourad Dextrose (SD) agar cultures in 0.9% saline respectively. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to 0.5 Mcfarland standard (1.5×10^{8} cfu mL^{-1}).

MH and SD agar plates (100 nm diameter) were seeded individually with bacterial or fungal.

Suspensions using a sterile cotton swab. In order to determine the relative minimum inhibitory concentration values, which are the minimum concentration of agents showing growth inhibition zone when examined visually, oil was dissolved in DMSO to make a concentration of 16.5 μg/disc. The essential oil was then diluted in a two-fold manner to make different concentration. Thirty microliter of the each diluted loaded on the paper disc and placed on the plates containing microorganisms. The plates were incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and 20-25°C for 48 h for *Candida albicans* and 72 h for *Aspergilus niger*.

In addition standard antibiotic discs such as Gentamycin, Amoxicillin and Nystatin and the solvent (DMSO) were used as positive and negative controls and the inhibition zones reported in millimeters. All the test was done in triplicates.

RESULTS AND DISCUSSION

The dried aerial parts of *S. spinosa* L. yielded 0.2% V/W of essential oil and the identified compounds were shown in Table 1.

Out of 19 peaks (representing 99.99% of the oil), 18 components were identified representing 98.59% of the total composition. The major compounds were 1,8-cineol (32.87%), (Z)-β-Ocimene (20.03%), Germacrene D(10.66%), 2-Butyl thiophene (9.83%), Trans caryophyllene (5.01) and 3-Butyl thiophene (3.49%).

Moreover, some of minor components were also detected of which sabinene (2.64%), p-cymene (1.25%), Alloocimene (2.73%), β-Bourbonene (1.93%), β-Gurjunene (1.6%), cis-α-Bisabolone (1.07%) and Hexadecane (1.99%). The oil showed a significant antimicrobial activity on some of the test microorganisms (Table 2). The MICs of the oil (Table 3) obtained against gram-positive bacteria was 3.75 μg/disc for *staphylococcus aureus* and 2.06 μL/disc for *Basillus subtilis*. *Salvia* oil tested in this study showed a potent antimicrobial activity against *Pseudomonas aeroginosa* (2.06 μL/disc). The oil showed no antifungal activity against *Candida albicans* and *Aspergillus niger*. in the oil of *S. spinosa* from Dizin with 29 Compounds (Baher Nik *et al*,. 2005) the (E)-β-ocimene (12.3%), β-Caryophyllene (10.2%), Isopentyl isovalerate (9.5%), α-Gurjunene (7.2%) and Isoamyl, 2-methyl butyrate (7.0%) were the major compounds. It is very interesting that 1,8-cineol was not detected in the oil from Dizin while it was the major component in the oil from Baraghan with 32.87%.

Table 1: Essential oil composition of *Salvia spinosa* L.

No.	Compounds	Rt*	(%)	RI**
1	Sabinene	6.64	2.64	972
2	P-Cymene	7.87	1.25	1021
3	Phellandrene	7.98	0.71	1026
4	1,8-Cineol (Eucalyptol)	8.05	32.87	1028
5	(Z)-β-ocimene	8.21	20.03	1035
6	2-Butyl thiophene	8.57	9.83	1045
7	3-Butyl thiophene	8.72	3.49	1054
8	Alloocimene	10.54	2.73	1126
9	β-Bourbonene	16.59	1.93	1384
10	Decan-3-methyl	16.80	0.60	1393
11	Trans-Caryophyllene	17.35	5.01	1419
12	β-Gurjunene	17.55	1.6	1428
13	Germacrene D	18.66	10.66	1481
14	Un known	18.88	1.40	1491
15	Bicyclo germacrene	18.95	0.97	1494
16	Cis-α-Bisabolone	19.15	1.07	1504
17	β-Cadinene	19.47	0.69	1521
18	Hexadecane	20.86	1.92	1593
19	Octadecane	24.51	0.59	1790

* Rt = Retention time ** RI = Retention Indices

Table 2: Antimicrobial activity of the essential oil of *Salvia spinosa* L.

Microorganism	Effect
Staphylococcus aureus (ATCC 6538)	+
Pseudomonas aeruginosa (ATCC 9027)	+
Basillus subtilis (ATCC 12711)	+
Escherichia coli (ATCC 8739)	–
Aspergilus niger (ATCC 16404)	–
Candida albicans (ATCC 10231)	

The oil of *S. spinosa* from Baraghan was found to contain 20.03% (Z)-β-ocimene, which was 2.5% in *S. spinosa* from Dizin (Baher Nik and Mirza, 2005), 1% in *S. multicaulis* (Tepe *et al*., 2004) and 0.9% in *S. cryptantha* (Tepe *et al*., 2004). Germacrene D was 10.66% of *S. spinosa* oil, from Baraghan which was 2.7% in *S. spinosa* from Dizin (Baher Nik and Mirza, 2005) and 1% in *S. tomentosa* (Zeki *et al*., 2001).

Salvia spinosa oil of Baraghan contains about 5.01% β-Caryophyllene, which was found about 4.2% in *S. multicaulis* (Tepe *et al*., 2004), 0.82% in *S. officinalis* (Miladinovie *et al*., 2000) and 10.2% in *S. spinosa* oil from Dizin (Baher Nik and Mirza, 2005). 2-Bultyl thiohenen (9.83%) and 3-Butyl thiophene (3.49%) were isolated for the first time from *S. Spinosa* oil from Baraghan.

All previous studies about antimicrobial activity of salvia species including *S. lanigera* (Al-Howiring,

Table 3: Minimum Inhibitory Concentrations (MICs) of the essential oil of *Salvia spinosa* L. determined by disc diffusion method

	MIC (µL/disc)			
Microorganism	Essential oil	Gentamycin	Amoxicillin	Nystatin
Staphylococcus aureus	3.75	0.62	0.029	-
Pseudomonas aeruginosa	1.88	2.49	-	-
Basillus subtilis	2.06	0.038	0.46	-
Escherichia coli	-	0.009	0.93	-
Aspergilus niger	-	-	-	0.02
Candida albicans	-	-	-	0.02

2003), *S. aucheri* var. *aucheri* (Tepe *et al*., 2004), *S. multicaulis* (Tepe *et al*., 2004), *S. tomentosa* (Zeki *et al*., 2001), *S. officinalis* (Miladinovie *et al*., 2000), Showed significant antimicrobial activity on gram-positive bacteria (*Sthaphylococcus aureus* and *Basillus subtilis*) but all of them were inactive on *Pseudomonas aeruginosa*.

The significant antimicrobial activity of *S. spinosa* oil against *Pseudomonas aeruginosa*, showed for the first time, may be due to its different oil composition with other *Salvia* species.

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