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Chemical composition and antimicrobial activity of essential oil of *Salvia officinalis* L. and *Salvia virgata* Jacq

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ABSTRACT

Background & Aim: The genus *Salvia* (commonly known as sage) is a broad genus belonging to the family Lamiaceae. This study is aimed at assessing the essential oil composition and antimicrobial activities of two Salvia species.

Experimental: The chemical composition of the essential oils of *Salvia officinalis* L. and *Salvia virgata* Jacq, cultivated in Estahban (Fars, South Iran), was studied by means of GC-MS analyses. Antimicrobial activity was tested against a panel of microorganisms including one Gram-positive (*Staphylococcus epidermidis* PTCC NO. 1435) and one Gram-negative (*Escherichia coli*) and three fungal strain (*Alternaria alternata* PTCC NO. 5224, *Penicillium funiculosum* PTCC NO. 5301 and *P. funiculosum* PTCC NO. 5169) using the disk diffusion and agar-well diffusion methods and the Minimum Inhibitory Concentration (MIC) technique.

Results: In all, 57 compounds were identified, 42 for *S. officinalis*, accounting for 98.94% of the total oil, 29 for *S. virgata* (98.81%). The major components of *S. officinalis* essential oil were α-thujone (37.18%), 1,8-cineole (12.71%), β-thujone (9.10%) and the major components of *S. virgata* essential oil were caryophyllene oxide (30.23%), β-caryophyllene (22.63%), sabinene (11.82%). The antimicrobial activity of the total essential oil evaluated by the agar-well diffusion method, the results showed that the highest active against *S. epidermidis* and also the least active against *E. coli.* Inhibition of growth was tested by the disk diffusion method, the results showed that essential oil of *S. officinalis* and *S. virgata* were highest active against *E. coli* and *P. funiculosum* (PTCC NO. 5301), respectively. Also the least active against *A. alternata.*

Recommended applications/industries: The results showed that *S. officinalis* oil had higher antimicrobial activity compare to *S. virgata*.

1. Introduction

Throughout human history, medicinal and aromatic plants have been used for flavor enrichment in culinary and medicinal purpose in folk medicine. The family of Lamiaceae has been of great importance due to the unique aroma and nutritional value. One of the most important members of the Lamiaceae family is salvia (Naghibi *et al.*, 2010). Several species of Salvia are cultivated for their aromatic characteristics and reported to have wide range of biological activities, such as antibacterial, fungistatic, astringent, antiseptic, antifungal and digestive effects. Leaves are used in antiseptic and astringent herbal mixtures. Some compounds present in the essential oil are microbiologically active (Lu and Foo, 2002; Fellah *et al.*, 2006; Farhat *et al.*, 2009). The antimicrobial effects of essential oils derived from Medicinal and aromatic plants are the basis of copious applications, in various revenue generating sectors such as pharmaceutical, nutraceutical, cosmetic, perfume, agronomy, and sanitary industries (Raut and Karuppayil, 2014). Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens (Friedman *et al.*, 2002). The most antibacterial effects of the essences and their components refer to hydrophobia traits in the essential oils which are caused to infiltrate the lipidic walls of bacterial cells and can be changed its structures (Skaltsa *et al.*, 2003) and consequently many ions and cellular substrates percolate toward to outside of cells and be ultimately dying of bacteria (Carson *et al.*, 2002). The antifungal and antibacterial activity exhibited by the extracts and essential oils of medicinal plants has been demonstrated by several researchers (Ghasemi Pirbalouti *et al.*, 2009; Ghasemi Pirbalouti *et al.*, 2010). The antimicrobial activity of the essential oils is due to its major compounds. The minor compounds possess also an important role on anti-microbial activity (Ceylan and Fung, 2004). Velickovic *et al.* (2003) reported sage ethanol extract possesses antibacterial activity against standard strains of Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeroginosa* and *Salmonella enteritidis*) and the result showed the inhibitory effect of sage ethanol extract with MIC= 10 mg/mL for *S. aureus*, MIC= 6 mg/mL for *B. subtilis*, MIC= 60 mg/mL for *E. coli*, MIC= 60 mg/mL for *P. aeroginosa* and MIC= 50 mg/mL for *S. enteritidis*. Investigation of Javidnia *et al.* (2008) on the antibacterial activity of the essential oil of *Salvia reuterana* and *Salvia multicaulis* districted that essential oil are more active against Gram-negative bacteria. The essential oil of *S. officinalis* is also effective against several bacteria, *e.g.*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, all recognized foodborne pathogens (Delamare, 2007; Klaus *et al.*, 2009). The aim of the present investigation was to study essential oil composition and antimicrobial activities of *Salvia officinalis* L. and *Salvia virgata* Jacq.

2. Materials and Methods

2.1. Plant material

The aerial parts of two *Salvia* species including *S. officinalis* and *S. virgata* were collected from Estahban (Fars province) in South Iran (29° 07′ N and 54° 02′ E) about 1700 m above sea level during 2014.

2.2. Essential oil extraction

Harvested fresh aerial parts of two *Salvia* species were dried at room temperature (25 ± 5 °C). Dried plant material was powered (100 gm, and subjected to hydro–distillation (1000 ml distillated water) for 3 hrs using a Clevenger-type apparatus according to the method recommended in BP (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulfate and kept in amber glass vials at 4°C ± 1°C until use.

2.3. Identification of the oil components

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Hewller-packard 6890 GC-MS system. HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas with flow rate of 1.0

mL/min. The injector temperature was set at 280°C. The oven temperature was kept at 50 °C for 4 min and programmed to 280 °C at a rate of 5 °C /min, and kept constant at 280 °C for 5 min. MS were taken at 70 eV. Mass range was from m/z 35 to 450. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams, 2007).

2.4. Bacterial and fungal strains

Extracts were individually tested against a panel of microorganisms including one Gram-positive (*Staphylococcus epidermidis* PTCC NO. 1435) and one Gram-negative (*Escherichia coli*) and three fungal strain (*Alternaria alternata* PTCC NO. 5224, *Penicillium funiculosum* PTCC NO. 5301 and *P. funiculosum* PTCC NO. 5169) were all clinical isolates obtained from the Food Medicine Faculty, (I.A.U.) Islamic Azad University of Jahrom Branch, Iran. Stock cultures of the bacteria were kept in 10% glycerol PBS (phosphate buffered saline) at 37°C. The yeast was cultured overnight at 30°C in Sabouraud dextrose agar (SDB) (Merck, Germany).

2.5. *Antimicrobial test*

These experiments were performed by the disc diffusion method and agar-well diffusion method with some modification. The extracts were dissolved in dimethyl sulfoxide (DMSO, 20 µl) before testing for antimicrobial activity. Normal saline was used for the preparation of inoculants having turbidity equal to 0.5 McFarland standards.

Disc assay was applied on nutrient agar media with adjusting pH at 7.0. Bacteria were spread on the media and the plates were allowed to dry for 5 minutes. The fungus was maintained on Potato Dextrose Agar (PDA) at 25 ± 1°C. Molten PDA (20 ml) was poured into sterilized petri dishes and seven concentrations of oil including (0.312, 0.625, 1.25, 2.5, 5, 10 and 20 µl/disc) were added into the medium. Bacterial plates were incubated at 30°C for 24 hours while fungal was incubated at 28°C for 48 hours. Diameter of inhibition zones was measured to the nearest millimeter for five replicates and the average diameter were calculated.

3. Results and discussion

3.1 Composition of the essential oils

Table 1 shows the chemical composition of the two essential oils; a total of 42 and 29 compounds were identified in the essential oil from the aerial parts of *S. officinalis* and *S. virgata*, respectively. The yield of *S. officinalis* oil is 2.4% (v/w), and its main components were α-thujone (37.18%), 1,8-cineole (12.71%), β-thujone (9.10%), camphene (5.54%) and viridiflorol (5.33%) (Fig. 1). The yield of *S. virgata* oil is 1.6% (v/w), and the main components were caryophyllene oxide (30.23%), β-caryophyllene (22.63%), sabinene (11.82%), 1-octan-3-ol (6.64%) and thujene (6.28%) (Fig. 2).

3.2 Antimicrobial activity

The results of antimicrobial activity of the essential oils obtained from *S. officinalis* and *S. virgata* are shown in (Table 2-5). The results showed that the essential oil of *S. officinalis* and *S. virgata* were most active against *S. epidermidis* and also the least active against *E. coli* (Table 2 and 3). Correlation coefficient between essential oil concentration of *S. officinalis* and diameter of inhibition zones by the agar-well diffusion method in *S. epidermidis*, *A. alternata*, *P. funiculosum* (PTCC NO. 5301), *P. funiculosum* (PTCC NO. 5169) and *E. coli* were 90%, 76%, 15%, 76% and 90%, respectively. Correlation coefficient between essential oil concentration of *S. virgata* and diameter of inhibition zones by the agar-well diffusion method in *S. epidermidis*, *A. alternata*, *P. funiculosum* (PTCC NO. 5301), *P. funiculosum* (PTCC NO. 5169) and *E. coli* were 72%, 75%, 82%, 92% and 92%, respectively.

The results showed that the essential oil of *S. officinalis* and *S. virgata* were most active against *E. coli* and *P. funiculosum* (PTCC NO. 5301), respectively. Also the least activity was observed against *A. alternata* (Table 4 and 5).

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Table 1. Chemical composition of the essential oils of *S. officinalis* and *S. virgata*

No	Compound	RI	*S. officinalis*(%)	*S. virgata* (%)
1	Cis-Salvene	852	0.43	-
2	Trans-Salvene	859	0.06	-
3	Tricyclene	915	0.19	-
4	Thujene	928	0.11	6.28
5	α-Pinene	937	5.20	1.14
6	Camphene	955	5.54	-
7	Sabinene	974	0.05	11.82
8	β-Pinene	978	2.03	-
9	1-Octan-3-ol	985	0.15	6.64
10	Myrcene	992	0.74	-
11	3-Octanol	995	0.03	-
12	α -Phellandrene	999	0.08	-
13	Cyclotetrasiloxane	1003	-	1.76
14	α-Terpinene	1008	0.20	1.04
15	*p*-Cymene	1021	1.07	-
16	Limonene	1025	1.75	1.45
17	1,8-Cineole	1029	12.71	2.09
18	Cis -Ocimene	1030	0.01	-
19	γ-Terpinene	1044	0.30	1.12
20	Cis- Sabinene hydrate	1050	0.11	0.23
21	Linalool oxide	1076	0.12	-
22	α-Terpinolene	1083	0.13	-
23	Trans-Sabinene hydrate	1093	0.09	0.32
24	Linalool	1101	0.42	-
25	α-Thujone	1112	37.18	-
26	β-Thujone	1115	9.10	-
27	trans-p-Menth-2-en-1-ol	1117	-	0.75
28	α-Campholene aldehyde	1119	0.07	-
29	Camphore	1126	0.15	-
30	trans-Pinocarveol	1136	-	0.34
31	Borneol	1155	6.47	-
32	Terpinene-4-ol	1177	0.64	5.25
33	α-Terpineol	1184	0.24	-
34	Thymol	1290	-	0.75
35	Trans-Sabinyl acetate	1291	0.28	-
36	Carvacrol	1300	0.11	-
37	Carvacryl acetate	1352	0.07	-
38	*β*-Caryophyllene	1418	1.69	22.63
39	α-Humulene	1454	1.72	0.95
40	Naphthalene	1480	0.05	-
41	γ-Curcumene	1483	-	1.08
42	Ledene	1499	0.05	-
43	γ-Elemene	1524	-	0.24
44	Bicyclogermacrene	1565	-	0.53
45	Spathulenol	1577	-	0.17
46	Caryophyllene oxide	1581	0.71	30.23
47	Viridiflorol	1590	5.33	-
48	β-Selinene	1604	0.18	-
49	Humullene epoxide II	1606	0.93	-
50	α–Cadinol	1626	-	0.23
51	Heptadecane	1700	-	0.19

52	Octadecane	1800	-	0.46
53	Manool	2056	2.45	-
54	Heneicosane	2100	-	0.34
55	Docosane	2200	-	0.23
56	Octacosane	2800	-	0.36
57	Nonacosane	2900	-	0.23
	Total		98.94	98.81
	Essential oil yield (%)		2.4	1.6

RI = Retention indices in elution order from DB-5 column

Table 2. Antimicrobial activity of the essential oil of *S. officinalis* determined by the agar-well diffusion method.

	Essential oil concentration (µl/disc)						
Microorganisms	**0.312**	**0.625**	**1.25**	**2.5**	**5**	**10**	**20**
Staphylococcus epidermidis (PTCC NO. 1435)	0	0.7	1	1.2	1.4	1.8	2.2
Alternaria alternata (PTCC NO. 5224)	0.1	0.2	0.4	1	0.7	1	1.5
Penicillium funiculosum (PTCC NO. 5301)	0	0.4	1	1	1.1	1.4	1
Penicillium funiculosum (PTCC NO. 5169)	0	0.3	0.8	1	1	1.2	1.6
Escherichia coli	0	0	0	0.4	0.6	0.8	1

Table 3. Antimicrobial activity of the essential oil of *S. virgata* determined by the agar-well diffusion method.

	Essential oil concentration (µl/disc)						
Microorganisms	**0.312**	**0.625**	**1.25**	**2.5**	**5**	**10**	**20**
Staphylococcus epidermidis (PTCC NO. 1435)	0	0	0.4	1	1.5	1.7	2
Alternaria alternata (PTCC NO. 5224)	0	0	0.2	0.5	1	1.2	1.4
Penicillium funiculosum (PTCC NO. 5301)	0	0	0.3	0.8	1.2	1.7	2
Penicillium funiculosum (PTCC NO. 5169)	0	0	0.3	0.6	1	1.4	1.9
Escherichia coli	0	0	0	0.4	0.6	0.8	1

Table 4. Antimicrobial activity of the essential oil of *S. officinalis* determined by disc diffusion method.

	Essential oil concentration (µl/disc)						
Microorganisms	**0.312**	**0.625**	**1.25**	**2.5**	**5**	**10**	**20**
Staphylococcus epidermidis (PTCC NO. 1435)	0	0.2	0.5	0.8	1.2	1.5	2
Alternaria alternata (PTCC NO. 5224)	0	0.1	0.1	0.5	0.6	0.7	1
Penicillium funiculosum (PTCC NO. 5301)	0	0.5	0.8	0.8	1	1	1.2
Penicillium funiculosum (PTCC NO. 5169)	0	0.2	0.4	0.7	1	1.3	2
Escherichia coli	0	0.7	0.7	1	1	1.2	3.1

Table 5. Antimicrobial activity of the essential oil of *S. virgata* determined by disc diffusion method.

	Essential oil concentration (µl/disc)						
Microorganisms	**0.312**	**0.625**	**1.25**	**2.5**	**5**	**10**	**20**
Staphylococcus epidermidis (PTCC NO. 1435)	0	0	0.3	0.8	1.2	1.4	1.7
Alternaria alternata (PTCC NO. 5224)	0	0	0.3	0.4	0.7	1	1.2
Penicillium funiculosum (PTCC NO. 5301)	0	0	0.2	0.7	1	1.2	1.8
Penicillium funiculosum (PTCC NO. 5169)	0	0	0.5	0.8	0.1	1.1	1.6
Escherichia coli	0	0	0.3	0.8	1.2	1.4	1.7

Correlation coefficient between essential oil concentration of *S. officinalis* and diameter of inhibition zones by the disk diffusion method in *S. epidermidis*, *A. alternata*, *P. funiculosum* (PTCC NO.

5301), *P. funiculosum* (PTCC NO. 5169) and *E. coli* were 84%, 81%, 71%, 94% and 91%, respectively. Correlation coefficient between essential oil concentration of *S. virgata* and diameter of inhibition zones by the disk diffusion method in *S. epidermidis*, *A. alternata*, *P. funiculosum* (PTCC NO. 5301), *P. funiculosum* (PTCC NO. 5169) and *E. coli* were 76%, 88%, 88%, 90% and 77%, respectively.

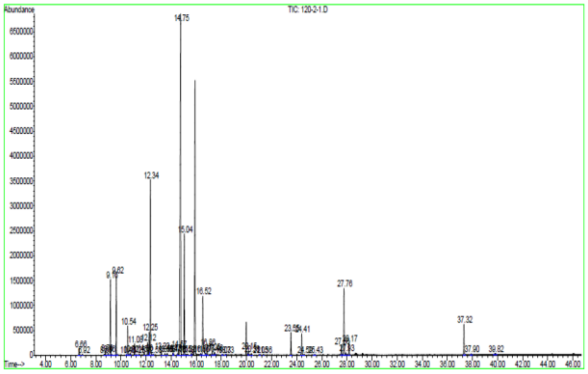

Fig. 1. The chromatogram of *S. officinalis* essential oil.

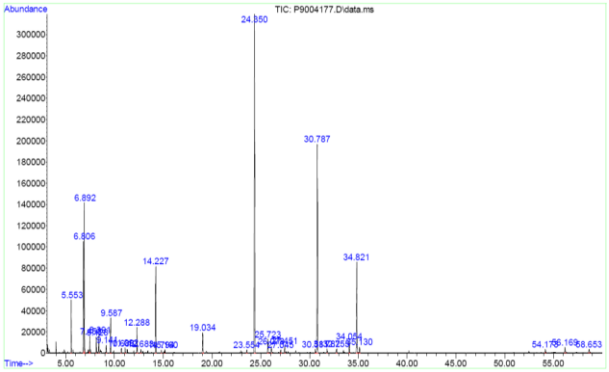

Fig. 2. The chromatogram of *S. virgata* essential oil

Many studies showed that herbs from the Lamiaceae family have a potent antioxidant and antibacterial activities. Among these, eugenol, carvacrol and thymol which are the major components of essential oils, are primarily responsible for their bactericidal/bacteriostatic properties. It was also observed, that the antimicrobial effect of plant extracts varies from one herb to another in different regions of the world. This may be due to many factors such as: the effect of climate, soil composition, the type of solvent used` in the extraction process, and also on the volume of inoculum used and culture medium or the type of strains within the same species of bacteria (Kozlowska *et al.*, 2015; Hadipanah and Khorrami, 2016). *Salvia officinalis* produces monoterpenes with a broad range of carbon skeletons, including acyclic, monocyclic, and bicyclic compounds. Three distinct monoterpene synthases are responsible for the first steps in the formation of the most characteristic monoterpenes of *S. officinalis* essential oil. The (+)-sabinene synthase catalyzes the production of sabinene, which undergoes further rearrangements leading to the two major monoterpenes, α- and β-thujone. The 1,8-cineole synthase produces in one-step 1,8-cineole. Finally, (+)-bornyldiphosphate synthase produces bornyl diphosphate, which is subsequently hydrolyzed to borneol and then oxidized to camphor (Wise *et al.*, 1998; Said-Al Ahl *et al.*, 2015). Golparvar and Hadipanah (2013) reported the major components *S. officinalis* cultivated in Isfahan (Iran) were camphor (17.75%), thujone (13.25%) and 1,8-cineole (13.03%). Sefidkon and Mirza (1999) reported the major components *Salvia virgata* cultivated in Tabriz (north of Iran) were β-caryophyllene (46.6%), germacrene-B (13.9%) and β-caryophyllene epoxide (13.2%).

Antimicrobial activity of essential oil is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo *et al.*, 2004). There has been no large scale, systematic investigation of the relationship between bacterial inhibition and the total phenolic content of spices and herbs. That oxygenated monoterpenes, exhibit strong antimicrobial activity, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility limits their diffusion through the medium. Hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen bound capacity and water solubility (Sokovic *et al.*, 2010). Compounds from *S. officinalis* essential oil have been shown to exhibit high antibacterial activity against *Staphyloccocus aureus*, *Escherichia*

coli, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Valero and Salmeron, 2003).

Khalil and Li (2011) during their study on the essential oil of *S. officinalis* against *E. coli*, *P. aeruoginosa*, *S. typhi*, *S. aureus*, *Streptococcus group D* and *C. albicans*, showed that *S. aureus*, *Streptococcus* and *C. albicans* were most susceptible than *E. coli*, *S. typhi* and *P. aeruoginosa*.

In most of the studies, the antimicrobial activity of *Salvia* species was considered to be related to 1,8-cineole, β-caryophyllene, thujone, camphor, borneol in their contents (Akin *et al.*, 2010; Tenore *et al.*, 2010). However, the role of other minor compounds should not be neglected. Some studies have concluded that whole essential oils have a greater antibacterial activity than the major components mixed. Chemical compounds have direct activity against many species of bacteria such as terpenes and a variety of aliphatic hydrocarbon (alcohols, aldehydes and ketones) (Bachir Raho *et al.*, 2016).

4. Conclusion

The composition of the essential oil of *S. officinalis* and *S. virgata* growing in Iran has been analyzed and its antimicrobial activity investigated. The results indicate that the oil may be used in the treatment of diseases caused by the microorganisms tested. Antimicrobial activity of essential oil is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin.

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