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Comparative study of the antimicrobial activity of essential oil and two different extract from *Salvia urmiensis* Bunge

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ABSTRACT

Objective: In this study, antimicrobial activity of essential oil, ethyl acetate and ether extracts of *S. urmiensis* Bunge were screened against some species of bacteria and fungi. Also, the essential oil of the aerial part of *S. urmiensis* Bunge was examined by GC and GC–MS. **Methods:** The oils obtained by hydrodistillation in a Clevenger apparatus from fresh and dried aerial parts of *S. urmiensis* Bunge were analyzed by GC and GC–MS to investigate the variations of oil components. Ethyl acetate and ether extracts of *S. urmiensis* Bunge were obtained using powdered aerial part and appropriate amounts of each solvent (ethyl acetate, ether) by maceration method. The minimum inhibitory concentration (MIC) of essential oil and extracts against the bacteria and fungi was determined using broth microdilution method. **Results:** In the essential oil of *S. urmiensis* Bunge 27 Compounds have been identified. Benzyl benzoate (60.3 %), n–hexyl benzoate (16.7 %), Amyl benzoate (5.2 %) and 2– octyl benzoate (4.2 %) were the main components of the essential oil. The essential oil analysis showed greatest antimicrobial activity against *Staphylococcus epidermidis* (5.3 μg/ml) and *S. cerevisiae* (9.3 μg/ml). The ethyl acetate showed greatest antimicrobial activity against *Bacillus subtilis* (106.7 μg/ml), *Candida albicans* (5.3 μg/ml) and ether extract showed greatest antimicrobial activity against *Klebseilla pneumoniae* (10.7 μg/ml) and *Saccharomyces cerevisiae* (10.7 μg/ml). **Conclusions:** we suggest that the antimicrobial activity of *S. urmiensis* may be due to its content of germacrene and linalool.

1. Introduction

Since ancient times the crude herbal extract and essential oil of aromatic plants have been in use for different purposes, such as food, drugs and perfumery [1]. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [2]. In the essential oil of aromatic plants, there are many compounds such as monoterpenes, sesquiterpenes, alcohols, aldehydes, phenols, esters, and ether, sulphurous and nitrogenous substances [3,4].

The genus Salvia with over 900 species is probably the largest member of the family Lamiaceae and is found in both subtropical and temperate parts of the world. Two largest centers of the genus are in America and in South–West Asia [5].

In Iran There is a high diversity of salvia species and accessions which includes 70 species that 40% of them are endemic [6]. *S. urmiensis* Bunge is an aromatic perennial woody sub–shrub of these species that are found in their natural in Iran. *S. urmiensis* Bunge is considered one of the most important Salvia species for medical (antimicrobial and antipestic) purposes [7]. Several reports have been published in recent years on the antimicrobial activity of some extracts and essential oils obtained from some species of Salvia [3,8–10]. Many reports was shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles [11]. The literature on the chemical constituents of the genus Salvia gave information for compounds such as germacrene, linalool, 1,8–cineole (eucalyptol), borneol, α–pinene, β–pinene, camphor and camphene [3,9]. There are no data concerning the essential oil composition and antimicrobial activity of *S. urmiensis* Bunge. The aim of this paper is to demonstrate the compounds and antimicrobial activities of essential oil, ethyl acetate and ether extract of *S. urmiensis* Bunge.

2. Materials and methods

2.1. Plant material

The aerial parts of *S. urmiensis* Bunge were collected from

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wild plant in Ardebil city (Northwest of Iran). The voucher specimen (No.7812) is deposited in the Herbarium of Science and Research Branch of Islamic Azad University, Tehran, Iran. The aerial parts were cut into pieces and air-dried for 10 Days at room temprature.70 g aerial parts were powdered, mixed with 500 ml of distilled water and the essential oil hydrodistilled in a Clevenger apparatus according to the British method for 3h. Ethyl acetate and ether extracts of *S. urmiensis* Bunge were obtained using 40 gr of powdered aerial part and150 ml of each solvent (ethyl acetate, ether) by maceration method.

2.2. Gas chromatography

GC analysis of the oil was conducted using a Thermoquest-Finnigan Trace GC instrument equipped with a DB-1 fused silica column (60 m x 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL min-1. The oven temperature was held at 60℃ for 1 min, then programmed to 250℃ at a rate of 4℃ min-1 and then held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280℃, respectively.

2.3. Gas chromatography-Mass spectrometry

GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-1 fused silica column (60 m x 0.25 mm i.d., film thickness 0.25 μm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The oven temperature was raised from 60 to 250 ℃ at a rate of 5℃ min-1 and then held at 250℃ for 10 min.; transfer line temperature was 250℃. The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of the components was based on comparison of their retention indices (RI), obtained using n-alkanes (C6-C25), and comparison of their mass spectra with those of Wiley library spectra and literature data. Additionally, the identity of all compounds was confirmed by comparison of the expected molecular weights with the results of available authentic samples.

2.4. Microorganisms

Five strains of bacteria and two strains of fungi were studied including *Bacillus subtilis* (ATCC 127111), Enterococcus faecalis (ATCC 29737), Staphylococcus aureus (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12229), Klebsiella pneumoniae (ATCC 10031), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763). Bacterial strains were cultivated on Mueller Hinton broth (Merck, Germany) and fungi were cultivated on Sabouraud liquid Medium broth (Merck, Germany). The minimum inhibitory concentration (MIC) of essential oil and extracts against the bacteria and fungi was determined using broth microdilution method.

3. Result

The essential oil of the aerial part of *S. urmiensis* Bunge was examined by GC and GC-MS. The constituents of the oil are showed in table 1. In the essential oil, 27 Compounds have been identified. Benzyl benzoate (60.3 %), *n*-hexyl benzoate (16.7 %), Amyl benzoate (5.24 %), 2- octyl benzoate (4.2 %) were the main components of the essential oil. According to the data shown in table 2, the essential oil of *S. urmiensis* Bunge had antimicrobial activity against all tested microorganisms. The MIC ranged from 5.3 to 298.7 μg/ml with highest inhibition values observed against *S. epidermidis*, *B. subtilis* (5.3 μg/ml) and *S. cerevisiae* (9.3 μg/ml) among bacteria and fungi. The antimicrobial activity against *S. epidermidis* (5.3 μg/ml) and *S. cerevisiae* (9.3 μg/ml). *B. subtilis* (170.7 μg/ml) and *C. albicans* (85.3 μg/ml) showed the greatest level of resistance to essential oil. The essential oil showed more antimicrobial activity on bacteria and fungi than ethyl acetate and ether extract. The ethyl acetate showed greatest antimicrobial activity against *B. subtilis* (5.3 μg/ml), *C. albicans* (106.7 μg/ml) and ether extract showed greatest antimicrobial activity against *K. pneumoniae* (10.7 μg/ml) and *S. cerevisiae*(10.7 μg/ml). S. epidermidis(149.3 μg/ml) and *S. cerevisiae* (298.7 μg/ml) showed the highest resistance to ethyl acetate extract and *S. epidermidis*(106.7 μg/ml) and *C. albbicans* (149.3 μg/ml) showed the highest resistance to ether extract. The observed antimicrobial (antibacterial and antifungal) effect of *S. urmiensis* Bunge against Bacteria and fungi is according to previous reports for other species of Salvia [3, 8-10].

Table 1

Chemical composition of identified compounds in the oil of *Salvia urmiensis Bunge*

No	Compound	RI	%
1	Benzyl aldehyde	932	0.1
2	Linalool	1082	2.2
3	2- Methyl butyl isovalerate	1089	0.1
4	2- Methyl propanoate	1128	0.5
5	Acetic acid octyl ester	1187	0.1
6	2- Methyl butanoate	1217	1.0
7	Hexyl isovalerate	1221	1.2
8	Benzyl isobutanoate	1267	0.3
9	Isobutyl benzoate	1301	0.3
10	n- Butyl isovalerate	1318	0.1
11	n- octyl isobutirate	1323	0.1
12	n- Butyl benzoate	1345	0.4
13	Benzyl valerate	1357	0.3
14	Benzyl isovalerate	1363	1.1
15	Amyl benzoate	1411	5.2
16	Isopentyl benzoate	1446	0.2
17	Germacrene D	1479	0.4
18	E,E- α -farnesene	1490	0.5
19	Benzyl hexanoate	1510	0.2
20	2-Allylpent-4, enoic acid, benzyl ester	1513	0.1
21	n- Hexyl benzoate	1553	16.7
22	β -Eudesmol	1639	0.4
23	α -Eudesmol	1644	0.1
24	Tert-butyl benzoate	1651	0.4
25	Benzyl benzoate	1738	60.3
26	2- ocetyl benzoate	1756	4.2
27	Bergamotol	1814	2.1

RI: Linear retention indices on column

Table 2

The minimum inhibitory concentration (MIC, μg/ml) of essential oil, ether ethyl acetate extract of *Salvia urmiensis Bunge* against microorganisms

Microorganism	Extract		
	Essential oil	Ether	Ethyl acetate
Enterococcus faecalis	106.7	74.7	42.7
Staphylococcus aureus	85.3	37.3	21.3
Staphylococcus epidermidis	5.3	106.7	149.3
Bacillus subtilis	170.7	85.3	5.3
Klebsiella pneumoniae	125	10.7	74.7
Candida albicans	85.3	149.3	106.7
Saccharomyces cerevisiae	9.3	10.7	298.7

The results are the conclusion of three replicate

4. Discussion

In the literature, the antimicrobial activity of some genus Salvia essential oils against microbes has been reported [3, 12]. It is due to the presence of chemical components that have antimicrobial activity such as germacrene, linalool. Germacrene is an important sesquiterpenes that occur widely in nature [13]. Antibacterial and antifungal activities of germacrene have been reported in previous studies [13,14]. Linalool is a major constituent of essential oil of some plants such as genus Lavandula. This componund has been used as an antimicrobial agent in some countries such as Greece [15,16]. The antibacterial and antifungal activity of terpenes such as linalool and germacrene has not been exactly understood but these compounds may cause disruption in membrane by lipophylic reactions [4]. It is very difficult to contribute antimicrobial activity of the essential oils to a few compounds because those contain different chemical compounds [17]. In conclusion, we suggest that the antimicrobial activity of *S. urmiensis* Bunge may be due to its content of germacrene and linalool.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Farjam MH, Khalili M, Rustayian A, Javidnia K, Izadi S. Biological activity of the n-butanolic extract of Stachys pilifera. *Afr J Microbiol Res* 2011; **5**: 5115-5119.

[2] Rustaiyan A, Javidnia K, Farjam MH, Mohammadi MK, Mohammadi N. Total phenols, antioxidant potential and antimicrobial activity of the methanolic extracts of Ephedra laristanica. *J Med Plants Res* 2011; **5**: 5713-5717.

[3] Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L., Echeverrigaray S. Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil. *Food Chem* 2007; **100**: 603-608.

[4] Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564-582.

[5] Salimpor F, Mazooji A, Akhoondi-Daezikolaei S. Chemotaxonomy of six Salvia species using essential oil composition markers. *J Med Plants Res* 2011; **5**: 1795-1805.

[6] Abdollahi J, Ebrahimi M, Ramshiri HA, Jaafari AA, Eftekhari M, et al. Seed germination as the major conservation issue of endemic Iranian salvia species. *J Med Plants Res* 2012; **6**: 37-46.

[7] Ghasempour HR, Kahrizi D, Borzo S. Plant micropropagation in wild sage (salvia multicaulis). *Plant Sci* 2007; **269**: 947.950.

[8] Akin M, Demirci B, Bagci Y, Baser KHC. D. Antibacterial activity and composition of the essential oils of two endemic Salvia sp. from Turkey. *Afr J Microbiol Res*. 2010; **9**: 2322 2327.

[9] Mohan L, Negi A, Melkani AB, Dev V. Chemical composition and antibacterial activity of essential oil from Salvia mukerjeei. *Nat Prod Commun* 2011; **6**: 1949-1952.

[10] Cardille V, Russo A, Formisano c, Rigano D, Senatore F, et al. Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *Asian J Ethnopharmacol* 2009; **126**: 265-272.

[11] Rustaiyan A, Javidnia K, Farjam MH, Aboee-Mehrizi F, Ezzatzadeh E. Antimicrobial and antioxidant activity of the Ephedra sarcocarpa growing in Iran. *J Med Plants Res* 2011; **5**: 4251-4255.

[12] Hayouni, EA, Chraief I, Abedrabba M, Bouix M, Leveau JY, Mohammed H, Hamdi M. Tunisian Salvia officinalis L. and Schinus molle L. essential oils: Their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat. *Int J Food Microbiol* 2008; **125**: 242-251.

[13] Adio AM. Germacrenes A-E and related compounds: thermal, photochemical and acid induced transannular cyclizations. *Tetrahedron* 2009; **65**: 1533-1552.

[14] Luna-Herrera J, Costa MC, González HG, Rodrigues AI, Castilho PC. Synergistic antimycobacterial activities of sesquiterpene lactones from Laurus spp. *J Antimicrob Chemoth* 2007; **59**: 548-52.

[15] Sato K, Krist S, Buchbauer G. Antimicrobial effect of vapours of geraniol, (R)-(−)-linalool, terpineol, γ-terpinene and 1,8-cineole on airborne microbes using an airwasher Flavour Fragr J 2007; **22**: 435437.

[16] Lis-Balchin M, Deans SG. And Eaglesham, E. Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Fragr J* 1998; **13**: 98-104.

[17] Ben-Amor IL, Neffati A, Sgaier MB, Bhouri W, Boubaker J, et al. Antimicrobial Activity of Essential Oils Isolated from Phlomis crinita Cav. ssp. mauritanica Munby. *J Am Oil Chem Soc* 2008; **85**: 845-849.