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Antibacterial, Antifungal Properties and Chemical Composition of Essential Oils of *Satureja hortensis* L. and *Satureja khuzestanica* Jamzad

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ABSTRACT

Background & Aim: The aim of this study was to investigate the antibacterial, antifungal properties and chemical composition screening of essential oils of *Satureja khuzestanica* Jamzad and *Satureja hortensis* L.
Experimental: For determination of antibacterial and antifungal activity of these essential oils, *Staphylococcus aureus* and *Candida albicans* were targeted, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each essential oil were determined individually. Also, chemical composition of essential oils was identified and characterized by gas chromatography armed by mass spectrometry (GC–MS).
Results: The total of 22 and 21 compounds were identified in the essential oils of aerial parts of *S. hortensis* and *S. khuzestanica,* respectively. The major components of *S. hortensis* essence were carvacrol (56.36%), γ-terpinene (24.75%), p-cymene (5.81%) and the major constituents of *S. khuzestanica* essence was reported carvacrol (69.62%), γ-terpinene (9.25%) and p-cymene (8.36%). The obtained results showed the antibacterial and antifungal activity of both extracted essential oils against the tested pathogens. The MIC and MBC of *S. hortensis* and *S. khuzistanica* essential oils against *S. aureus* were determined 0.1 and 0.5 µl/ml, and 0.1 and 0.2 µl/ml against *C. albicans*, respectively.
Recommended applications/industries: The results showed that *S.hortensis* oil had higher antimicrobial activity compare to *S. khuzistanica*.

1. Introduction

The genus *Satureja* belonging to the (Lamiaceae) family, subfamily Nepetoideae, tribe Mentheae, consists of more than 200 species of herbaceous perennials worldwide. Distribution of the genus *Satureja* overlaps the region of southern and southeastern Europe, Asia Minor, and northern Africa,

with the center of the genus area predominantly in the Mediterranean (Senatore et al., 1998). The genus of Satureja with name of "Marze" in Persian, consists of fourteen species has been reported in Flora Iranica (Rechinger, 1982). The species *Satureja khuzestanica* Jamzad is an endemic plant that widely distributed in the northern Khuzestan and southern Lorestan provinces of Iran (Mozaffarian, 2008). The essential oils extracted from some species of Satureja have showed wide range of biological activity including the antibacterial and antifungal activities (Sefidkon and Jamzad, 2000; Behravan *et al.,* 2004), analgesic and anti-inflammatory (Ghazanfari *et al.,* 2006), antispasmodic and antidiarrhoea (Hajhashemi et al., 2002), antioxidant, antidiabetic, antihyperlipidemic and reproduction-stimulatory activities (Abdollahi *et al.,* 2003). 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 and agronomy (Raut and Karuppayil, 2014). Carvacrol is a monoterpenoid phenol biosynthesized via aromatization of γ-terpinene to p-cymene and subsequent hydroxylation of p-cymene. This phenol along with its two precursors γ-terpinene and p-cymene appeared as the major components in numerous phenolic essential oils of the Lamiaceae family (e.g., in thymus, oregano, and savory oil) (De Vincenzi *et al.,* 2004). Hence carvacrol has a wide range of activities including antimicrobial, antioxidant, anticandidal, and anti-inflammatory properties which already investigated (Di Pasqua *et al.,* 2007). Many of the previous studies demonstrated that the members of the genus *Satureja* showed a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors (Gulluce*et al*., 2003; Sahin *et al.,* 2003). Also, several studies have been performed concerning the antimicrobial activity of essential oils of other *Satureja* species while antibacterial and antifungal effects of *S. khuzistanica* as an endemic plants in Iran has been never investigated.

The aim of the present investigation is evaluation of antibacterial and antifungal properties and also essential oil composition in *Satureja khuzestanica* Jamzad and *Satureja hortensis* L.

2. Materials and Methods

2.1. Plant material

The aerial parts (leaves and flowers) of two *Satureja* species including *Satureja khuzestanica* Jamzad and *Satureja hortensis* L.were harvested in July 2015 from Isfahan province in center Iran. Collection site information and soil physical and chemical characteristics field including pH and EC were determined (Table 1).

Table 1. Collection site information, some physical and chemical properties of soil collection site in the present work

Site no	Collection site city	Latitude	Longitude	Altitude (m)	PH	EC (ds/m)	Sand (%)	Silt (%)	Clay (%)
1	Isfahan	34° 48 ′N	48° 31 ′E	1550	7.31	4.15	17.57	77.66	5.63

2.2. Essential oil extraction

Air-dried plant material was subjected to hydro-distillation for 2 h using a Clevenger-type apparatus according to the method recommended in BP (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulfateand kept in amber glass vials at 4°C ± 1°C until use.

2.3. Identification of the oil components

Volatile compounds from the aerial parts of plants were analyzed by GC/MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system equipped with. HP-5MS column (30 m x 0.25 mm, 0.25 µm filmthickness). Helium was used as carrier gas with flow rate of 1.0 mL/min. 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, at split mode. The injector temperature was set at 280°C. 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. Antimicrobial assay

Essential oils were individually tested against a panel of microorganisms including one Gram-positive

bacteria, *Staphylococcus aureus* and one fungal strain *Candida albicans* collected from Isfahan province. 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 with some modification. For the experiments, antibacterial activity of the crude extract was investigated against *Staphylococcus aureus* bacterial strains by the paper disk diffusion technique. The extract was redissolved in methanol to make a 100 mg/ml solution and then filtered. From this solution, 40-µl aliquots were transferred onto blank paper disks with a diameter of 6 mm. Dried disks were placed onto Mueller Hinton agar medium (Merck) previously inoculated with a bacterial suspension (*ca.* 108 CFU/ml) and incubated at 35±1 ◦C for 24 h. antimicrobial activity of the crude extract was investigated against *C.albicans*fungal strains the disc diffusion method with some modification. The yeast was cultured overnight at 30°C in Sabouraud dextrose agar (SDB) (Merck, Germany). 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. The fungus was maintained on Potato Dextrose Agar (PDA) at 25 ± 1ºC. Disks were placed onto Mueller Hinton Broth and LB broth (MILLER) medium.

Plates were then examined for the presence of growth inhibition zones, and diameters were measured, if any. Clindamycin disks (20 µg), Amikacin disks (30 µg), Nalidixic acid disks (30 µg), Cefalexin disks (30 µg), Erythromycin disks (15 µg), Gentamicin disks (10 µg), Penicillin disks (10µg) as well as Sulfamethoxazole disks (30 µg) were used as positive controls, where appropriate. Means of the traits were compared by Duncan's multiple range test at $p < 0.01$ level.

3. Results and discussion

3.1. Composition of the essential oils

Qualitative and quantitative analysis of the essential oils volatile profile are listed in Table (2). A total of 22 and 21 compounds were identified in the essential oil from the aerial parts of *S. hortensis* and *S. khuzestanica* respectively.The yield of *S. hortensis* oil is 2.01% (v/w), and components corresponding to 98.07% and consisted mainly of oxygenated monoterpenes (60.05%) and monoterpene hydrocarbons (36.11%), with a small amount of sesquiterpene hydrocarbons (1.5%) and oxygenated sesquiterpenes (0.41%). The major constituents of *S. hortensis* oil were carvacrol (56.36%), γ-terpinene (24.75%) and *p*-cymene (5.81%) (Table 2).

The yield of *S. khuzestanica* oil is 1.21% (v/w), and components corresponding to 98.54% and consisted mainly of oxygenated monoterpenes (74.83%) and monoterpene hydrocarbons (21.18%) with a small amount of sesquiterpene hydrocarbons (1.85%) and oxygenated sesquiterpenes (0.68%). The major constituents of *S. khuzestanica* oil were carvacrol (69.62%), γ-terpinene (9.25%) and *p*-cymene (8.36%).

Table 2. Chemical composition of essential oils of two *Satureja* species cultivated from Iran.

No	Compound	RI	*S. hortensis*	*S. khuzistanica*
1	α-Thujene	929	0.82	0.16
2	α-Pinene	937	1.42	0.36
3	Camphene	952	0.31	tr
4	Sabinene	970	-	tr
5	β-Pinene	979	0.13	-
6	β-Myrecene	989	-	tr
7	α-phellandrene	1006	-	tr
8	δ-3-Carene	1011	tr	-
9	α-Terpinene	1018	2.73	2.32
10	ρ-Cymene	1025	5.81	8.36
11	Limonene	1028	tr	0.63
12	β-Phellandrene	1031	tr	tr
13	trans-β-Ocimene	1053	tr	-
14	γ-Terpinene	1060	24.75	9.25
15	trans-Sabinene hydrate	1074	-	tr
16	*cis*-sabinene hydrate	1085	tr	-
17	Linalool	1105	0.42	0.32
18	Borneol	1164	tr	0.93
19	Terpinene-4-ol	1176	0.58	3.25
20	Thymol	1290	2.64	0.71
21	Carvacrol	1295	56.36	69.62
22	Thymol acetate	1354	tr	-
23	β-caryophyllene	1416	0.98	1.21
24	Aromanderene	1444	-	0.22
25	α-Humulene	1453	0.21	-
26	β-Bisoblene	1509	0.31	0.42
27	Caryophyllene oxide	1584	0.41	0.68
	Monoterpene hydrocarbons		36.11	21.18
	Oxygenated		60.05	74.83

monoterpenes		
Sesquiterpene hydrocarbons	1.5	1.85
Oxygenated sesquiterpenes	0.41	0.68
Total	98.07	98.54
Oil yield (%)	2.01	1.21

RI = Retention indices in elution order from DB-5 column, tr, trace (< 0.1%).

3.2. Antimicrobial activity

The results of antimicrobial activity of the essential oils obtained from *S. hortensis* and *S. khuzistanica* are shown in Table 3-6. The results showed that the essential oil of *S. hortensis* were most active against *S. aureus* (Table 3). According to these results, MIC and MBC of *S. hortensis* and *S. khuzistanica* oil on *S. aureus* were estimated 0.1 and 0.5 µl/ml, respectively. MIC and MBC of *S. hortensis* and *S. khuzistanica* oil on *C. albicans* were estimated 0.1 and 0.2 µl/ml, respectively. The results of antimicrobial activity of the essential oils obtained from *S. hortensis* and *S. khuzistanica* determined by Mueller Hinton Broth medium were shown in Table 5 and 6. Correlation coefficient between essential oil concentration of *S. hortensis* and diameter of inhibition zones in *S. aureus* and *C. albicans* were 85% and 90%, respectively. Correlation coefficient between essential oil concentration of *S. khuzistanica* and diameter of inhibition zones in *S. aureus* and *C. albicans* were 96% and 92%, respectively. The dominant monoterpene produced in glandular trichomes on the surface of the leaves, geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol, is named after its dominant monoterpene.

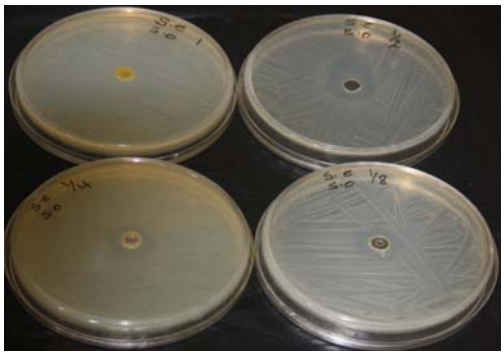

Figure 1. Inhibition diameter zones obtained by paper disk diffusion method.

Table 3. Antimicrobial activity of the essential oil of *S. hortensis* determined by the disc diffusion method.

Microorganisms	Essential oil concentration (µl/disc)					
	0.625	1.25	2.5	5	10	20
S. aureus	0	0.5	1	1.4	1.8	2.2
C. albicans	0	0.2	0.8	1.4	1.6	1.7

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

Microorganisms	Essential oil concentration (µl/disc)					
	0.625	1.25	2.5	5	10	20
S. aureus	0	0.4	1.2	1.3	1.6	1.9
C. albicans	0	0.1	1	1.1	1.4	1.8

Table 5. Antimicrobial activity of *S.hortensis* essential oil determined by Mueller Hinton Broth medium.

	Essential oil concentration (µl/disc)							
	0.05	0.1	0.2	0.5	1	2	4	8
S. aureus	3.2×10^{7}	0	0	0	0	0	0	0
C. albicans	3.1×10^{8}	3.5×10^{6}	1.6×10^{5}	0	0	0	0	0

Table 6. Antimicrobial activity of *S. khuzistanica* essential oil determined by Mueller Hinton Broth medium.

	Essential oil concentration (µl/disc)							
	0.05	0.1	0.2	0.5	1	2	4	8
S. aureus	4.1×10^{8}	1.7×10^{5}	2.3×10^{4}	0	0	0	0	0
C. albicans	1.3×10^{9}	3.2×10^{6}	0	0	0	0	0	0

The six monoterpenes are all produced from geranyl pyrophosphate via a series of changes in configuration and hydroxylation and have fairly similar molecular structures. A major distinction is the phenolic nature of carvacrol and thymol, and the nonphenolic nature of the four other monoterpenes (Thompson *et al.*, 2003). Carvacrol and thymol are structural isomers and have a phenolic hydroxyl at a different location on the phenolic ring. The hydroxyl group increased their hydrophilic ability, which could help them dissolve in microbial membrane and impair them (Sikkema *et al.*,1995).

According to Ghasemi Pirbalouti and Moalem (2013), analyzed the essential oil of the aerial parts of S. khuzistanicafrom different natural habitats in South-west Iran, results showed that the carvacrol contents in different ecotypes ranged (42.5 - 94.8 mg/ml) oil. Carvacrol and γ-terpinene were found in two oils but the amount of carvacrol in S. khuzestanica oil is higher than that of *S. hortensis* oil (Table 2).

Table 7. Comparingtested antibiotics effect on *S. aureus*.

	Clindamycin	Amikacin	Nalidixic acid	Cefalexin	Erythromycin	Gentamicin	Penicillin	Sulfamethoxazole
Zone diameter	-	14mm	10mm	-	16mm	-	-	14mm
Condition	R*	I***	I**	R	S*	R	R	I****

**** I(Semi sensitive), ***S(Sensitive), **R(Resistant).

Hassanzadeh-Khayyat *et al.* (2012), analyzed the essential oil of the aerial parts of *S. hortensis* from the Khorasan province, Northeast of Iran and twenty-one compounds were identified at which the main oil constituents were carvacrol (55.69 %), γ-terpinene (24.93 %) and p-cymene (4.07 %). Biological activity of essential oils depends on their chemical composition which is determined by the genotype and influenced by environmental and agronomic conditions. It is well known that yield and yield components of plants are determined by a series of factors, including plant genetic, climate, edaphic, elevation, and topography and also an interaction of various factors (Golparvar and Hadipanah, 2016; Ardalani *et al.*, 2017).

Previously, antimicrobial properties of essential oils from plants of the genus Satureja have been reported, although in variable degrees and spectrum of activity according to plant species and their composition.The previous study showed that essential oil and extract of *S. khuzistanica*exhibited antimicrobial activities against *Staphylococcus aureus* subsp. *aureus*, *Bacillus cereus*, *Escherichia coli*, S*almonella enterica subsp. enterica*, *Shigella flexneri*, *Candida albicans*, and *Aspergillus brasiliensis* (Hadian *et al.*, 2011; Saei-Dehkordi *et al.*, 2012; Ghodrati *et al.*, 2015; Mahboubiand Kazempour, 2017) and *Aspergillus flavus, A. niger, Penicillium* sp.*, Fusarium* sp.,*Alternaria* sp.*, Rhizopus* sp.*,* and *Mucor* sp(Sadeghi-Nejad *et al.*, 2010). The results of the antimicrobial activity of *S. hortensis* L. extract were published. The effect of the essential oil was investigated by the more or less precise disk diffusion method. It was limited to food borne pathogens (Oussalah *et al.*, 2007; Adiguzel *et al.*, 2007).

Most of the studies on the mechanism of this phenolic compound focused on its effects on cellular membranes which alters its function and, in some instances, structure, causing swelling as a result of its increased permeability. Increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, proton motive force and decreased ATP levels, resulting in the death of the cell (Ultee *et al.*, 2000). Also, synergism between carvacrol and its precursor *p*-cymene has been noted. *P-cymene* is a very weak antibacterial, and swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism p-cymene probably enables carvacrol to be more easily transported into the cell so that a synergistic effect is achieved when the two are used together. Carvacrol, which is the main component of *Satureja* species essential oils, has been considered as a biocidal, resulting in bacterial membrane perturbations that lead to leakage of intracellular ATP and potassium ions and ultimately cell death (Ultee *et al.*, 2002).Carvacrol and thymol have strong antibacterial activities against *Pseudomonas aeruginosa*, *in vitro* (Kotan *et al.*, 2013). Moreover, carvacrol exhibits antioxidant, antibacterial and antifungal activities (Ramak, 2013).

4. Conclusion

In the present work, the carvacrol, γ-terpinene and p-cymene were the major components of *S. hortensis* and *S. khuzistanica* oil. The essential oils of these Satureja species can be placed into the phenolic class with regard to their high contents of monoterpene phenols and their precursors (p-cymene and γ-terpinene). The antimicrobial effect of carvacrol is due to damage in membrane integrity with changing in pH hemostasis and also equilibrium of inorganic ions. P-cymene does not have antimicrobial activity but it increases the antimicrobial activity of carvacrol. The obtained data showed that the tested oil was active against all the tested microorganisms.

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