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Essential oil constituents and antimicrobial activities of Iranian *Satureja khuzistanica* Jamzad

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Abstract

Satureja khuzestanica Jamzad is an endemic medicinal plant that grows wild in Iran. Essential oil composition and antimicrobial activity of *S. khuzistanica* was investigated. The essential oils was obtained by hydro distillation using Clevenger and analyzed by gas chromatography/mass spectrometry (GC/MS). Forty-four components were identified in *S. khuzistanica* essential oil. The total represented 99.98% of the oil that major components were identified, carvacrol (92.16%). As study of antimicrobial effects of the essential oil of *S. khuzistanica* was against one Gram-negative bacteria (*Escherichia coli)* and yeast (*Candida albicans*) and three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus, Staphylococcus epidermidis*), in a test based on randomized complete design (RCD) factor with three replication at two method, micro-broth dilution and disc diffusion method. Results showed the highest antimicrobial activities of essential oil of *S. khuzistanica* on microorganism's was investigated. Antimicrobial activity of *S. khuzistanica* essential oil on *C.albicans* yeast more than *B. cereus* bacteria and it was less than *S. epidermidis*, *E. coli* bacterias. The inhibition zones values for bacterial strains, which the essential oils of *S. khuzestanica* were sensitive in the range of 6.34–54.4 mm and 0.097-0.390 mg/ml, showed effective the oil of *S. khuzistanica* on Gram-positive bacteria and yeast instead Gram-negative bacteria.

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Introduction

Medicinal and aromatic plants have been traditionally used as the important sources for treatment human diseases of Iran (Moalem *et al.*, 2011). The genus *Satureja* (Lamiaceae family) consists of about 235 species of herbs annual and perennials. Fiftheen species of this genus, which 10 species are endemic, are found in Iran (Rechinger, 1982). *Satureja khuzistanica* Jamzad (Marzeh-e-Khuzestani in Persian) is one of the endemic *Satureja* species that it grows in the foothills and limestone gap and the south-west part of Iran (Farsam *et al.*, 2004), The active substances of *S. khuzistanica* is essential oil that the amount varies from 1% to 5% (Alizadeh, 2013). The aerial parts of this species were used for treatment of some digestive diseases such as Acute and chronic diarrhea, as antiparasitic, carminative, Facilitates digestion and diuretic (Alizadeh, 2013). The essential oil and extract of *S. khuzistanica* have biological properties including antibacterial (Ghasemi Pirbalouti *et al.*, 2011; Saei-Dehkordi *et al.*, 2012; Skocibusic *et al.*, 2004)), antifungal (Zarrin *et al.*, 2010; Sadeghi-Nejad *et al.*, 2011; Saei-Dehkordi *et al.*, 2012), antioxidant (Abdollahi *et al.*, 2003; Rezvanifar *et al.*, 2010; Vosovgh-Ghanbari *et al.*, 2010; Saei-Dehkordi *et al.*, 2012; Hashemi *et al.*, 2012; Ahmadvand *et al.*, 2012), anti-inflammatory (Ghazanfari *et al.*, 2006), anti-diabetic (Abdollahi *et al.*, 2003; Vosovgh-Ghanbari *et al.*, 2010), anti-leishmanial (Kheirandish *et al.*, 2011; Sadeghi-Nejad *et al.*, 2011), anti-hyperlipidemic (Abdollahi *et al.*, 2003; Haeri *et al.*, 2006), anti-spasmodic (Alizadeh, 2013). Ghasemi Pirbalouti and Moalem (2013) reported that the major component in essential oil of *S. khuzistanica* was the carvacrol (90.8%). Majd *et al.*, (2008) reported that different ontogenesis conditions affected in essential oil yield in *S. khuzistanica* essential oil and the maximum essential oil yield was observed in before flowering stage. The aim of this study is essential oil composition and antimicrobial activity of *S. khuzistanica* essential oil against some important human pathogens.

Material and methods

Plant material

The aerial parts of *S. khuzestanica* were collected in full-flowering stage from Estahban, Fars province, Iran (29°632' N, 54°142' E; 1760 m above sea level). The plant driedin shade at room temperature(25°C) completely.

Essential oil extraction and analysis

Dried plant sample of *S. khuzestanica* was subjected to hydro-distillation using a Clevenger-type apparatus for3 hr. according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The essential oil obtained by dehydration of anhydrous sodium sulphate was particularly essential oil in small bottles (vials)were collected at various stages of testing before analysis of microbial andthen kept in vials at 4°C (Cruz *et al.*, 2006).

Analysis Gas chromatography (GC)

The essential oil was analyzed using into the gas chromatography(GC) model Agilent Technologies 7890Agas chromatography with a HP-5MS capillary column (30m × 0.25 mm Internal Diameter (ID) × 0.25 micron Film Thickness (FT)), Oven temperature was kept at 60 °C for 4 minand from 60 to 210 °C with Rate: 3 °C/min then increased to240 °C with Rate 20 °C/min and the final temperature kept for 8.5 min Run Time:60 min the election ionization energy was70 eV in the electronic ionization (EI); ion source temperature, 230 °C and 1µl samples were injected manually in the split ratio equal to 1:50,Helium was used as carrier gas at a flow rate of 1ml/min (Adams, 2007), The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as the mean value of injections from essential oil (Dejenane *et al.,* 2011).

Analysis Gas chromatography/mass spectrometry (GC/MS)

The essential oil was analyzed using into the gas chromatography / mass spectrometry(MS / GC) model Agilent Technologies 5975C mass selective with a HP-5MS capillary column (30m× 0.25mm Internal Diameter (ID) × 0.25 micron Film Thickness (FT) and operating under the same conditions as

above was described, the temperature was 280 ºC(Adams, 2007).

Micro-organisms

Clinical isolates of one Gram (-) *Escherichia coli*PTCC1399(ATCC 25922), one fungal *Candida albicans* ATCC 10231 BBL and 3 Gram (+) *Staphylococcus aureus* PTCC 1112 (ATCC 6538), *Staphylococcus epidermidis* PTCC 1435 (CIP81.55), *Bacillus cereus* PTCC 1247 were used in this experiment. All microorganisms strain was obtained from Microbiology Laboratory, Shiraz Medical Sciences University, Shiraz, Iran.

Preparation of standard solution

Suspensions were prepared from the tested microbes. For measure of suspension concentration was used of Spectrophotometer to model (JENWAY6305) in OD_{600}=0.1,In the case of soluble microbial population of approximately10^8 CFU/ml for bacteria and 10^6 CFU/ml (0.5 McFarland) spore for fungi strain (NCCLS , 2006), from 8 different essential oil concentrations (20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16 µl/disc) using dimethyl sulfoxide (DMSO), and the final volume of essentialoil and solvent was 20µl/disc, which were then used in the tests.

Antibacterial /antifungaltest by the disc diffusion method

In vitro antimicrobial activity of the essential oil of *S. khuzestanica* was evaluated by the disc diffusion method. Minimum inhibition concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism detected visually. Nutrient agar medium (Merck, Germany) was used to prepare the culture medium and autoclaved at 121°C for 15 min. A thin layer of bacterial suspensions were grown on medium.Sterile paper discs (6 mm in diameter) were impregnated different concentration with 20 µl essential oil and incubated at 37°C for 48 h. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC)

The minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) were performed by broth microdilution method, with were according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001), micro-dilution technique using 96-well microtiter plates. The microplates were incubated for 48h at 37 °C. The lowest concentrations without visible growth (by the binocular microscope) were defined as MICs. The minimal bactericidal or fungicidal concentration (MBC / MFC) were determined by serial subculture of 2µl into microtiter plates containing 100 µl of broth per well and further incubation 48 h at 37°C. The lowest concentration with no visible growth was defined as MBC/MFC indicating 99.5% killing of the original inoculum. Each experiment was repeated in triplicate (Najafzadeh, 2007).

Statistical analysis

All data were statistically expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA by the software SAS (version 9.2 for windows). Software, using a completely randomized design(CRD).Means of the traits were compared by Duncan'smultiple range test at $p< 0.01$ level.

Results and discussion

Chemical composition of the essential oil

The result of the chemical analysis of the oil from *S. khuzestanica* is presented in (Table 1). The oil was intensively yellow, with a characteristically strong and pleasant odor. The chemical constituents identified by GC-MS. We identified forty-four components of the *S. khuzestanica* oil, representing 99.98% of total oil. The main components of *S. khuzestanica* essential oil was carvacrol (92.16 %). Other components were present in amounts less than 2%. In our study the number of major components in *S. khuzestanica* essential oil was higher than previous reports in this plant. The results reported in previous shows, forty-three

components of the *S. khuzestanica* essential oil, representing 99.96% of total oil were identified. The major constituents of the oil were carvacrol (77.21%), γ-terpinene (6.43 %), α-Farincen (2.30%) and p-cymene (2.24%) (Shirali *et al.*, 2013). These differences in the essential oil compositions can be attributed to several environmental factors such as climatic, seasonal and geographical or ontogenesis variations (Alizadeh *et al.*, 2011). The other factors play a significant share of the results: plant harvest time (Majd *et al.*, 2008), Error in the analysis of essential oils, Time between essential oil extraction and analysis of compounds forming (Sedaghat, 2008).

Table 1. Essential oil components of *S. khuzestanica*.

No.	Compound	RI[a]	Concentration
1	α-Thujene	925	0.06
2	α-Pinene	932	0.06
3	Camphene	946	0.007
4	1-Octen-3-ol	975	0.04
5	3-Octanone	984	0.03
6	dehydro-1,8-Cineole	989	0.41
7	n-Decane	998	0.07
8	α-Phellandrene	1004	0.01
9	α-Terpinene	1015	0.09
10	p-Cymene	1022	0.50
11	Limonene	1025	0.01
12	β-Phellandrene	1026	0.02
13	1,8-Cineole	1029	0.01
14	Benzene acetaldehyde	1041	0.009
15	γ-Terpinene	1056	0.29
16	cis-Sabinene hydrate	1064	0.23
17	Terpinolene	1086	0.02
18	Linalool	1098	0.89
19	n-Nonanal	1102	0.09
20	cis-p-Menth-2-en-1-ol	1119	0.05
21	trans-p-Menth-2-en-1-ol	1137	0.03
22	Borneol	1163	0.12
23	Terpinene-4-ol	1175	0.42
24	α-Terpineol	1189	0.28
25	n-Dodecane	1197	0.27
26	Carvacrolmethy ether	1241	0.18
27	Thymol	1291	0.51
28	Carvacrol	1300	92.16
29	α-Terpinyl acetate	1347	0.06
30	Eugenol	1355	0.27
31	n-Tetradecane	1397	0.09
32	(E)-Caryophyllene	1417	0.19
33	Unknown	1441	0.07

34	Geranyl acetone	1451	0.06	
35	β-Bisabolene	1506	1.02	
36	(E)-γ-Bisabolene	1538	0.09	
37	Spathulenol	1575	0.02	
38	Caryophyllene oxide	1580	0.04	
39	n-Hexadecane	1597	0.09	
40	β-Eudesmol	1647	0.03	
41	α-Cadinol	1652	0.16	
42	α-Bisabolol	1681	0.02	
43	6,10,14-trimethyl-2-Pentadecanone	1841	0.20	
44	n-Hexadecanoic acid	1963	0.70	
	Total			99.98

RI[a], retention indices in elution order from HP-5 column.

Antimicrobial activity

The essential oil were evaluated for antimicrobial activity against pathogenic strains ofagainst3 Gram-positive bacteria *Staphylococcus aureus* PTCC 1112 (ATCC 6538), *Staphylococcus epidermidis* PTCC 1435 (CIP81.55), *Bacillus cereus* PTCC 1247 , a Gram-negative bacteria *Escherichia coli* PTCC1399 (ATCC 25922), *Candida albicans* (ATCC 10231 BBL) as yeast using the disc diffusion method. Essential oil of *S. khuzestanica* showed that *C. albicans* yeast has a high sensitivity than other Gram-positive bacteria, respectively, Gram-negative bacteria are resistant to *S. khuzestanica* oils. In the results, the inhibition zone of *E. coli* and *S. epidermidis* bacteria did not differ and same resistance against different concentrations. MIC for all microorganisms equal in 0.16 µl/disc concentrations. Which shows essential oil of *S. khuzestanica* at low concentrations is tested against microbes (Table 2).

Table 2. The mean diameter of the tested microorganisms atdifferent concentrations of essential oil of *S. khuzestanica*.

Essential oil concentration (µl/disc) microorganisms	20	10	5	2.5	1.25	0.63	0.31	0.16
Escherichia coli	40.74± 3.17^{cd}	19.48± 1.14^{klm}	17.25± 0.92^{lmn}	14.36± 0.65^{mno}	11.57± 0.31^{mnop}	9.78± 2.15^{qr}	9.24±0.69 ^{qr}	8.22± 0.31^{rstu}
Candida albicans	55.6±1.19^{a}	51.12± 0.52^{b}	36.82± 1.58^{de}	18.31± 0.83^{lmn}	14.23± 0.95^{mno}	8.13± 0.74^{rs}	8.0± 0.6 ^{rstu}	7.37± 0.31^{stuv}
Staphylococcus aureus	36.36±1.16^{de}	31.96± 1.58^{efg}	29.58±1.10^{gh}	17.59± 0.47^{lmn}	14.5± 1.09^{mno}	9.77±1.47 ^{qr}	8.96±0.37 ^{rst}	7.9±0.82 ^{stu}
Staphylococcus epidermidis	42.97±1.87^{c}	26.8±0.41^{hij}	14.46±0.65^{mno}	12.73±0.91^{mnop}	9.64±0.55^{qr}	8.6±0.26 ^{rs}	7.2±0.05 ^{stuv}	6.74±0.51^{uv}
Bacillus cereus	36.81±1.24^{de}	33.72±0.63^{ef}	32.51±0.23^{efg}	28.27±2.13^{hi}	23.53±1.57^{jk}	8.43±0.21^{rs}	7.99±0.46^{rst}	7.18±0.50^{stuv}

Each value in the table was obtained by calculating the average of three experiments ± standard deviation
Means with different letters were significantly different at the level of $p < 0.01$.

Study of *S. khuzestanica* essential oil on microorganisms shows that their interactions with each other was conducted at 1%level of Duncan test by broth micro dilution method. Significant differences were observed in antimicrobial activities on essential oils. The results are given in Tables 3 show that the lowest concentration of the MIC, the essential oil of *S. Khuzestanica* is greatest impact on Gram-positive bacteria *S. epidermis's* and *B. cereus* and Minimum Inhibitory effect of essential oils from

microorganisms between is to the Gram-negative bacteria *E. coli* also essential oil of *S. khuzestanica* in different concentrations of microbes in the test indicated that Most MBC, minimum bactericidal effect on the bacteria, The worst effect of essential oils of *S. khuzestanica* were identified on *S. epidermis* and *B. cereus* bacteria at concentrations of 25 µl/ml.The MBC lowest concentration of the bacterium is for *E. coli*, *S.aureus* bacteria and the yeast *C. albicans* that the most Bactericides and effective at low concentrations (Table 3).

Table 3. antibacterial activity of *S. khuzestanica* essential oil.

Test microorganism	MIC[a](µl/ml)	MBC[b](µl/ml)
Escherichia coli	0.19	0.19
Candida albicans	0.19	0.19
Staphylococcus aureus	0.19	0.19
Staphylococcus epidermidis	0.10	25
Bacillus cereus	0.10	25

a: Minimum InhibitoryConcentration

b: Minimum Bactericides Concentration.

Recent studies on the essential oils of many Lamiaceae show that these plants have a broad range of biological activities, notably their antimicrobial potency (Baratta *et al*., 1998), 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. khuzestanica*, could be attributed to high amount of carvacrol in essential oil. Many reasons can influence the phenolic compounds on gram-positive bacteria, Gram-negative and yeast. According to research conducted by Arvin (2003), Inhibitory effects *S. khuzestanica* oils were reported on *C. albicans* yeast 0.1 µl/ml but MBC for *E. coli*, *S. aureus* bacteria and *C. albicans* yeast were 0.20-0.26 µl/ml. The essentialoil of *S. khuzestanica* is the cell wall of yeast and bacterial species, even creating a high sensitivity to oil compounds was including carvacrol, but essential oil of *S. khuzestanica*was on *S. epidermidis* and *B. cereus* bacteria bactericide activity very weak. In previous research, Inhibitory effect of *S. khuzestanica* essential oil were reported on *E. coli* and *S. aureus* bacteria that the MIC of the *E. coli* bacteria is 2.5 µl/ml and MBC 5 µl/ml and the MIC and MBC for *S. aureus* bacteria were observed resistant 1.25 µl/ml and 2.5 µl/ml (Maghsoudlou *et al*., 2003).

It is somewhat difficult to compare results from different studies reported that Probably due to differences in methods, the antibacterial properties of essential oils, different properties of different bacterial strains and culture medium is used.

The *S. khuzestanica* oil impact on Gram-negative and Gram-positive microorganisms differ from what may be a reflection of differences in their cell wall structure. Cell wall of gram-positive bacteria, form peptidoglycan wall of several layers that a major part of it and Cell wall of Gram-negative bacteria, is a complex and multi-layered construction with lipopoly saccharide outer wall of the structure is hydrophilic. Due to the presence of abundant purines proteins pass through the small hydrophilic solutes easily But this wall as a barrier against hydrophobic compounds is a large molecule and Since most of the compounds in the oil are hydrophobic compounds So these are not easily able to pass through walls, That is why it is resistant Gram-negative bacteria than Gram-positive bacteria.

Conclusion

In the present study we demonstrated, Savory species because of carvacrol important, According to the results and the unique properties of carvacrol savory plants, cultivation of this Savory specie to extract the

valuable combination is better, Savory plant southwest province as having the best selection of high carvacrol in the oil and savory alternative are to species that endemic to Iran.

References Abdollahi M, Salehnia A, Mortazavi SH, Ebrahimi M, Shafiee A, Fouladian F, Keshavarz K, Sorouri S, Khorasani R, Kazemi A. 2003. Antioxidant, antidiabetic, anti-hyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Saturejakhuzestanica* in rat in vivo :a toxicopharmacological study .Med .Sci .Monitoring **9,** 331-335.

Adams RP. 2007. Identification of essential oil components by gas chromatography /mass spectrometeryAllured Publishing Corporation, Carol Stream, IL, 4th Ed, 456.

Ahmadvand H, Tavafic M, Khalatbary AR. 2012. Hepatoprotective and hypolipidemic effects of *SaturejaKhuzestanica* essential oil in alloxan-induced type 1 diabetic rats . Iranian Journal of Pharmaceutical Research **11,** 1219-1226.

Alizadeh A, Alizadeh O, Sharafzadeh SH, Mansoori S. 2011. Effect of different ecological environments on growth and active substances of garden thyme. Advances in Environmental Biology **5,** 780-783.

Alizadeh A. 2013. Iranian endemic medicinal plants. Estahban Branch, Islamic Azad University. Lecture notes.

Arvin A. 2003. Phytochemistry, Effects of logging debris and antibacterial and antifungal activity of Saturejakhuzestanicaisnative already has. Faculty of Pharmacy.Medical Sciences University. Tehran branch. Dissertation **1382,** 4360.

Baratta MT, Dorman HJD, Deans SG, Figueiredo AC, Barroso JG, Ruberto G. 1998. Antibacterial and anti-oxidant properties of some commercial essential oils. Flavor and Fragrance Journal **13,** 235-244. http://dx.doi.org/10.1002/(SICI)10991026(1998070)

Cruz T, Cabo M, Castillo M. 2006. The spasmolytic activity of the essential oil of *Thymus baeticus*boiss in rats. Phytotherapy Research **7,** 92. http://dx.doi.org/10.1002/ptr.2650030308

Djenane D, Yangüela J, Montañés L, Djerbal M, Roncalés P. 2011. Antimicrobial activity of Pistacialentiscus and Saturejamontana essential oils against Listeria monocytogenes CECT 935 using laboratory media: Efficacy and synergistic potential in minced beef. Food Control **22,** 1046-1053. http://dx.doi.org/10.1016/j.foodcont.2010.12.015

Farsam H, Amanlou M, Radpour MR, Salehinia AN, Shafiee A. 2004. Composition of the essential oils of wild and cultivated SatureujakhuzistanicaJamzad from Iran. Flavor and Fragrance Journal **19,** 308-10.

GhasemiPirbalouti A, Hamedi B, Malek Poor F, Rahimi E, NasriNejhad R .2011. Inhibitory activity of Iranian endemic medicinal plants against *Vibrio parahaemolyticus* and *Vibrio harveyi* . Journal of Medicinal Plants Research **5,** 7049-7053. http://dx.doi.org/10.5897/JMPR11.1256

Ghasemi Pirbalouti A, Rahimmalek M, Malekpoor F, Karimi A. 2011. Variation in antibacterial activity, thymol and carvacrol contents of wild populations of *Thymus daenensis*subsp . *daenensis*Celak .Plant Omics Journal **4,** 209–214 .

GhasemiPirbalouti A, Moalem E. 2013. Variation in antibacterial activity of different ecotypes of *SaturejakhuzestanicaJamzad*, as an Iranian endemic plant. Indian Journal of Traditional Knowledge **12(4),** 623-629.

Ghazanfari GH, Minaie B, Yasa N, Nakhai L, Mohammadirad A, Nikfar SH, Dehghan GH, Boushehri V, Jamshidi H, Khorasani R, Salehnia A, Abdollahi M. 2006. Biochemical and

histopathological evidences for beneficial effects of *Saturejakhuzestanica*Jamzad essential oil on the mouse model of inflammatory bowel diseases . Toxicology Mech. Methods **16,** 365-372.
http://dx.doi.org/10.1080/15376520600620125

Haeri S, Minaie B, Amin GH, Nikfar SH, Khorasani R, Esmaily H, Salehnia A, Abdollahi M. 2006. Effect of *Satureja khuzestanica* essential oil on male rat fertility, Fitoterapia **77,** 495-499.
http://dx.doi.org/10.1016/j.fitote.2006.05.025

Kheirandish F, Delfan B, Farhadi S. 2011. The effect of *Satureja khuzestanica* essential oil on the lesions induced by *Leishmania major* in Balb/c mice. African Joutnal of Pharm Phara **5**, 648–653.
http://dx.doi.org/10.5897/AJPP11.130

Maghsoudlou1 Y, Asgharpoor A, Ariaiee P. 2013. Effect of *Satureja khosestanica* essential oil on bacterial, chemical and sensory properties of frankfurter sausages. JRIFST **2(3),** 279-294.

Majd A, NejadSattari T, KhavdriNejad RA, Doosti B. 2008. Quantitative and qualitative changes Pharmaceutical Ingredients manufacturer of essential oil of Khuzestan Savory (*Satureja khuzestanica* J) Antimicrobial properties of plant essential oils during the development of the situation in vitro. Journal of Sciences, Islamic Azad University (JSIAU) **18 (1/70),** 51-60.

Moallem E, GhasemiPirbalouti A, Yousef-Naanaie S. 2011. Micro-morphology of fruit and pollen in *Satureja khuzistanica* Jamzad . Journal Herbal Drugs **2,** 193-201.

Najafzadeh H. 2007. Antibiotics and antimicrobial drugs (basic concepts and clinical applications). ahvaz.trava Publications1.

NCCLS–National Committee for Clinical Laboratory Standards. 2001. Performance standards for anti-microbial susceptibility testing: eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standard, Wayne, PA, USA.

NCCLS/CLSI (National Committee for Clinical Laboratory Standards). 2006. Performance standards for antimicrobial disk susceptibility tests .Approved standard, document M2-A9.

Rechinger KH. 1982. *Satureja* .Flora Desiranischen Hoclandes and der Umrahmenden Gebirge. Akademische DrukuVerlagsAntalt Graz, Austria **150**, 495–504.

Rezvanifar MA, Farshid AA, Sadrkhanlou RA, Ahmadi A, Rezvanfar MA, Salehnia A, Abdollahi M. 2010. Benefit of *Saturejakhuzestanica* in subchronically rat model of cyclophosphamide-induced hemorrhagic cystitis .Exp Mol Pathol **62,** 323–330.
http://dx.doi.org/10.1016/j.etp.2009.05.005.

Sadeghi-Nejad B, Saki J, Khademvatan S, Nanaei S. 2011. In vitro antileishmanial activity of the medicinal plant *Satureja khuzestanica* Jamzad . Journal Medicinal Plants Research **5,** 5912-5915.

Saei-Dehkordi S, Fallah AA, Heidari-Nasirabadi M, Moradi M. 2012. Chemical composition, antioxidative capacity and interactive antimicrobial potency of *Satureja khuzestanica* Jamzad essential oil and antimicrobial agents against selected food-related microorganisms .Internatinal Journal Food Scieance Technology **47,** 1579–1585.
http://dx.doi.org/10.1111/j.1365-2621.2012.03006.x

Sedaghat S. 2008. Essential oil chemistry. Tehran North Branch. Islamic Azad University. P 163.

Shirali R, BabadaeiSaman R, Alizadeh A. 2013. Essential oil composition and antimicrobial activity of medicinal plant of *Satureja khuzistanica* Jamzad. The first national conference on the use of medicinal plants and traditional medicine in life style Torbat Heydarieh University. November 27.

Skocibusic M, Bezic N, Dunkic V. 2004 . Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis .growing in Croatia, Food Chemistry **21(6),**164.
http://dx.doi.org/10.1016/j.foodchem.2005.01.051

Vosovgh-Ghanbari S, Rahimi R, Kharabaf S, Zeinali S, Mohammadirad A, Amini S, Yasa N, Salehnia A, Toliat T, Nikfar S, Larijani B, Abdollahi M. 2010. Effects of *Satureja khuzestanica* on serum glucose, lipids and Markers of oxidative stress in patients with type 2 diabetes mellitus :A double -bind randomized controlled trial . Evidence Based Complement and Alternate Medicin7, 465-470.
http://dx.doi.org/10.1093/ecam/nen018

Zarrin M, Amirrajab N, Sadeghi- Nejad B. 2010. In vitro antifungal activity of *Saturejakhuzestanica* .Pakistan Journal of Medical Sciences **26,** 880-882.