Comparison of Chemical Compounds and Antioxidant and Antibacterial Properties of Various *Satureja* Species Growing Wild in Iran

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Received: 21 Apr. 2016 **Accepted:** 12 June 2016

Abstract

Background: Many members of the genus *Satureja* L. (Lamiaceae) have interesting pharmacological and biological properties.

Objective: In this research, major essential oil (EO) compositions, rosmarinic acid (RA) content, and antioxidant and antibacterial properties of the leaf extracts of nine *Satureja* species (*S. bachtiarica, S. mutica*, *S. sahandica, S. macrantha, S. atropatana, S. edmondi, S. spicigera, S. isophylla* and *S. intermedia*) were assessed.

Methods: The chemical composition of EO was determined using Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC-MS) analysis. RA content of the extract was detected using a High-Performance Thin-Layer Chromatography (HPTLC) method, and the 2,2-diphenyl-2-picrylhydracyl hydrate (DPPH) radical scavenging procedure was used to study of antioxidant capacity.

Results: According to the analytical results, EO of the most samples were mainly composed of *p*-cymene, γ-terpinene, thymol, and carvacrol. There was a wide variability for RA content among the studied species ranging from 0.03 mg g^{-1} RA (*S. sahandica*) to 1.11 mg g^{-1} RA (*S. isophylla*). The lowest IC50 in the radical scavenging assay was shown by *S. sahandica* extracts as compared to the other samples. This activity was followed by *S. spicigera* and *S. edmondi*. The minimum inhibitory concentration (MIC) varied from 0.06 to 32 mg ml^{-1}, however, the minimum bactericidal concentration (MBC) value differed from 0.06 to >64 mg ml^{-1}. Moreover, *S. sahandica* revealed the lowest inhibitory activity against all microorganisms tested particularly on *E. coli.*

Conclusion: In conclusion, the extracts of certain *Satureja* species have the potential to be considered as alternatives for synthetic food preservatives and antibiotics.

Keywords: ***Satutreja* sp., Antibacterial, Antioxidant, Extracts, Rosmarinic acid**

Introduction

The genus *Satureja* L. (Lamiaceae) comprises approximately 200 species of herbs, shrubs and mainly aromatic plants with wide distribution in the Mediterranean area, Asia and boreal America [1]. Many of them are used as flavouring agents and for medicinal purposes. In Iranian folk medicine, for example, the aerial parts of some *Satureja* species are used to treat various diseases such as gastroenteritis, upper respiratory tract infections, urinary tract infections, diarrhoea and wounds [2]. Therefore, there is a great interest in continuing researches on the extracts of these plants from points of view of the chemical compounds to biological properties.

Lamiaceae species are known to produce a diverse array of secondary metabolites, such as volatile and non-volatile terpenes, hydroxybenzoates, hydroxycinnamates and flavonoids, among others. Also, many members of the genus *Satureja* have aromatic and medicinal properties. The essential oil composition and antimicrobial activity of some *Satureja* species has been previously studied [3, 4, 5]. These studies revealed that *Satureja* species have antimicrobial activity against human, food and plant pathogens due to the presence of phenolic components such as thymol and carvacrol.

In recent years, antimicrobial and antioxidant activity of different spices have been demonstrated and demands for these natural food preservatives have increased in modern food industry [6, 7]. Moreover, the discovery of natural antimicrobials, especially those of plant origin, has become a worldwide trend. This is not only due to development of microbial resistance against conventional food preservatives but also increasing awareness of their residual toxicity or possible side effects [8].

Up to now, many studies have performed on various s pecies of *Satureja* based on chemical compositions and biological activities as seperately. For example, It has been reported that chemical composition of the essential oils of *S. mutica*, collected from Khorasan proviance are carvacrol (30.9%), thymol (26.5%), γ-terpinene (14.9%), and *p*-cymene (10.3%) [9]. Gohari et al. [10] also reported thymol (62.6%), *p*-cymene (9.4%), and carvacrol (6.6%) were the main components of *S. mutica* from Guilan province. Previous studies have shown that the main constituents of the essential oils of eight populations of *S. sahendica* were thymol (19.6 – 41.7%), *p*-cymene (32.5–54.9%) and γ-terpinene (1.0–12.8%) [11]. According to Rustaiyan et al. [12] thirty-nine components were identified in the essential oil of *S. mutica* plants grown in Iran, comprising 95.1% of the total essential oil. Menthol (37.4%), menthone (17.2%) and 1,8-cineol (9.3%) were the main components in the essential oils.

However, in this experiment the major essential oils components and rosmarinic acid content, as well as antioxidant and antibacterial efficacy of the extracts from nine *Satureja* species growing wild in Iran were assessed against four Gram-positive and Gram-negative pathogenic bacteria.

Materials and Methods

Plant materials

Aerial parts of the nine *Satureja* species including *S. bachtiarica*, *S. mutica*, *S. sahandica*,

S. macrantha, *S. atropatana*, *S. edmondi*, *S. spicigera*, *S. isophylla* and *S. intermedia* were collected from the wild growing plants in natural habitats (Table 1) at the full flowering stage. Voucher specimens of all species were deposited at the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran (Table 1).

Isolation of essential oils

The essential oil of all air-dried samples (100 g) was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus accordingto the method recommended in British Pharmacopoeia [13]. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 ˚C until analysis.

GC and GC/MS analysis

GC analysis was performed by using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out using fused silica capillary DB-1 column (60 m × 0.25 mm; film thickness 0.25 µm). The operating conditions were as follows: injector and detector temperatures were 250°C and 300°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL/min; oven temperature programmes, 60°–250°C at the rate of 5°C/min, and finally held isothermally for 10 min.

GC/MS analysis was performed by using Thermoquest-Finnigan gas chromatograph equipped with above mentioned column, used under the same conditions as above and coupled to a TRACE mass. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200°C and 250°C, respectively. Mass range was from *m/z* 43–456. Gas chromatographic conditions were as given for GC.

Identification of compounds

The constituents of the oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6-C24) and the oil on a DB-1 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [14]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Table 1- Geographical origin of different tested *Satureja* species.

species	Voucher number	Habitat	Altitude (m)	Longitude (E)	Latitude (N)
S. sahandica *	TARI 83144	Zanjan, Garmab, Ghar-e-Katal khor	1692	46° 08′ 45″	35° 32′ 06″
*S. intermedia**	TARI24346	Gilan, Khalkhal, Gardan-e-almas	2803	48° 68′ 22″	37° 56′ 15″
S. mutica	TARI 78411	Khorasan-e-Jonobi, Bojnoord	1210	57° 52′ 51″	38° 20′ 24″
*S. isophylla**	TARI43677	Mazandaran, Valad, Niknamdeh	1678	58° 42′ 29″	34° 23′ 12″
*S.atropatana**	TARI76973	Azarbayjan-e-Sharghi, Tabriz	1365	46° 16′ 25″	38° 05′ 46″
*S. bachtiarica**	TARI69555	Fars, sepidan	1754	52° 29′ 51″	29° 38′ 06″
S. spicigera	TARI 78410	Gilan, Roostamabad	189	49° 24′ 57″	36° 48′ 34″
S. edmondi	TARI75585	Ilam, Baneroshan	1382	46° 24′ 55″	33° 38′ 05″
S. macrantha	TARI78409	Azarbayjan-e-Gharbi, Uromieh	1344	45° 04′ 43″	37° 32′ 59″

* Endemic to Iran

Preparation of the methanol extracts

The air-dried and finely powdered 500 mg shoot tissues were Soxhlet-extracted as described by Van Den Berghe et al. [15]. Plant samples from each treatment were separately soaked in 5 ml of methanol (90 %) at 50 ˚C for 8 h. Then, obtained solution was filtered through Whatman filter paper and concentrated by a rotary evaporator at 45 ˚C for 5 min. The yields of extracts were calculated by dividing the dry weight of the extract filtrate by the dry weight of the plant sample.

Rosmarinic acid measurement

Rosmarinic acid content of *Satureja* species extracts was determined using a High-Performance Thin-Layer Chromatography (HPTLC) method [16].

Briefly, Separation was performed on 20 cm × 10 cm silica gel TLC Aluminum plates with fluorescence indicator F254 (Merck, Darmstadt, Germany) with toluene-ethyl acetate- formic acid (5:4:1) as mobile phase. Sample solutions for HPTLC analysis were applied by means of a CAMAG Linomat. The determination was carried out by using the densitometry absorbance mode at 329 nm using a CAMAG TLC Scanner 3. The amount of rosmarinic acid in *Satureja* species extracts were determined according to a calibration curve (Y=92.24 X×1154.1) with 0.9903 as correlation coefficient. The linearity of the method was in the range of 5–400µg/mL. LOD and LOQ values were 1.57 and 4.80 µg/mL.

Antioxidant activity, DPPH assay

Radical scavenging activity of *Satureja* species extracts was determined using a published 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay method [17] with minor modifications. Briefly, stock solutions (10 mg/ml each) of the extracts and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions are made to obtain concentrations ranging from 1 to 5 $\times 10^{-10}$ mg/ml. Diluted solutions (2 ml each) were mixed with 2 ml of freshly prepared 80 mg/ml DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. Ultraviolet (UV) absorbencies of these solutions were recorded on a spectrometer at 517 nm using a blank containing the same concentration of oils or extracts or BHT without DPPH. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$I\% = [(A_{blank} \_ A_{sample})/A_{blank}] \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

Bacterial strains and antibacterial assays

The *in vitro* antibacterial activity of nine *Satureja* extracts were assessed against the following bacterial strains: *Stapylococcus aureus* (PTCC1431) and *Bacillus cereus* (PTCC 1015) as Gram-positive and *Eschericha coli* (PTCC1399) and *Pseudomonas aeruginosa* (PTCC1430) as Gram-negative bacteria.

Antibacterial activity of each extract was assessed by broth dilution susceptibility tests. Broth micro-dilution method was carried out according to the standard protocols recommended by National Committee for Clinical Laboratory Standards (NCCLS) [18] with some modifications to determine the minimum concentration of each antimicrobial agent required for inhibition (MIC) of visible growth of tested bacterium.

Briefly, two-fold serial dilutions of each extract were prepared in a concentration range of 0.03-64 mg/ml in 96 wells trays containing MHB. Then inoculants of each bacterial strain were prepared from freshly cultured bacteria that were adjusted to 0.5 McFarland standard turbidity using sterile normal saline, and were further diluted (1:100) by sterile Mueller-Hinton broth (MHB) just before adding to the microplates containing a serial dilution of each extracts. MICs were recorded after 20 hrs incubation at 37 °C. Resazurin was used as indicator of bacterial growth in the case of any turbidity which could not be refer to the bacterial growth easily and accurately.

Minimum bactericidal concentrations (MBC) were determined by sub-culturing of 100 µL from each negative well and from the positive growth control onto a nutrient agar plate. MBCs were defined as the lowest concentration that could kill 99.9 % of the test strains.

Chloramphenicol was used as standard antibiotic and all experiments were done in triplicate.

Statistical analysis

Data analysis of variance was performed by ANOVA using SAS (version 9.2 for windows) software. Significant differences between means were determined by Duncan new multiple-range test at the level of $P < 0.05$. Relationships among samples and species were investigated by principal component analysis (PCA). PCA was performed using SPSS statistics software. Mean values were used to create a correlation matrix from which standardized principal component (PC) scores were extracted the dendrogram was constructed through SAHN clustering program using the unweighted pair group method using arithmetic means (UPGMA). All parameter values tested were obtained by calculating the average of three replicates ± SD (standard deviation).

Results

Major essential oil constituents

According to the results of GC and GC-MS analysis, all essential oils samples consisted of four major constituents including *p*-cymene, γ-terpinene, thymol, and carvacrol (Table 2).

As shown, the highest content of *p*-cymene (38.3 %), γ-terpinene (12.2 %), thymol (62.1%), and carvacrol (29.3%) were achieved in essential oil obtained from *S. sahandica*, *S. spicigera*, *S. atropatana*, and *S. mutica*, respectively. Also, the other major constituents of essential oil including caryophyllene oxide (26.4% in *S. spicigera*), borneol (11.6% in *S. bachtiarica*) and linalool (13.1% in *S. spicigera*) were belongs to terpenoid phenols. Interestingly, α-eudesmol was the sole compound present in appreciable amount (47.7%) in the essential oil of *S. isophylla* so that did not detect in other species. The other main constituents of essential oil in studied *Satureja* species were presented in table 2.

Table 2- Major essential oil content (%) of different *Satureja* species

species	*p*-cymene	γ-terpinene	thymol	carvacrol	β-eudesmol	α-eudesmol	linalool	borneol	caryophylene oxide	elemicin	spathulenol
S. mutica	15.14	3.67	29.78	29.35	-	-	-	0.39	4.0	-	0.17
S. sahandica	38.34	4.94	23.47	6.47	-	-	1.56	0.73	4.73	-	0.85
S. macrantha	25.8	0.6	8.1	0.4	0.2	-	-	1.3	0.7	-	2.2
S. atropatana	6.1	3.3	62.1	1.5	-	-	-	1.0	-	-	5.2
S. edmondi	36.1	4.7	8.2	6.0	-	-	1.8	7.2	4.3	-	3.3
S. bakhtiarica	1.47	0.1	27.99	13.21	-	-	9.61	11.64	16.96	-	2.36
S. spicigera	11.12	12.29	18.23	18.53	-	-	13.12	-	26.45	-	26.26
S. isophylla	1.3	3.78	0.1	0.1	9.19	47.77	-	-	0.65	-	-
S. intermedia	14.7	3.3	32.3	1.0	-	-	-	0.1	0.1	4.8	0.2

Rosmarinic acid content

The content of rosmarinic acid for methanol extracts of all *Satureja* species is presented in Table 3. According to the results obtained, all samples were principally comprised of rosmarinic acid. The maximum (1.11 mg g^{-1} RA) and minimum (0.03 mg g^{-1} RA) rosmarinic acid contents were observed in *S. isophylla* and *S. sahandica*, respectively. In addition, rosmarinic acid content of other species ranged from 0.09 to 7.54 mg g^{-1} RA (Table 3).

Cluster analysis and scatter plot

UPGMA cluster analysis based on Euclidean distances from main essential oil compositions matrix grouped the studied nine species into two main clusters including A and B (Figure 1), each representing a chemotype. The first cluster (A) formed by eight species (*S. bachtiarica, S. mutica*, *S. sahandica, S. macrantha, S. atropatana, S. edmondi, S. spicigera,* and *S. intermedia*) and the second cluster (B) formed by one species namely *S. isophylla*. The dendrogram showed that *S. isophylla* was set apart from all other studied species because its major essential oil constituents were β-eudesmol and α-eudesmol. Also, *S. atropatana* formed sub-individual cluster (A_1) separating from other sub-individuals of A group (A_2), mainly due to high level of thymol. Species *S. mutica* and *S. sahandica* were the most closest among all the other species because their major essential oil compounds (e.g. γ-terpinene, thymol, β-eudesmol, α-eudesmol, borneol, elemicin, spathulenol, caryophylene oxide) were the roughly same. Also, biplot created according to the PC1 and PC2 reflected relationships among the studied species. Results of biplot supported the results of UPGMA cluster analysis, in which species were clustered into two groups according to chemotype properties (Figure 2).

Table 3 - Rosmarinic acid (RA) content and antioxidant capacities of different *Satureja* species.

Source of plant extracts	Rosmarinic acid content (mg g^{-1} RA)	Antioxidant activity IC_{50} value (µg ml^{-1})
S. mutica	0.15±0.02	26.54±1.1
S. sahandica	0.03±0.01	1.43±0.1
S. macrantha	0.09±0.02	27.82±0.9
S. atropatana	0.27±0.04	28.12±1.2
S. edmondi	0.29±0.02	20.58±0.4
S. bachtiarica	0.75±0.08	25.87±0.5
S spicigera	0.73±0.07	3.287±0.1
S. isophylla	1.11±0.09	22.55±0.6
S. intermedia	0.33± 0.04	26.32±0.8
Standard antioxidant (BHT*)		23.65±0.4

* BHT; Butylated hydroxytoluene. Values expressed are means ± SD (standard deviation) of three parallel experiments.

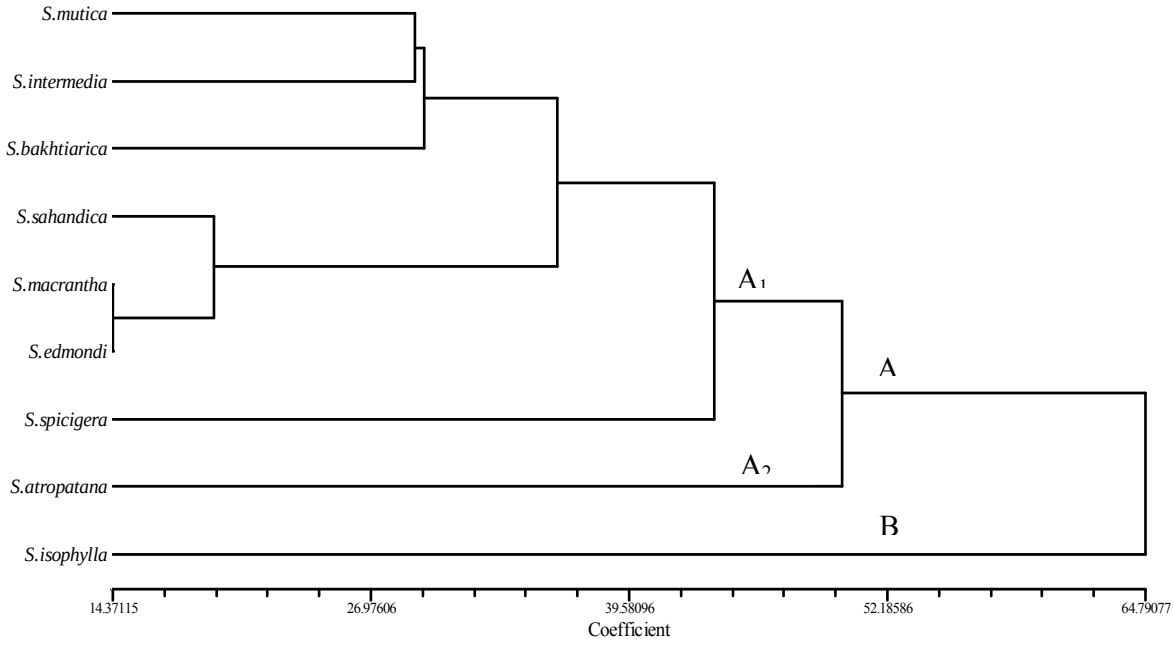

Figure 1- The UPGMA dendrogram for different studied *Satureja* species based on major essential oil compounds using Euclidean distance.

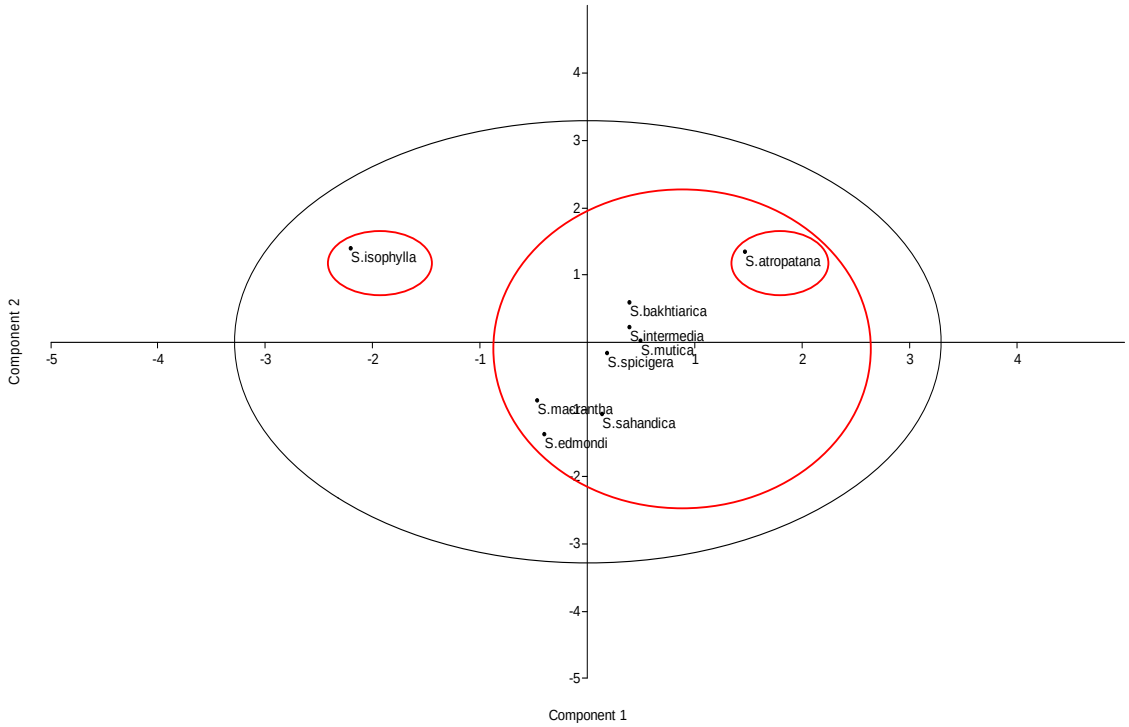

Figure 2- Biplot of the first two principle components (PCs) for different studied *Satureja* species based on major essential oil compounds.

Antioxidant capacity

Free radical scavenging capacities of the corresponding extracts were determined based on their ability to quench the synthetic 2, 2-diphenyl-1-picryl hydrazyl (DPPH), and the results are shown in Table 3. The IC_{50} value was defined as the concentration of sample that scavenging 50% of the DPPH radical. Since IC_{50} is the amount of *Satureja* extract need to reduce 50% the amount of free radical of DPPH solution, lower values indicate higher antioxidant potential. According to the results obtained, *S. sahandica* extracts was found the most active ones with an IC_{50} value of 1.43 µg ml^{-1}. This activity was followed by *S. spicigera* and *S. edmondi* with the IC_{50} values of 3.28 and 20.58 µg ml^{-1}, respectively. The IC_{50} value of synthetic antioxidant, butylated hydroxytoluene (BHT), was also determined in parallel experiment. Samples from *S. isophylla* showed similar activity to the positive standard; however, none of other samples exhibited activity as strong as the standard (Table 3). The current study showed that some of Iranian *Satureja* species including *S. sahandica* and *S. spicigera* have strong radical scavengers, which could be considered as a good source of natural antioxidant for pharmaceuticals, food and medicinal purposes.

Antibacterial activity

The methanol extracts of employed *Satureja* species were tested *in vitro* for antibacterial activities using micro-dilution method, and the results are given in Table 4. As can be seen, all extracts exhibited significant inhibitory activity against both Gram-positive and Gram-negative bacterial strains, although in different degrees. The maximum activity of the all extract was observed against *Bacillus cereus* with a range of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values from 0.06 to 4 mg ml^{-1}, therefore, this pathogenic bacterium was found as the susceptible ones. Also, the extract of *S. bachtiarica* showed higher activity than other eight *Satureja* species against *Bacillus cereus*. However, *S. sahandica* sample revealed the lowest inhibitory activity against all microorganisms tested particularly on *Eschericha coli* with the MIC and MBC values of 16 and higher than 64 mg ml^{-1}, respectively. On the other hand, *Eschericha coli* with the highest MIC and MBC values was found as the most resistant bacterium followed by *Pseudomonas aeruginosa*, *Stapylococcus aureus* and *Bacillus cereus*. Generally, the extracts of *S.bachtiarica* and *S. mutica* appeared to be more active on both type of Gram positive and Gram negative bacteria than those of other species (table 4).

Table 4- Antibacterial activities of the leaf extracts in different *Satureja* species.

Source of plant extracts	Pathogenic bacteria							
	***Stapylococcus aureus* *PTCC*1431**		***Eschericha coli* *PTCC*1399**		***Pseudomonas aeruginosa* *PTCC*1430**		***Bacillus cereus* *PTCC*1015**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
S. mutica	1	2	4	8	4	8	0.5	0.5
S. sahandica	4	8	16	>64	8	32	4	4
S. macrantha	4	4	8	16	4	16	2	2

Table 4- Continued

Source of plant extracts	Pathogenic bacteria							
	*Stapylococcus aureus PTCC*1431		*Eschericha coli PTCC*1399		*Pseudomonas aeruginosa PTCC*1430		*Bacillus cereus PTCC*1015	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. atropatana	2	2	8	32	4	16	0.5	1
S. edmondi	2	2	8	32	8	16	0.5	0.5
S. bachtiarica	2	2	16	16	2	16	0.06	0.06
S spicigera	2	4	4	16	4	16	0.5	0.5
S. isophylla	1	2	16	32	2	32	1	1
S. intermedia	1	2	8	16	4	32	2	2

The values indicate minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), expressed as μg ml^{-1}.

Discussion

In our experiment, there was a high variability in chemical compounds of different studied *Satureja* species. Variation in biological and chemical characters of *Satureja* species from different origins has been previously reported [4, 19, 20, 21], in which many different chemotypes can be detected.

In the present study, eleven compounds including *p*-cymene, γ-terpinene, thymol, carvacrol, β-eudesmol, α-eudesmol, linalool, borneol, caryophylene oxide, elemicin and spathulenol were the major constituents comprising about 98.5 % of essential oil in studied *Satureja* species. These compounds have been reported as major components of essential oil in related species growing wild in other regions such as *S. bachtiarica* [22], *S. hortensis* [23], *S. khuzistanica* [24], *S. montana* [25] and S. *pilosa* [5]. Also, Menthone (24.2% and 35.7%), isomenthone (29.7% and 25.1%) and pulegone (10.7% and 8.7%) were identified as the major compounds of two *Satureja* species (*S. boliviana* and *S. brevicalix*, respectively) growing in Peru [1].

Various species of the genus *Satureja* have been investigated for the essential oil variability [26]. In this investigation on thirty accessions of *S. hortensis*, twenty nine components were identified in the essential oils, and carvacrol (42.0-83.3%), γ-terpinene (0.5-28.5%), and *p*-cymene (1.0-17.1%) were the major components.

Analysis of the essential oils of 69 sampled individuals from seven populations of *S. khuzistanica* showed that all samples have the high percentage of carvacrol (89.59-95.41%) as main component [24].

In a sudy by Karimi et al. [27], on *S. mutica* population from north of Iran, Thymol (6.5-74.6%), carvacrol (0.9-70.4%), borneol (0.1-10.9%), *p*-cymene (0.30-14.2%), and γ-terpinene (0.1–9.9%) were identified as the major predominant constituents of essential oils for the studied individual plants.

It is acknowledged that variability in the essential oil constituents of medicinal and aromatic plants as well as their biological activities may be related to parameters such as genetic factors, geographical origin, developmental stage, climatic conditions, etc. [3, 24, 28].

According to the literature, carvacrol, thymol, and their precursors, *p*-cymene and γ-

terpinene are the major components of essential oil in various *Satureja* species. 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). Carvacrol has a wide range of activities including antimicrobial, antioxidant, anticandidal, and anti-inflammatory properties [29, 30].

Although there is no information on the mode of inheritance of carvacrol and thymol in *Satureja* species, it has been reported that biosynthesis of these two phenolic monoterpenes in Thymus vulgaris is controlled by an epistatic series of several biosynthetic loci [31]. However, biosynthetic pathway of carvacrol and thymol seems to be different and less complicated. It has been reported that the essential oil and carvacrol content of *Satureja* is genetically controlled [21].

Rosmarinic acid as a natural phenolic compound contains two phenolic rings, both of which have two ortho-position hydroxyl groups. In Lamiaceae family it is present in the subfamily Nepetoideae in different concentrations [32].

Tepe and Sokmen [33] reported a concentration of 2.5% rosmarinic acid in ethanolic extract of wild *S. hortensis*. Gabor et al. [34] reported rosmarinic acid content of several species belongs to Nepetoideae family varied between 0.001% and 0.93% and it was 0.26% for *S. montana* L. Also, Hadian et al. [4] reported that rosmarinic acid content of *S. hortensis* varied between 0.06 and 0.69%. Also, rosmarinic acid content of *S. khuzistanica* was reported with range of 0.59-1.81%. Zgorka and Glowniak [35] determined the concentration of rosmarinic acid in several Lamiaceae species. The highest concentrations were observed in the aerial parts of *S. hortensis* (1.2%) and *Ocimum basilicum* (1.1%), as well as in the leaves of Melisia officinalis (1.0%) and Rosmarinus officinalis (0.7%). Skoula et al. [36] reported genetic variation of volatiles and rosmarinic acid in populations of *Salvia fruticosa*. It has been reported that the rosmarinic acid content of plant extracts varied depending on the species, genotype, growth stage, and environmental conditions. The multifunctional caffeic acid ester, rosmarinic acid, is known to have astringent, antioxidant, anti-inflammatory, antimutagenic, antibacterial, and antiviral activities [32].

We showed, using DPPH method, a high radical scavenging activity in the methanol extract similar to the synthetic compound BHT. The DPPH method usually is on the base of hydrogen atom transfer reaction. Synthetic antioxidants such as BHT, butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate have been widely used around the world for decades. However, they are being scrutinized for possible toxic and carcinogenic effects. As a result, an intense new area of research has been developed concerning the search for identification and characterization of naturally occurring antioxidants. Natural antioxidants are more ideal as food additives, not only for

their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus they are more readily acceptable to the modern consumers. Similar to *Satureja* species numerous aromatic, spicy and medicinal plants have been reported for their antioxidative potential [37, 38].

Several groups of secondary metabolites in higher plants have the capacity to react as antioxidants, and a primary group of antioxidants is the phenolics [39]. Also, it has been reported that rosmarinic acid is considered as a defense compound against pathogens and herbivores and has been revealed several biological activities, the most important one a high antioxidant capacity [40]. However, in present study we did not observe any correlation between rosmarinic acid content and antioxidant activity of the extracts; it could be due to the fact that other metabolites contribute in antioxidative processes. The biological activities, flavor and other features of crude extracts and essential oils depend on concentration of components present and overall composition. Comparing with the results of other studies, antioxidant activity of essential oil of some *Satureja* species including *S. bachtiarica* [41], S. *hortensis* [42], *S. Montana and S. cuneifolia* [43] has been previously reported. Moreover, differences observed in the chemical profile and antioxidant activity of medicinal plants is influenced by various parameters such as genetic factors, geographical origin and climatic conditions. The cardinal mode of action of natural antioxidants is their ability to scavenge free radicals before they can initiate free radical chain reactions in cellular membranes or lipid-rich matrices in foodstuffs, cosmetics or pharmaceutical preparations [44].

To date, little has been reported concerning the antimicrobial activities of plant extracts to compare with our current study. However, antimicrobial activity of essential oil of some *Satureja* species on pathogenic bacteria or fungi or other types of microorganisms has been previously studied. Sonboli et al. [45] reported that the antimicrobial activity of essential oil of *S. laxiflora* which thymol was the main component of the essential oil proposed to be responsible for its antimicrobial activity.

The effect of *S. montana* and *S. subspicata* oils were also reported and essential oils extracted from *S. spicigera*, *S. biflora*, *S. masukensis* and *S. pseudosimensis* showed a high inhibition against a wide range of microorganisms [46, 47]. It has been proposed that the essential oils which are rich in phenolic compounds may exert a high antimicrobial activity [48].

In the present study, the extracts of *S. bachtiarica* and *S. mutica* appeared to be more active on both type of bacteria, Gram positive and Gram negative, than those of other species, which was in consistent with those reported by Hadian et al. [24]. Confirming previous reports, we found that the strength and spectrum of activity varied among investigated *Satureja* species and type of bacteria. Thus, *Satureja* species extracts could be considered as potential candidate for raw material antibacterial phyto-preparations. 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 [24]. This was extended in this study to the extracts of plants of the genus *Satureja*. As evident from table 4, the Gram-negative bacteria showed more resistance than the other type towards all the extracts tested, which attributed to the hydrophilic cell wall structure of these microorganisms.

Conclusion

There was a wide variability in essential oil and rosmarinic acid contents of different tested *Satureja* species. From all findings in present investigation, the methanolic extract of some *Satureja* species including *S. bachtiarica*, *S. sahandica* and *S. spicigera*, showed good antibacterial and antioxidant activity as well as high chemical contents. Also, data indicate the possibility of *Satureja* extracts as an efficient medicinal properties such as antibacterial and antioxidant agents. However, it is important to educate consumers on the benefits of the varying medicinal plants consumption, choosing those that have highest biological capacity in order to promote a healthy diet.

Acknowledgment

This study received financial support from Arak University under contract number of 93. 12215. The authors also would like to thank Dr. Abdollah Khadivi-Khub for statistical analysis of data.

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