

A STUDY ON ESSENTIAL OIL CHEMICAL COMPOSITIONS, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF NATIVE AND ENDEMIC SATUREJA SPECIES GROWING IN IRAN

F. Jafari,¹ F. Farmani,¹ K. Zomorodian,^{2,3} M. Moein,^{1,4} P. Faridi,⁵ and M. M. Zarshenas^{1,5,*}

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In this study, chemical compositions of the essential oil and antioxidant activities of four *Satureja* species, namely *S. hortensis* (SH), *S. bachtiarica* (SB), *S. laxiflora* (SL), and *S. intermedia* (SI) were evaluated. GC and GC/MS were employed to analyze the essential oil samples extracted via hydrodistillation. DPPH and FRAP assays were used to evaluate the radical scavenging and antioxidant properties as well as antimicrobial activities. The GC and GC/MS analysis revealed and identified a total of 25 compounds. The most abundant chemical constituents were ρ -cymene (13.07 – 17.50%) in all four species, γ -terpinene in SL, SH, and SI (11.35 – 37.60%), thymol in SL, SB, and SI (24.54 – 38.75%), and carvacrol in SB and SH (32.07 – 42.51%). The FRAP assay showed that none of the methanol and dichloromethane extracts possessed significant antioxidant activity as compared to quercetin. In addition, the radical scavenging activity of SB and SI methanol extracts were higher than that of SL and SH (DPPH assay). However, all extracts did not show potent radical scavenging activities. It should be noted that this study was the first to evaluate the possible antioxidant or radical scavenging properties of SI and SL. The antimicrobial assay demonstrated a potent activity of all samples against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus fumigatus*, and *Epidermophyton floccosum* (minimum inhibitory concentrations, MIC = 0.06 – 8 $\mu\text{g}/\text{mL}$). Due to rich content of thymol and carvacrol isomers in *Satureja* species, they can be suggested for use in various pharmacological applications.

Keywords: essential oil; antioxidant; Lamiaceae; *Satureja*.

1. INTRODUCTION

Around 230 genera and more than 7000 species have been authenticated within the family Lamiaceae [1]. *Satureja* (Savory) is one of the most popular Lamiaceae members with nearly 200 different herbs or shrub species which are distributed in Asia, Mediterranean area, and some parts of the United States [2]. Botanical parts of *Satureja* species

have been traditionally and ethnopharmacologically used for treating various human ailments from long time ago [3]. Due to the presence of various classes of metabolites in this genus, many pharmacological and clinical assessments have been performed on many of the medicinal species of *Satureja* [4, 5]. *Satureja* species mostly contain essential oil in their botanical compartments. The extracted essential oil frequently yields more than 5% in different species [6].

In Iran, 14 species has been reported for *Satureja* (Persian name of “Marze”), eight of those being endemic. These species, currently used by natural healers and traditional practitioners [7], are mainly found in mountainous north and west areas [8]. In particular, *Satureja hortensis* L. (SH) is one of the main *Satureja* species throughout the world with different medical and nutritional applications. The herb has been evaluated for several pharmacological properties such as antispasmodic, antimicrobial, antidiarrheal, and sedative [9]. Culinary and pharmaceutical applications of SH include

¹ Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

² Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

³ Department of Medical Mycology and Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

⁴ Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

⁵ Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

* e-mail: zarm@sums.ac.ir

but not limited to the antioxidant and antimicrobial properties [10]. Similar to SH, *S. bachtiarica* Bunge (SB) is also an advantageous species. The herb is traditionally used as a flavor in food and an analgesic, antiseptic and expectorant by local healers [11]. Current medical knowledge revealed the antibacterial and antileishmanial properties of SB [12, 13]. Despite numerous phytochemical and pharmacological evaluations on many of *Satureja* species, some studies have been performed on two endemic species, *S. laxiflora* K. Koch (SL) and *S. intermedia* C. A. Mey (SI). In spite of extensive folk and traditional applications, the only performed studies on these species include two essential oil analyses and antimicrobial and cytotoxic assessments [14–16].

In view of the high content of phenolic compounds in SL and SI, extending the pharmacological and experimental assessments on these two species should be given consideration. Accordingly, the present work was aimed at the chemical analysis of SL, SI, SH, and SB essential oils and the evaluation of antioxidant, radical scavenging, and antimicrobial properties of these species.

2. EXPERIMENTAL PART

2.1. Plant Collection

Aerial parts of SH, SB, SI, and SL at full flowering stage were collected from Alborz, Fars, Ardebil, and Ardebil provinces of Iran. Plant material was subsequently dried in the shade and authenticated by Miss Khademian, botanist of Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences (Shiraz, Iran). Later, samples were specified by voucher names and deposited in the Shiraz School of Pharmacy herbarium.

2.2. Physicochemical Methods

Preparation of extracts. Each sample was subjected to both hydrodistillation and static maceration in methanol and dichloromethane to yield the essential oil and respective extracts. The hydrodistillation was performed for 3 h and the collected essential oils were dehydrated and kept in amber glass screw vials at -20°C . In addition, each sample was macerated in methanol (1 : 20) for 48 h at room temperature. Dried residues were then washed with dichloromethane (1 : 20, three times) in an ultrasonic apparatus for 15 min at room temperature. The obtained extracts were concentrated, dried, and kept at -20°C for further steps.

GC and GC/MS analysis of samples. GC/FID analysis of essential oil samples was performed on a gas chromatograph (Agilent Technologies Model 7890A) with an HP-5 column (25 m length \times 0.32 mm i.d.; film thickness 0.52 μm) connected to a flame ionization detector (FID). Nitrogen was used as the carrier gas (flow rate, 1 mL/min; split ratio, 1 : 30). Temperatures of injector and detector were adjusted at 250 and 280°C , respectively. The column tempera-

ture was programmed linearly from 60 to 250°C (ramp, ~ 5 K/min) and consequently held at 250°C for 15 min. Essential oils of four species were diluted in *n*-hexane ($\sim 1\%$) and injected to the GC system.

The GC/FID method and conditions were employed for GC/MS analysis. The process was carried out on a gas chromatograph (Agilent Technologies Model 7890A) equipped with an HP-5MS capillary column (phenyl methyl siloxane, 30 m \times 0.25 mm i.d.), connected to a mass detector (Agilent Technologies Model 5975C). The carrier gas (helium) flow rate was selected as for the GC/FID. In a mass range of 30–600 *m/z*, the mass spectrometer was used in the EI mode (70 eV). The temperature of the interface was adjusted at 280°C .

The data from GC and GC/MS were used to identify constituents of the samples. This process was based on comparison of the Kovats indices (KIs) calculated for a homologous series of *n*-alkanes C_8 – C_{22} as well as MS data of the components to those reported in the related literature [17–36].

Evaluation of the antioxidant and radical scavenging properties. To study the positive or negative radical scavenging activities, both methanol and dichloromethane extracts were subjected to 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. According to this, DPPH free stable radical (100 mM) in methanol was mixed with various concentrations (16–3200 $\mu\text{g/mL}$) of methanol and dichloromethane extracts (100 mL). The reaction mixture was incubated in the dark at room temperature for 30 min. By using a microplate reader, the DPPH radical inhibition was determined at 490 nm. The procedure was performed in triplicate and EC_{50} for each extract was calculated and presented as mean \pm SD (standard deviation) [36].

The possible antioxidant activity was evaluated via ferric-reducing antioxidant power (FRAP) assay. For this purpose, 2, 4, 6-tripyridyl-S-triazine (TPTZ) solution (0.01 mol/L) in HCl (0.04 mol/L), acetate buffer (0.3 mol/L, pH 3.6), and FeCl_3 solution (0.02 mol/L) were prepared. Acetate buffer, FeCl_3 and TPTZ solutions were mixed together before use and the mixture was heated to 37°C . Approximately 20 mL of each essential oil (EO) sample and 180 mL of FRAP reagent were mixed on a 96-well microplate reader and then incubated at 37°C for 10 min. The absorbance of each sample was measured at 593 nm [37].

2.3. Evaluation of Antimicrobial Activity

Bacterial and fungal strains. Four American Type Culture Collection (ATCC) strains including *Candida albicans* (ATCC 10261), *A. fumigatus* (ATCC 14110), *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 25922) as well as a clinical strain of *Epidermophyton floccosum* isolated from *Tinea cruris* and identified by phenotypic methods (slide culture and colony morphology) were used in this study.

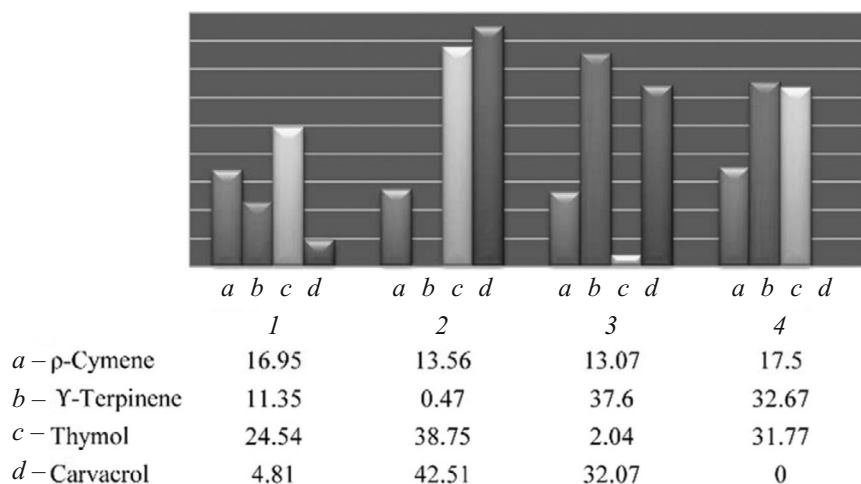


Fig. 1. Histogram of the most abundant volatile constituents in *Satureja* species studied: (1) SB; (2) SL; (3) SH; (4) SI.

Determination of minimum inhibitory concentration (MIC). MIC values were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI), with some modifications. Briefly, for determining the antimicrobial activity against fungi, serial dilutions of EOs (0.03 – 32.0 µL/mL) were prepared in 96-well microtiter plates using RPMI-1640 medium (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). To determine the antibacterial activities, serial dilutions of EOs (0.03 – 32.0 µL/mL) were prepared in Muller – Hinton medium (Merck, Darmstadt, Germany). Test fungi or bacteria were suspended in the media and the cell densities were adjusted to 0.5 McFarland standards at 530 nm using a spectrophotometric method (this yielded stock suspension of $(1 - 5) \times 10^6$ CFU/mL for yeasts and $(1 - 1.5) \times 10^8$ cells/mL for bacteria). To each well of the microtiter plate, 0.1 mL of the working inoculum was added and the plates were incubated in a humid atmosphere at either 32°C for 24 – 48 h (fungi) or 37°C for 24 h (bacteria). Two hundred microliters of un-inoculated medium was used as a sterility control (blank). In addition, growth control samples (medium with inoculum, but without EO) were also prepared. The growth in each test well was compared to that in the growth control well. MICs were visually determined and defined as the lowest concentration of the EO producing ? 95% growth reduction compared to the growth control wells. Each experiment was performed in triplicate.

In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany). Wells with bacteria showing no visible growth on Muller – Hinton agar medium were used to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC), respectively.

3. RESULTS AND DISCUSSION

3.1 Essential Oil Analysis

The yield of essential oil extracted upon hydrodistillation varied within 0.6 – 2.1% (V/W). The yield (%) order for *Satureja* species were SB (2.1%) > SI (2.0%) > SH (1.8%) > SL (0.6%). The results of GC and GC/MS analysis revealed and identified a total of 25 compounds (Table 1). The most abundant chemical constituents were ρ-cymene (I) in all samples, γ-terpinene (II) in SL, SH, and SI, thymol (III) in SL, SB, and SI, and carvacrol (IV) in SB and SH (Fig. 1). To compare the species together, SB, SL and SI showed to have III as a major constituent with lack or low amount of IV. SI essential oil was found to contain no IV. At the same time, IV (42.51%), III (38.75%), and I (31.77%) were the most abundant components in SB. Unlike other samples, II was also present as trace in this species.

To compare with previous literature, SB showed to have IV (45.5%) and III (27.9%) as major constituent in recent investigation [38], where the amount of I in the investigated SB sample was much lower than that in our study. In contrast, I was reported as a major constituent in some other SB samples [39], whereas this component was not significant in our case. Moreover, in the sample from Ardebil province (Northwest of Iran), I was found in highest amount (26.4%) [40]. The maximum amount of III was reported SB sample from Fars province (65.1%) [41], while in other investigations, IV was the major ingredient and reported in highest amount (66.5%) [42]. It is commonly accepted that location and climate as well as the harvesting time and type of extraction are factors deeply influencing the essential oil composition [43]. Study of the SB harvesting time and essential oil composition revealed the reduction of III (19.2% to 0.5% from pre-flowering to post-flowering stage) and the appearance of

mentone (18.5%) after flowering stage [44]. The latter compound was not reported in aforementioned investigations.

There are numerous studies on the chemical composition of SH essential oil. A sample from Isfahan province (center of Iran) demonstrated that IV (59.7%), II (12.8%) and I (9.3%) were major constituents [5], which is rather similar to our sample. A report from a Syrian sample showed similar chemical and concluded that full blooming stage results in the highest yield [45]. Another study indicated that the amount of II may increase in harvested samples [46]. III was also showed as a major constituent in another sample [47].

Concerning the volatile components of SI and SL, SI showed to have thymol as the major constituent [14, 16]. However, our sample showed II in highest amount. It is obvious that the collection times for our sample and those two mentioned above were different. On the other hand, SL showed to contain III on the highest level (24.54%) among other major constituents in our study. However, there was only one report on the chemical compositions of SL essential oil, in which III was the major component (63.9%) [15].

Although these species are from the Lamiaceae family, the profiles of volatile constituents are very similar to those

TABLE 1. GC/MS Data on Chemical Constituents of *Satureja* Species Studied*

Peak no.	Compound**	SL (%)	SB (%)	SH (%)	SI (%)	RI _{Cal}	RI _{Ref}	Ref.
1	α -Thujene	2.1	-	0.59	-	922	923	19
2	α -Pinene	1.98	-	1.24	0.36	927	929	20
3	β -Pinene	1.72	-	0.80	-	970	974	20
4	β -Myrcene	4.08	-	1.59	1.04	990	992	21
5	α -Phellandrene	1.03	-	0.40	0.34	1002	1003	22
6	3-Carene	0.32	-	-	-	1006	1005	20
7	α -Terpinene	11.5	-	4.63	4.72	1015	1014	23
8	ρ -Cymene	16.95	13.56	13.07	17.50	1025	1021	24
9	β -Phellandrene	2.33	-	-	-	1026	1026	24
10	γ -Terpinene	11.35	0.47	37.60	32.67	1057	1056	25
11	Linalool	-	1.07	-	-	1106	1106	21
12	Borneol	-	0.50	-	0.34	1170	1171	26
13	Terpinen-4-ol	0.28	-	-	-	1181	1178	27
14	Thymol	24.54	38.75	2.04	31.77	1310	1306	28
15	Carvacrol	4.81	42.51	32.07	-	1323	1317	29
16	Thymyl acetate	3.25	-	-	-	1361	1362	30
17	Carvacryl acetate	-	-	1.31	-	1377	1372	31
18	trans-Caryophyllene	5.57	1.82	1.57	5.0	1414	1416	32
19	Aromadendrene	0.85	-	-	1.33	1432	1431	20
20	α -Humulene	0.33	-	-	-	1447	1448	33
21	Ledene	0.48	-	-	1.54	1489	1489	34
22	β -Bisabolene	5.26	-	3.09	0.59	1507	1506	32
23	δ -Cadinene	-	-	-	0.81	1519	1521	35
24	Caryophyllene oxide	-	1.32	-	-	1579	1578	29
25	Spathulenol	-	-	-	0.52	1581	1581	36
Monoterpenes (%)		86.24	96.86	94.03	88.74			
Sesquiterpenes (%)		12.49	3.14	5.97	9.79			
Total		98.73	100	100	98.53			

* SB: *S. bachtiarica*; SH: *S. hortensis*; SL: *S. laxiflora*; SI: *S. intermedia*. Bold figures refer to compounds that are the most abundant fractions.

**Identification of compounds is based on considering both the mass spectra and retention indices (RIs). RIs were calculated against C₈–C₂₄ n-alkanes on the given column (RI_{Cal}: calculated RI values for each constituent; RI_{Ref}: calculated RI values reported in literature). Compounds have been classified with respect to RIs for DB-5 MS capillary column.

in some species from other genera and families. A screening study on different *Trachyspermum ammi* sprague species (from Apiaceae) showed that III, I, and II were main components in ten samples, [43]. Accordingly, many of the undertaken pharmacological assessments on the essential oil of those medicinal plants can be also performed for these species.

3.2. Radical Scavenging and Antioxidant Activities

All *Satureja* samples were evaluated for their possible antioxidant and radical scavenging activities in comparison to each other. In contrast to previous reports on the antioxidant properties of SH and SB [3, 48], the FRAP assay in current study revealed that none of the methanol and dichloromethane extracts possessed significant antioxidant activity as compared to that of quercetin (Table 2). In addition, the radical scavenging activity of methanol extracts of SB and SI were higher than that of SL and SH (DPPH assay). However, none of those extracts showed acceptable effectiveness in comparison to quercetin ($IC_{50} = 26.51 \pm 0.06 \mu\text{g/mL}$). In respect of the radical scavenging assay, all dichloromethane extracts were also inactive in comparison to quercetin (Table 2).

3.3. Antimicrobial Activity

The antibacterial activities of the essential oils extracted from *Satureja* species and tested against selected microorganisms are presented in Table 3. All samples inhibited the growth of *S. aureus* at concentrations within $0.5 - 4 \mu\text{L/mL}$. In addition, the oils possessed bactericidal activity against Gram-positive cocci at concentrations similar to the corresponding MICs. The *E. coli* strain was susceptible to all essential oil samples at concentrations of $0.12 - 1 \mu\text{L/mL}$. Furthermore, oil samples exhibited bactericidal activity (MBC) against this Gram-negative bacterial strain at concentrations ranging within $0.25 - 8 \mu\text{L/mL}$.

TABLE 2. Results of DPPH and FRAP Assays on *Satureja* Species Studied*

Sample	DPPH ($IC_{50} \approx \mu\text{g/ mL}$)	FRAP (%)
SB methanol extract	181.76 ± 0.18	96.55 ± 0.37
SI methanol extract	195.61 ± 0.71	81.03 ± 0.22
SL methanol extract	287.07 ± 1.2	151.46 ± 0.36
SH methanol extract	361.76 ± 0.9	136.76 ± 0.39
SB dichloromethane extract	900.20 ± 1.19	
SI dichloromethane extract	551.43 ± 0.33	
SL dichloromethane extract	1759.69 ± 0.9	
SH dichloromethane extract	1887.35 ± 0.16	
Quercetin	26.51 ± 0.06	8.69 ± 0.03

* SB: *S. bachtiarica*; SH: *S. hortensis*; SL: *S. laxiflora*; SI: *S. intermedia*.

The antifungal activities of essential oils from *Satureja* species against fungi are also shown in Table 3. For the clinical and standard fungal strains tested, the MICs were within $0.06 - 0.5 \mu\text{L/mL}$. No significant differences in MICs were found between oil samples against *C. albicans*. In addition, the oils exhibited fungicidal activities against test fungi at concentrations ranging within $0.25 - 32 \mu\text{L/mL}$.

Due to rich content of III and IV isomers in *Satureja* species, they can be suggested for various pharmacological applications. Regardless of the antioxidant activities reported for SB and SH, the present work did not support previous studies. In addition, results of the present work revealed that SI and SL, which are currently used in folk medicine in Iran, cannot be evidently presented as antioxidants for food additives and nutritional preparations. It should be emphasized that the present study was the first to evaluate the possible

TABLE 3. Antimicrobial Activity of Essential Oils Distilled from *Satureja* Species Studied*

Sample	<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>A. fumigatus</i>		<i>E. floccosum</i>	
	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC
SH	0.12	8	2	2	0.06	0.12	0.12	32	0.06	0.5
SI	0.25	0.5	4	4	0.06	0.25	0.25	32	0.06	0.5
SB	0.12	0.25	2	2	0.06	0.06	0.06	32	0.06	0.12
SL	1	1	0.5	0.5	0.06	0.25	0.5	32	0.25	1

* SB: *S. bachtiarica*; SH: *S. hortensis*; SL: *S. laxiflora*; SI: *S. intermedia*. MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration; MIC and MBC values are given in $\mu\text{L/mL}$

antioxidant and radical scavenging properties of SI and SL species. Concerning the results of antimicrobial assessment, it seems that these essential oils can be good candidates as new antimicrobial agents for clinical studies.

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