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Chemical Composition Analysis of the Essential Oil of New Species of *Satureja* L., *Satureja kermanica*, from South-East of Iran

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Article information

Available online: 15 Sep. 2021

Abstract

Satureja L. is one of the typical plants of the Lamiaceae family, and its essential oil has a wide variation of composition depending on the spices, growing area and climate conditions. Recently, *Satureja kermanica* (Kerman savory or ezgen) has been identified as a new species from south-east of Iran using morphological and molecular phylogenetic analyses. The essential oils have many applications in the pharmaceutical, food and cosmetic industries. The analysis and identification of the chemical composition of essential oils (EOs) was performed using the (GC-MS). Fifteen components were identified representing 99.6% of the entire essential oil composition, 95.39% of monoterpenes, 2.35% of sesquiterpenes and 1.86% of other compounds. The EOs of *S. kermanica* were dominated by the presence 51.30% thymol and 25.88% *γ*-Terpinene as major constitutes accounting for 77.81% of total oil components. The other compounds of essential oils were p-cymene (5.45%) carvacro (4.28%), α- terpinene (3.23%), (*E*)-caryophyllene (2.35%), *β*-pinene (1.75%), *a*- thujene (1.53%) and n-nonadecane (1.13%). The presence of thymol, γ-Terpinene, p-cymene, carvacrol, α- Terpinene and (E)-caryophyllene, as main essential oils in lamiaceae family, in this new species indicate to its significant activities in food, cosmetics and medicine industries.

Keywords:
Ezgen
Satureja kermanica
Thymol
γ-Terpinene

1. Introduction

The genus *Satureja* L. (Lamiaceae) contains about 200 species of herbs, shrubs and mainly aromatic plants with distribution in Caucasus and W Asia (Saudi Arabia, Iran and Iraq), the Mediterranean region, N Africa (Morocco and Libya) and north America (Ghorbanpour *et al.*, 2016, Bordbar *et al.*, 2020). The plant height is ranged from 50–100 cm. The flowers at least in a part of inflorescence are pedicellate and some cymes are pedunculated. The species produces flowers and fruits in autumn (Bordbar *et al.*, 2020).

Because of the aromatic characteristics, leaves and aerial parts of this genus possess distinctive but pleasant tastes (Jafari *et al.*, 2016). Some members of *Satureja* are of economic importance since they have been used as culinary herbs, flavoring agents in perfumery and cosmetics (Pirbalouti and Moalem, 2013). In traditional Iranian medicine, the aerial parts of some *Satureja* species are used for treatment different diseases such as upper respiratory tract infections, diarrhea, urinary tract infections, gastroenteritis and ulcers. Therefore, there is a great interest in continuing researches on the extracts of these plants for their chemical compounds and biological features. In addition, the demands for these natural food preservatives have increased in modern food industry. This is not only due to increasing microbial resistance against conventional food preservatives but also due to increasing awareness of their residual toxicity or possible side effects (Ghorbanpour *et al.*, 2016)

Essential oils are complex aromatic and natural substances (secondary metabolites) with low molecular weight, secreted by plants to protect them against predators and harsh environmental conditions. Essential oils are present in secretory cells, hair, secretory sacs and secretory ducts in surface and internal parts of different organs like leaves, flowers, fruit and shoots of plants. They are an integral part of our everyday life. Volatile oils are outspread in the Lamiaceae family, so many species are used as aromatic herbs and spices since ancient times. Nowadays, apart from the food industry as food enrichments and preservatives, essential oils are widely used in the production of cosmetics, perfumes, aromatherapy, and holistic medicine (Mohtashami *et al.*, 2018; Chambre *et al.*, 2020). İn addition, the essential oils and phenolic

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compounds have usuful function as antibacterial, antifungal, anti-inflammatory, antitumor, antiviral, antioxidant and sedative (Memarzadeh *et al.*, 2020).

Terpenes play important roles in almost all the basic processes, including plant growth, development, reproduction and defense. Mono- and sesqui-terpenes protect plants against herbivores and pathogens. Two monoterpenes of the Lamiaceae attracting much attention are thymol and carvacrol. These two phenolic components are known for their anti-herbivore, antimicrobial, and antioxidant activities. The oxidized monoterpenes, thymol and carvacrol, are most likely derived from one of the initial cyclic products, γ-terpinene, by oxidation. Since p-cymene itself was not accepted as a substrate, it is likely that γ-terpinene is directly converted to thymol and carvacrol with p-cymene as a side product (Naghdibadi *et al*., 2017)

As seen in review studies and literature, Essential oils of most savory (*Satureja*) species have been identified. The main components of *S. hortensis* essential oil are thymol, carvacrol, *γ*-terpinene, *p*-cymene, caryophyllene, α-terpinolene, *β*-pinene, α-thujene and α-pinene, which have antibacterial, antiviral, antifungal activities (Chambre *et al.*, 2020). The leaves of *S. bachtiarica* are used as herbal medicine, spice, and food industr. The essential oils from the aerial parts, especially its leaves contains a rich source of two phenolic compounds i.e. carvacrol and thymol and other terpenes such as *ρ-cymene* and *γ*-terpinene (Memarzadeh *et al.*, 2020). A total of 47 components were recorded in the essential oils of *S. rechingeri* at different developmental stages. The main compods of all samples were carvacrol (83.6%-90.4%), p-cymene (0.8%-2.9%) and γ-terpinene (0.6%-2.4%) (Alizadeh, 2015).

However, there is no published study on essential oils of *S. kermanica* distributed in south east of Iran. This species has recently been identified and named by (Bordbar *et al.*, 2020). The present study was conducted to investigate the essential oils in *S. Kermanica*.

2. Material and Methods

The fresh shoots of *S. kermanica* were collected from Godar sabz area in Sarduiyeh (Kerman province, Iran) in vegetative phase. The freshly collected samples were dried at room temperature and then stored at -20°C until using.

Dried samples were hydrodistilled using the Clevenger apparatus. The obtained distillate was extracted using acetone as the solvent and dried over anhydrous sodium sulphate. Thermo-UFM Ultra-Fast gas chromatograph equipped with a DB-5 fused silica column (10 m × 0.1 mm i.d., film thickness 0.40 μm) with Flame ionization detector (FID) was used for Gas chromatography (GC) analysis. The oven temperature program was 3 minutes isothermally at 60 ^{o}C , enhancing to 260 ^{o}C at the rate of 40 ^{o}C /min and was held isothermally for 5 minutes. Detector (FID) temperature and the injector temperature were 280 ^{o}C. Helium was used as carrier gas at a flow rate of 0.5 mL/min. The essential oils were injected manually into the GC instrument without dilution.

The analysis of the volatile compounds was carried out on a Varian 3400 gas chromatograph combined with a Saturn II mass spectrometer (ion trap) operating at 70 eV ionization energy. It was equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed to increase from 50 to 260 ^{o}C at a rate of 4 ^{o}C /min; the injector and transfer line temperature were 260 and 280 ^{o}C, respectively. The carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio of 1:60 and flow rate 1 mL/min. Mass spectra were scanned every 1 s in the range of 30-450 amu.

The identification of the essential oil compounds was performed by comparing their mass spectra to those reported in Wiley GC/MS library. The identification was further confirmed by comparing their calculated retention indices obtained using a homologous series of n-alkanes (C8-C22) with those of authentic compounds in Adams reference. This was further supported by co-injection of the available authentic compounds or standards such as α-pinene, β-pinene, γ-terpinene and limonene. Components relative concentrations were obtained by the peak areas normalization. No response factors were calculated (Adelifar and Rezanejad, 2021).

3. Results

S. kermanica, known as ezgen in local name is perennial and has the appearance of bush. The plant preferably grows on sandy and rocky sediments of seasonal streams (Fig 1). The local people use this species as green tea, flavoring yogurt, buttermilk and bread. It is also used to treat digestive problems and colds.

The gas chromatography (GC) and gas chromatography = mass spectrometry (GC=MS) analysis of the essential oils revealed the identification of 15 components accounting for 99.6% of the oil. The essential oils from *S. kermanica* showed a high content of monoterpenes (95.39%) and low contents of sesquiterpene (2.35%). The predominant compounds among the essential oils components were thymol (51.30%) and *γ*-Terpinene (25.88%) from monoterpens while the amount of all other components were 22.42% (Table 1). As seen in Table 1, there is only one sesquiterpene, (*E*)-caryophyllene, showing 2.35% of essential oils. In addition, the other compounds of essential oils, each containing more than one percent, wew p-cymene (5.45%), carvacrol (4.28%), α- terpinene (3.23%), *β*-

pinene (1.75%), *α*- thujene (1.53%) and n-nonadecane (1.13%) accounting for 17.37% of essential oils. The remaining compouns make up about 2.72%, each containing less than one percent (Table 1).

Fig. 1 Structure and habitat of *S. kermanica* (Lamiaceae)

4. Discussion

Results of the present study using GC-MS showed that the main components in essential oils of *S. kermanica* were were thymol (51.30%) and *γ*-Terpinene (25.88%) from monoterpens (Table 1). The only compound recorded from sesquiterpenes was (*E*)-caryophyllene (2.35%). These two major monoterpens constitute 77.18% of total essential oils in *S. Kermanica* (Table 1). There is no published study on the composition of essential oils of *Satureja* species in Sarduiyeh area. İn addition, there is no report on these components in *S. kermanica*. On the whole, the composition and amount of major components obtained in this study are similar to most results reported in the literature on *Satureja* species. However, compositional differences in essential oils of plants are common and the the changes of type and content of constituents may be explained based on genetic features, geographical location, harvest time, environmental conditions, cultivation handling, developmental stages, period and storing conditions (Mossi *et al*., 2011; Lombrea *et al*., 2020). According to most reports, main components in this genus and even in lamiaceae family are carvacrol, thymol, *p*-cymene, and *γ*-terpinene, although their levels are different between various species and populations as well as in different developmental stages.

Table 1 Chemical composition of the essential oils of *Saturja kermanica*

Sr. Nos.	Components	RT[a]	RI[b]	Concentration (%)
1	α- Thujene	1.9	932	1.53
2	α- Pinene⁎	1.96	936	0.55⁎
3	β- Pinene⁎	2.14	980	1.75
4	α- Terpinene	2.31	1015	3.23
5	P-cymene	2.36	1021	5.45
6	γ-Terpinene	2.49	1063	25.88
7	*Cis*-sabinene hydrate	2.56	1072	0.27
8	Linalool⁎	2.62	1100	0.42
9	Terpinene-4-ol	3.04	1183	0.36
10	Thymol methyl ether	3.13	1230	0.37
11	Thymol	3.42	1293	51.30
12	Carvacrol	3.46	1305	4.28
13	(E)-caryophyllene	3.94	1423	2.35
14	N-nonadecane●	5.20	1900	1.13
15	N-heneicosane	5.70	2100	0.73
-	MT (Ns. 1- 12)	-	-	95.39
-	ST (N. 13)	-	-	2.35
-	OT (Ns. 14, 15)	-	-	1.86
-	Total oil composition	-	-	99.6

RT[a]= Retention Time.
RI[b]= Retention indices (DB-5 column) using normal alkane series (C_8-C_{22}).
RI[c]= Retention indices reported in the literature[20].
The compounds observed predominantly in two or three species were marked with the symbol (✶) whereas the compounds found predominantly in one species were shown with the symbol (●).
MT: Monoterpenes; ST: Sesquiterpenes; NT: Nonterpenes.

Table 2 The comparison of the chemical composition of the essential oils of different species of *Saturja* (obtained from literature and the present study (*S. kermanica*). The main components have been shown in bold.

Components	Concentration (%)					
	S. kermanica[1]	*S. bakhtiarica*[2]	*S. khozestanica*[3]	*S. hortensis*[4]	*S. thymbra*[5]	*T. vulgaris*[6]
α- Thujene	1.53	-	0.28	1.93	1.55	-
α- Pinene	0.55*	0.7	-	1.49	1.48	2.95
β- Pinene	1.75	-	-	2.32	0.7	1.97
α- Terpinene	3.23	1.89	0.49	2.31	3.26	-
P-cymene	5.45	18.82	3.11	8.05	7.17	18.35
γ-Terpinene	25.88	6.47	1.24	42.35	39.23	-
cis-sabinene hydrate	0.27		-	0.87	0.23	0.76
Linalool	0.42	2.25	0.91	-	0.34	0.6
Terpinene-4-ol	0.36		0.65	036	0.36	
Thymol methyl ether	0.37		-	-	-	2.16
Thymol	51.30	19.43	0.19	3.42	25.16	51.34
Carvacrol	4.28	34.65	90.88	32.83	4.18	2.03
(*E*)-caryophyllene	2.35	1.75	0.15	2.22	2.76	4.26
Carvacrol methyl ether	0.37		-	0.09	3.33	-
Borneol	-	4.21	0.35	-	-	-
N-nonadecane•	1.13	-	-	-	-	-
N-heneicosane	0.73	-	-	-	-	-

1-The results of present study, 2-6- Taken from the following papers: 2- (Memarian *et al*., 2020), 3- (Pirbalouti and Moalem, 2013), 4- (Chambre *et al*., 2020), 5- (Giweli *et al*., 2012), 6- Al (Maqtari *et al*., 2011).

Futhermore, there are reports showing the presence of borneol as one of major components in few species of *Satureja* too. The presence of (*E*)-caryophyllene as one of main sesquiterpenes has been repoted in this species although its content is not very high (2-4%) (Table 2, Giweli, 2012; Chambre *et al*, 2020; Pirbalouti and Moalem, 2013; Memarian *et al*., 2020).

As seen in Table 2, there are various differences between different species; furthermore, according to the table, there are high similarity between *Satureja* and *Thymus vulgaris* particulary in the content of carvacrol, thymol, *p*-cymene and (*E*)-caryophyllene as main components of *S. kermanica* and other *Satureja* species (Table 2). However, the level of γ-terpinene is low in *S. khozestanica* and *T. vulgaris.* According to the literature, carvacrol and thymol, and their precursors, *p*-cymene and γ- terpinene are the major components of essential oil in various *Satureja* species. Carvacrol and its isomer, thymol, are monoterpenoid phenols that are biosynthesized via aromatization of γ-terpinene to *p*-cymene and subsequent hydroxylation of *p*-cymene. These two phenol along with their two precursors γ-terpinene and *p*-cymene appeared as the major components in numerous phenolic essential oils of the Lamiaceae family. 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 *T. vulgaris* is genetically controlled by an epistatic series of several biosynthetic loci that seems to be different (Ghorbanpour *et al*., 2016). However, (Naghdi Badi *et al*., 2017) demonstrated that *γ*-terpinene is directly converted to thymol and carvacrol with *p*-cymene as a side product (Naghdi Badi *et al*., 2017).

Due to the conversion of *γ*-terpinene to thymol and carvacrol and their separate biosynthetic pathways, as well as the formation of p-cement as one of the products of this pathway, it is supported why their levels are different in different species and population as well as in different genera. Thus, the amount of each of these compounds can be changed based on sample collection time, the time of sample storage and analysis method. Therefore, the amount of each of these compounds varies based on the biosynthetic pathway assigned to each of them too.

Carvacrol and thymol are produced to protect plants against pathogens, pests, herbivores, or environmental stresses and other biological and environmental stresses. They have antibacterial, antifungal, insecticidal, and anti-oxidative properties which are the basis for the wide use of these compounds in the cosmetic, food, and pharmaceutical industries (Naghdi Badi *et al*., 2017; Lombrea *et al*., 2020).

The antibacterial activity of the essential oils results from the high level of thymol and carvacrol which tie to membrane proteins and increases the permeability of the bacterial cell membrane. Their antifungal activity is based on the disturbance of the fungal cell wall integrity and with the interference of ergosterol synthesis. The antioxidant activity of essential oils in various species of lamiaceae family is attributed to the presence of carvacrol, thymol, and *p*-cymene, each one having the property to form chemical complexes with metal ions and free radicals. İn addition, the derivatives with carvacrol and thymol-based scaffolds have gained much attention owing to their anti-inflammatory, anticancer, antitumor, anti-HIV, antiviral, antipyretic, anticonvulsant, and antidepressant properties. (Zielińska-Błajetand and Feder-Kubis, 2020). The application of this bioactivities can be valued in food and pharmaceutical industries, as a safer alternative to the synthetic antioxidants (Lombrea *et al*., 2020).

β-caryophyllene (BCP) is one of main sesquiterpens extracted from spice and food plants, such as black pepper, rosemary, cinnamon. Pre-clinical studies have revealed that BCP is a modulator of nervous system and exerts beneficial effects on numerous neurodegenerative and inflammatory pathologies. Moreover, it is able to act on the liver and bones, and has antibiotic properties (Francomano *et al*., 2019)

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