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Chemotaxonomy of Two *Satureja* L. (Lamiaceae) Species from Different Localities of Turkey

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Abstract: In this study, two *Satureja* L. species from different localities of Turkey (*Satureja hortensis* L. and *Satureja boissieri* Hausskn. ex Boiss.) have been studied to determine their taxonomic classification based on chemical characters. For this purpose, aerial parts of the studied taxa were investigated by HS-SPME/GC-MS. 28, 30 and 29 compounds were identified in *S. boissieri* from Şanlıurfa, Bingöl and Malatya accounting from 90.80%, 91.31% and 92.67% of the whole oil, respectively. 26, 30 and 28 compounds were identified in *S. hortensis* from Adıyaman, Diyarbakır, Elazığ accounting from 93.20%, 92.85% and 92.42% of the whole oil, respectively. γ-terpinene (30.4%, 26.5% and 32.1%), carvacrol (26.4%, 25.2% and 23.3%) and *p*-cymene (10.5%, 13.2% and 10.4%) were determined as main compounds of *S. boissieri* in Şanlıurfa, Bingöl and Malatya species, respectively. Carvacrol (25.0%, 34.1% and 32.1%), thymol (28.2%, 20.2% and 28.1%) and γ-terpinene (10.1%, 11.3% and 9.4%) were found as main constituents of *S. hortensis* in Adıyaman, Diyarbakır and Elazığ samples, respectively. The chemical distributions of the essential oil compounds in the *Satureja* pattern were discussed in respect to their chemotaxonomy and natural products.

Key words: *Satureja hortensis, Satureja boissieri*, essential oil, chemotaxonomy, Turkey.

1. Introduction

Turkey is regarded as an important genetic-centre for the family Lamiaceae [1]. *Satureja* L. is a genus of the well-known aromatic plant belonging to Lamiaceae and comprises many species growing wild in the Mediterranean area. The aerial parts of these species have distinctive tastes and can be added to stuffing, meat, pies and sausages as a seasoning. *Satureja* is also used as a condiment and herbal tea, owing to its stimulating, tonic and carminative effect [2]. Most aromatic plants that belonging to the family Lamiaceae, such as *Satureja*, *Origanum*, *Thymus*, etc., are used as herbal tea in Turkey [3]. With two endemic *Satureja* species found, *Satureja* now is represented by 16 taxa in Turkey [4, 5].

Many *Satureja* species are used as flavouring agents or for medicinal purposes. The therapeutic properties of these species can mainly be attributed to their essential oil that includes different amounts of biologically active compounds such as carvacrol and thymol. The essential oils isolated from various species of *Satureja* have been shown to have high chemical polymorphism [6] and various biological activities such as antibacterial and antifungal [7, 8], antioxidant [1], analgesic and anti-inflammatory [9], antispasmodic and antidiarrhoea [10], antidiabetic, antihyperlipidemic and reproduction-stimulatory activities [11], and improvement of fertility [12], treatment of recurrent aphthous stomatitis [13], protection against organophosphorus compounds [14] and treatments of cardiovascular diseases and thrombosis [15], muscle pain, intestinal and stomach disorders [10].

In Turkey folk medicine, some *Satureja* species are used to treat various diseases such as antispasmodic, cold and flu [16] and menstural, abdominal pains [17]. Therefore, there is a great interest in continuing researches on the essential oil of these plants from

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points of view of the chemical composition to biological properties. Recently, 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 [18]. Essential oils and their constituents, whose inhibitory effects against a wide range of microorganisms are documented, have received particular attention mainly due to their relatively safe status, wide acceptance by consumers and enrichment by a variety of structurally different active components [19, 20].

Because of the frequent occurrence of chemotypes in the family Labiatae, quite different patterns in the composition of oils of the same species are common. Some other factors such as collection time, plant maturity, drying conditions, mode of distillation, geographic and climatic factors also play a role in the oil composition. But generally the main compounds of plant do not change. Therefore, in this study, aerial parts of two *Satureja* species from different localities of Turkey were analyzed by HS-SPME/GC-MS and chemical properties were compared and examined for natural products and chemotaxonomy.

2. Materials and Methods

2.1 Plant Harvesting and Analysis of Gas Exchange

Information about plant materials has been shown in Table 1. Plant materials were identified with Flora of Turkey and East Aegean Islands [4].

The extraction of dried aerial part of five grams powder of plant samples were carried out by a HS-SPME (headspace solid phase microextraction) method using a DVB/CAR/PDMS fiber, with 50/30 lm film thickness (Supelco, Bellafonte, PA, USA); before the analysis, the fiber was preconditioned in the injection port of the GC as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed into a 40 mL vial, the vial was equipped with a “mininert” valve (Supelco, Bellafonte, PA, USA). The vial was kept at 35 °C with continuous internal stirring and the sample was left to equilibrate for 30 min and then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35 °C. After sampling the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyzes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time, were studied on the extraction efficiency as previously reported by Verzera, Ziino, Condurso, Romeo and Zappala [21].

A Varian 3,800 gas chromatograph directly interfaced with a Varian 2,000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used. Injector temperature, 260 °C, injection mode, splitless, column, 60 m, CP-Wax 52 CB 0.25 mm, i.d., 0.25 lm film thickness (Chrompack Italy s.r.l., Milan, Italy). The oven temperature was programmed as follows: 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C /min, and to 240 °C at 2 °C/min. The carrier gas was helium used at a constant pressure of 10 psi; the transfer line temperature, 250 °C, the ionisation mode, electron impact (EI); acquisition range, 40-200 m/z; scan rate, 1 us^{-1}. The compounds were identified using the NIST (National Institute of Standards and Technology) library (NIST/EPA/NIH Mass Spectra Library, version 1.7, USA), mass spectral library and verified by the retention indices, which were calculated as described by Van den Dool and Kratz [22]. The relative amounts were calculated on the basis of peak-area ratios. The chemical composition of studied samples is seen in Table 2.

3. Results and Discussion

The chemical composition of the essential oil of dried aerial parts of the two *Satureja* species from different localities of Turkey, were analyzed by HS-SPME (Headspace Solid Phase Microextraction

Table 1 Information of plant materials.

S. hortensis	Adıyaman: east of Turuş village field sides, 700-1,000 m., 18.VI.2012, Kilic, 3004.
S. hortensis	Diyarbakır: west of Pirinçlik village, road sides, 850-1,000 m., 15.VI.2012, Kilic, 3001.
S. hortensis	Elazığ: North of Örnek village, slopes, 1,300-1,400 m, 10.VII.2012, Kilic, 3027.
S. boissieri	Şanlıurfa: Siverek, north of Duydum village, rocky slopes, 900-1,000 m., 20.VI.2012, Kilic, 3005.
S. boissieri	Bingöl: Alatepe village (Arçuk) slopes, 1,300-1,350 m., 21.VI.2013, Kilic, 3090.
S. boissieri	Malatya: west of Akçadağ teacher school, fiel sides, 1,050-1,100 m., 20.V.2013, Kilic, 3050.

Method) extraction technique combined with the GC-MS (gas chromatography-mass spectrometry) system. 28, 30 and 29 compounds were identified in *S. boissieri* from Şanlıurfa, Bingöl and Malatya accounting from 90.80%, 91.31% and 92.67% of the whole oil, respectively. 26, 30 and 28 compounds were identified in *S. hortensis* from Adıyaman, Diyarbakır, Elazığ accounting from 93.20%, 92.85% and 92.42% of the whole oil respectively. γ-terpinene (30.4%, 26.5% and 32.1%), carvacrol (26.4%, 25.2% and 23.3%) and *p*-cymene (10.5%, 13.2% and 10.4%) were detected as main compounds form *S. boissieri* in Şanlıurfa, Bingöl and Malatya samples, respectively. Carvacrol (25.0%, 34.1% and 32.1%), thymol (28.2%, 20.2% and 28.1%) and γ-terpinene (10.1%, 11.3% and 9.4%) were identified as main components from *S. hortensis* in Adıyaman, Diyarbakır and Elazığ samples, respectively. The identified components of the studied *Satureja* taxa are listed in Table 2 and the main constituents of *Satureja* taxa from literature are listed in Table 3.

Table 2 Main constituents of studied *Satureja* L. species from different localities of Turkey (%).

Compounds		*S. boissieri*			*S. hortensis*		
	RRI	Şanlıurfa	Bingöl	Malatya	Adıyaman	Diyarbakır	Elazığ
α-thujene	933	0.7	1.1	0.3	0.8	0.6	1.4
α-pinene	937	0.6	0.7	0.5	0.4	0.8	0.5
Camphene	950	-	0.2	1.2	0.1	-	0.3
Mrycene	978	0.2	-	-	-	1.1	-
β-pinene	985	1.4	0.1	0.3	2.2	1.0	0.2
α-phellandrene	1,005	-	0.2	-	0.1	0.2	-
δ-3-carene	1,010	0.1	-	1.2	-	0.3	0.6
p-cymene	1,022	10.5	13.2	10.4	8.2	7.1	5.5
α-terpinene	1,023	3.2	4.3	2.5	1.8	3.6	3.1
Trans-sabinene-hydrate	1,048	-	0.1	-	-	0.3	-
γ-terpinene	1,055	30.4	26.5	32.1	10.1	11.3	9.4
Linalool	1,098	1.3	-	0.6	1.2	-	0.4
Limonene	1,107	-	1.1	-	0.8	0.1	-
β-ocimene	1,142	0.1	-	-	0.2	-	-
Eucalyptol	1,154	-	0.3	1.3	-	-	0.2
Trans-2-caren-4-ol	1,158	-	-	0.2	-	0.4	-
Borneol	1,160	2.5	1.5	-	4.2	2.7	-
Terpineol-4-ol	1,177	-	-	0.3	-	1.1	0.2
Camphor	1,185	0.3	-	1.1	0.2	-	0.5
Pulegone	1,195	-	0.2	-	-	0.7	-
Carvacrol methy ether	1,248	2.5	3.1	1.9	4.2	0.9	2.9
Carvacrol	1,299	26.4	25.2	23.3	25.0	34.1	32.1
Eugenol	1,336	-	-	0.1	-	1.2	0.2
α-copaene	1,365	1.3	0.4	-	-	-	-
β-caryophyllene	1,433	0.3	-	2.4	0.2	-	0.6
Aromadendrene	1,452	-	0.4	-	-	0.3	-
γ-muurolene	1,478	1.2	-	0.3	-	0.6	0.2

(table 2 continued)

Compounds		*S. boissieri*			*S. hortensis*		
	RRI	Şanlıurfa	Bingöl	Malatya	Adıyaman	Diyarbakır	Elazığ
Germacrene D	1,480	0.1	1.2	-	2.1	0.1	1.4
β-bisabolene	1,485	-	0.4	0.5	-	0.5	-
α-humulene	1,490	-	-	0.1	-	-	0.2
Trans-carveol	1,510	-	0.2	-	0.4	0.3	-
α-amorphene	1,515	1.2	-	0.3	-	-	0.3
δ-cadinene	1,528	-	0.2	-	0.1	-	-
α-terpineol	1,555	0.4	-	-	-	0.2	-
Bicyclogermacrene	1,562	0.2	-	0.5	-	1.1	0.3
Carvacrol methyl ether	1,580	0.5	0.9	1.2	0.1	0.7	1.6
β-cubebene	1,680	-	-	1.3	-	-	-
Thymol	1,715	3.6	6.4	8.9	28.2	20.2	28.1
Geranyl acetate	1,725	0.2	1.3	-	-	-	0.4
Geraniol	1,790	-	0.5	0.4	0.3	-	-
Isoledene	1,809	0.1	0.2	0.3	-	0.7	0.6
Caryophylleneoxide	1,930	0.1	-	0.1	0.9	-	0.5
Hexadecanoic acid	1,942	-	0.1	-	0.2	0.1	-
Benzene-1-methyl-4	1,960	-	0.2	0.5	-	0.3	-
Spathunelol	1,969	0.2	-	-	0.6	-	0.6
α-cadinol	2,025	1.2	0.1	-	-	0.3	-
β-eudesmol	2,062	-	1.1	0.2	0.6	-	0.3
Total		90.80	91.31	92.67	93.20	92.85	92.42

Table 3 Main constituents of *Satureja* taxa from literature (%).

Main constituents	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Carvacrol	40.8	67.0	-	5.4	9.2	1.5	6.6	4.0	53.5	52-56	45.7	4.1	30.9	0.4	1.0	44.9	13.2	68.8	29.4	77.0
γ-terpinene	26.4	15.3	23.9	0.4	14.5	3.3	3.4	13.7	4.2	5.9	8.1	-	14.9	6.4	29.3	4.3	-	5.5	3.7	2.7
p-cymene	14.5	6.7	7.3	12.3	14.6	6.1	9.4	22.1	17.4	17.0	12.6	3.8	10.3	25.8	14.7	21.6	3.5	7.3	15.1	4.7
Thymol	-	0.2	44.5	6.2	37.3	62.1	62.6	35.1	0.2	2.2	3.9	3.6	26.5	8.1	32.3	9.01	28.0	0.5	29.8	0.7

1: *S. boissieri* [23]; 2: *S. hortensis* [24]; 3: *S. bachtiarica* [25]; 4: *S. macrantha*; 5: *S. spicigera* [26]; 6: *S. atropatana*; 7: *S. mutica* [27]; 8: *S. spicigera* [28]; 9: *S. pilosa*; 10: *S. icarica* [29]; 11: *S. Montana*; 12: *S. cuneifolia* [30]; 13: *S. mutica*; 14: *S. macrantha*; 15: *S. intermedia* [31]; 16: *S. cuneifolia* [32]; 17: *S. bachtiarica*; 18: *S. khuzistanica*; 19: *S. mutica*; 20: *S. rechingeri* [33].

Carvacrol was detected as one of the major compound in the two studied *Satureja* species from all localities (Table 2). Similarly, carvacrol was found as major compound of *S. boissieri* (40.8%) [23], *S. hortensis* (67.0%) [24], *S. pilosa* (53.5%) and *S. icarica* (52-56%) [29]; *S. bachtiarica* (13.2%), *S. khuzistanica* (68.8%), *S. mutica* (29.4%) and *S. rechingeri* (77.0%) [33]. It is noteworthy that in the composition of *S. bachtiarica* Bunge, carvacrol was not determined [25] or determined with very low amounts in study pattern of *S. atropatana* Bunge [27]. *S. macrantha* C.A.Mey and *S. intermedia* C.A.Mey [31].

γ-terpinene (30.4%, 26.5% and 32.1%) was determined as main compound from *S. boissieri* in Şanlıurfa, Bingöl and Malatya species. Also same component (10.1%, 11.3% and 9.4%) was found as main constituent from *S. hortensis* in Adıyaman, Diyarbakır and Elazığ samples, respectively. As we can see in the Table 3, γ-terpinene was the main compounds of some *Satureja* taxa. *p*-cymene was found principal constituents of *S. boissieri* (10.5%, 13.2% and 10.4%) from Şanlıurfa, Bingöl and Elazığ samples, respectively (Table 2). Like our results, this compound also has been detected as principal constituent in *S. spicigera* (22.1%) [25]. *S. icarica*

(17.0%) [29] and *S. cuneifolia* (21.61%) [32] oils (Table 3). On the other hand, this compound is not the principal constituent in *S. hortensis* from Adıyaman, Diyarbakır and Elazığ samples (Table 2), *S. cuneifolia* [30] and *S. bachtiarica* [33] species (Table 3). Thymol was determined a high percentage of *S. hortensis* from Adıyaman (25.0%), Diyarbakır (34.1%) and Elazığ (32.1%) samples (Table 2). It is interesting that this compound has not been detected in *S. boissieri* [23] and was determined low percentages in three different localities of *S. boissieri* from Turkey (Table 2). The main differences between the studied samples is a high percentage of *p*-cymene in *S. boissieri* than *S. hortensis* and high percentage of thymol in *S. hortensis* than *S. boissieri* samples (Table 2).

In conclusion, the studied *Satureja* species synthesized many similar compounds in their essential oils that could be justified by the similar ecological conditions of their habitat. However, taking into account of the differences referred to in some minor constituents major constituents (carvacrol, γ-terpinene) of *Satureja* taxa in literature and studied samples are generally the same. So chemical taxonomic results of literature species could be confirmed by our chemical data. The comparison between studied samples from different localities of Turkey evidenced a similarity at least with reference to the presence of the main constituents: carvacrol and γ-terpinene was among the principal one in all studied taxa. Also the percentages of *p*-cymene and thymol were comparable. The only differences between the studied taxa were substantially due to *p*-cymene: found only high percentages in *S. boissieri* samples of *Şanlıurfa* (10.5%), Bingöl (13.2%) and Malatya (10.4%) samples, also only high percentages in *S. hortensis* samples of Adıyaman (28.2%), Diyarbakır (20.2%) and Elazığ (28.1%) samples (Table 2). It is possible to say that, *S. boissieri* and *S. hortensis* showed carvacrol and γ-terpinene chemotype in Eastern Anatolian region of Turkey. Although the chemical composition of the essential oil of *Satureja* taxa showed differences in respect to some constituents owing to the genetic, local, climatic and seasonal factors etc., but major compounds of *Satureja* taxa are generally the same in currently studied as in the literature samples (Tables 2 and 3).

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