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Chemical Composition of The Essential Oil of *Satureja Spicigera* at Different Developmental Stages from Central Black Sea Region, Turkey

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Satureja spicigera (Lamiaceae) is an aromatic perennial plant and grows wildly in Black Sea Region of Turkey. This species traditionally has been used in traditional medicine, to treat various ailments and infectious diseases. The aim of this study was to compare the chemical composition the essential oil that was obtained from *Satureja spicigera* in different developmental stages in Central Black Sea Region, Turkey. Plant material was harvested at three different phenological stages (pre- flowering, full- flowering and post- flowering period) of the life span of the *Satureja spicigera*. The essential oil of *S. spicigera* was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The hydrodistillation of aerial plant that were collected at pre- flowering, full- flowering and post- flowering period resulted in essential oil yields of 0.64%, 1.17% and 0.12%, respectively. Thirty-six compounds representing 90.83–97.81% of the oils were characterized. Throughout the growth stage of the plant, the main components were found to be carvacrol (32.77-49.11%), thymol (3.04 – 13.21%), γ-terpinene (3.11-13.33%) and ısothymol methyl ether (3.65-11.98%). While carvacrol content decreased during vegetation period, isothymol methyl ether level increased. Thymol and γ-terpinene reached that its highest values during full-flowering stage. The results obtained from this study showed differences compared to pre-flowering, full-flowering and post- flowering period on essential oil composition.

Key words: Salıpazarı, Essential oil, Distillation, Satureja spicigera, Carvacrol, Thymol

Turkey hosts many plant species thanks to its geographical location and has an important position in the export of essential oil rich plants. It ranks the top in the world in terms of thyme, one of the most important of these plants. Thyme, an important medicinal and aromatic plant that contains essential oils, belongs to the *Lamiaceae* family and has the species of *thymus, origanum,* and *satureja.* There are about 45-50 types of phenolic monoterpene compounds, mainly carvacrol and thymol, in the composition of thyme. Therefore, the active ingredient in thyme varies depending on its type and environmental and climatic factors (Kan, 2008; Baydar, 2013). The genus Saturaje (*Lamiaceae*), a perennial, horizontally-growing plant in the form of a shrub, is seen in the temperate and subtropical climates of the Mediterranean, Europe, West Asia, North Africa, Canary Islands, South America, and Iran-Turan Region. It is used extensively in the industries of food, medicine, and cosmetics thanks to its thymol and carvacol content (Montaz and Abdollahi, 2010; Estaji *et al.,* 2018). Satureja has 30 main species and is mostly seen in a limited area extending to the Mediterranean and Iran-Turan phytogeographical regions. *Satureja* belongs to the tribe *Mentheae* within the subfamily *Nepetoideae* and includes about 284 genera in the world. The genus of satureja belonging to the family *Lamiaceae* has 15 species in total in Turkey, and 5 of them are endemic (Baydar, 2013). Satureja species can be grown in almost every region of Turkey; therefore, it is popularly known by many local names such as *sivri kekik* (pointed thyme), *kara kekik* (black thyme), *Trabzon kekiği* (Trabzon thyme), and *süpürge kekiği* (broom thyme) (Satıl *et al.,* 2008; Katar *et al.,* 2011). The species "*satureja spicigera*" is seen in rocky areas, flat parts of mountainous areas, and scrublands at up to 1500 meters above sea level (Eminagaoglu *et al.,* 2007). Thanks to the variability in their chemical and aromatic properties depending on species and regional differences, *Satureja species* are widely used in the industries of food (spices, aroma in beverages, etc.), medicine, and cosmetics, and in the treatments of muscle pain, cramps, nausea, indigestion, stomach and intestinal disorders (Cavar *et al.,* 2008; Momtaz and Abdollahi, 2010). Carvacol and thymol, which are produced extensively by *satureja species*, have antibacterial, antifungal, antihelmintic, insecticidal, analgesic, antiseptic, and antioxidant effects (Baytop, 1999). Moreover, thanks to the antibacterial and antifungal properties of their essential oil and extract, *satureja species* exhibit a strong inhibition effect on a wide variety of bacteria and fungi that affect humans, foods, and plants (Gulluce *et al.,* 2003; Baydar *et al.,* 2004; Boyraz and Özcan, 2006).

Essential oil and extract contents of *Satureja species* vary depending on genetic factors, seasonal transitions and regional climatic factors in the process of growing in nature. Several studies in the literature reported some results confirming the effect of these factors (Beaulieu *et al.,* 2007; Garcia *et al.,* 2008; Sefidkon *et al.,* 2007; Runyoro *et al.,* 2010; Hadian *et al.,* 2011). *Satureja species*, seen in the regions starting from temperate coasts to inlands at an altitude range of 20-1500 m, are sensitive to drought and bitter cold. It was reported that the *Satureja spicigera* species collected from Artvin region predominantly contained carvacol (42.5%), but a small amount of thymol (0.3%), which proves that this species is compatible with the conditions of Black Sea region, a temperate climate, and that the carvachol content increases depending on the climate (Davis, 1982; Eminagaoglu *et al.,* 2007; Sotto *et al.,* 2008). There is a limited number of studies on *S. spicigare* species in the Black Sea region. Considering the fact that today synthetic and chemical drugs cause irreversible damage to people, and essential oil and its compounds can be used in the treatment of many serious diseases such as cancer; microbiological and pharmacological studies should be carried out to identify the active ingredients of plants to be used in natural drugs, instead of synthetically produced drugs (Sotto *et al.,* 2008; Dagcı and Dıgrak, 2005). Recent years witnessed an increasing interest in extracts and essential oils of medicinal plants, which can be an alternative (as a preservative) in the treatment of many diseases and in the production of healthy foods.

The purpose of this study was to examine the essential oil content and compounds of *Satureja spicigera*, a naturally grown plant in the central Black

Sea region of Turkey that is used in medicine thanks to the antioxidant properties of its essential oils, at different growth stages (pre-flowering period, flowering period, and post-flowering period) considering the climatic and regional differences.

MATERIALS AND METHODS

Plant Material:

The above ground parts of *Satureja spicigera* were collected at different developmental stages including pre-flowering, flowering, and post-flowering in 2018 in the vicinity of Salıpazarı valley (about at 500 m height), Samsun, Turkey. The taxonomic identification of the plant material was confirmed by Department of Biology, Gazi University, Ankara, Turkey. The collected plant material was dried in shadow and ground in a grinder with a 2 mm in diameter mesh. The collected samples were dried in a semi-shaded, airy room at room temperature to be used in analysis of essential oil, and 100 g sample bags were placed on each sample for analysis, and information such as collection time, place and altitude was recorded and labeled.

Isolation of the essential oil:

Essential oil analysis was carried out in the laboratory of the Department of Field Crops, Faculty of Agriculture, Atatürk University. The air-dried and ground aerial parts of the plants were distilled in water using a Clevenger-type apparatus for 3 hours. The obtained essential oil (EO) was dried over anhydrous sodium sulphate and, after filtration, stored at +4 ^{0}C until being tested and analyzed (Linskens and Jackson, 1997).

GC-MS analysis conditions:

The fatty acids components of the obtained essential oils were determined in the Agilent Technologies 7890B GC system and Angilent Technologies MSD/5977B gas chromatography (with Ms detector) in the Central Laboratory of Bayburt University. An Agilent J&W GC column (60.0 m f x 0.25 mm, 0.25 µm film thickness) was used, and helium (10 psi flow rate) was used as the carrier gas. Primarily obtained essential oil samples were diluted with hexane at a ratio of 1:50 for analysis. The diluted essential oil samples were injected as 1 µL with a split ratio of 1:40. The injection block temperature was set at 240 ^{o}C and the detector temperature at 250 ^{o}C. In order to realize the fatty acid component separation, the column temperature program was set at 60 °C (10 minutes), then increased from 60 °C to 220 °C by 4 °C min^{-1}, and held at 220 °C (10 minutes). An electron ionization system with a scanning range (m/z) of 35-450 amu and an ionization energy of 70 eV was used for GC-MS detection. The data of Wiley7n, Oil Adams and Nist05 libraries were taken as a basis in the diagnosis of essential oil components of the samples. The percentages of the obtained components were defined using an FID detector and an MS detector.

Essential Oil Yield:

Essential oil contents of the *Satureja spicigera (C. Koch)* Boiss. collected from nature at different stages of development including pre-flowering, post flowering, and post-flowering were found to be 0.67%, 1.17%, and 0.12%, respectively.

Statistical Analysis:

The data obtained from the study was subjected to Duncan multiple comparison testing using SPSS package program. The statistical data obtained were presented as mean ± standard error.

RESULTS AND DISCUSSION

Table1 gives the GC/MS analysis results for 36 essential oil components representing the essential oil content range of 97.81-90.83% of the *satureja spicigera* collected at different growth stages from the north of Turkey. The most important component of the *Satureja spicigera* species was found to be carvacrol with 49.41%, 39.16%, and 32.77%; followed by γ-terpinene with 13.15%, 13.33%, and 3.11%; thymol with 3.04%, 13.21%, and 9.62%; and caryophyllene with 7.25%, 3.94%, and 9.25% at pre-flowering, flowering, and post-flowering periods, respectively (Figure 1).

Carvacrol, which determines the feature of thyme especially in *Satureja spicigera* species, was found to exist with the highest content of 49.41% in the pre-flowering period, while its content decreased in the flowering (39.16%) and post-flowering (32.77%) periods. The contents of the thymol and γ-terpinene components showed a certain increase until flowering period and then decreased. The content of caryophyllene increased in the pre-flowering period, decreased in the flowering period, and then increased again in the post-flowering period. These results show that the big difference between the essential oil components was between the pre-flowering and flowering periods. *Saturaje spicigare* species are categorized into two: carvacrol and thymol types. The *S. spicigare* species in Iran was found to have a tyhmol content of 35.1% in the oil component. On the other hand, carvacrol was reported to be the main component in the species in Turkey. So, it was shown that there were differences between different *Satureja* types in terms of chemical composition of oil (Sefidkon and Jamzad, 2005). In the literature, the mean carvacrol content was reported to be within the range of 42.69-51.15% (Tumen and Başer, 1996; Kurcuoğlu *et al.,* 2001; Eminagaoglu *et al.,* 2007; Grosso *et al.,* 2009; Bahtiyarca Bağdat *et al.,* 2010; Farzaneh *et al.,* 2015). Our results were similar to those in the literature. The chemical composition of *Satureja spicigera* species varies depending on the genotype of the plant, ecological conditions of the region where they grow, climate, and seasonal factors. Actually, it was reported in the previous studies that the content of carvacrol/γ - terpinene changed under water stress (Baher *et al.,* 2002), the carvacrol content (42.5%) of the *S. spicigare* species collected from Artvin region was high (Eminagaoglu *et al.,* 2007), and the environmental conditions during the plant growth stages affected the physiological characteristics of the plant, and the aging of plant organs due to the prolongation of the vegetation period affected the chemical components of the plant (Lakusic *et al.,* 2013; Mammadov, 2014). Since the carvacrol content was found to be high in the *Satureja spicigera* species collected from the Black Sea region that has a rainy climate, it can be asserted that the rainfall positively affected the carvacrol component. Recent studies showed that carvacrol component, which is present in *Satureja spicigera* species with a high rate, has some antioxidant, antibacterial, antifungal, anticancer, hepatoprotective, spasmolytic, and vasorelaxant effects (İpek *et al.,* 2005; Suntres *et al.,* 2015; Sharifi-Rad *et al.,* 2018; Khoshbakht *et al.,* 2020).

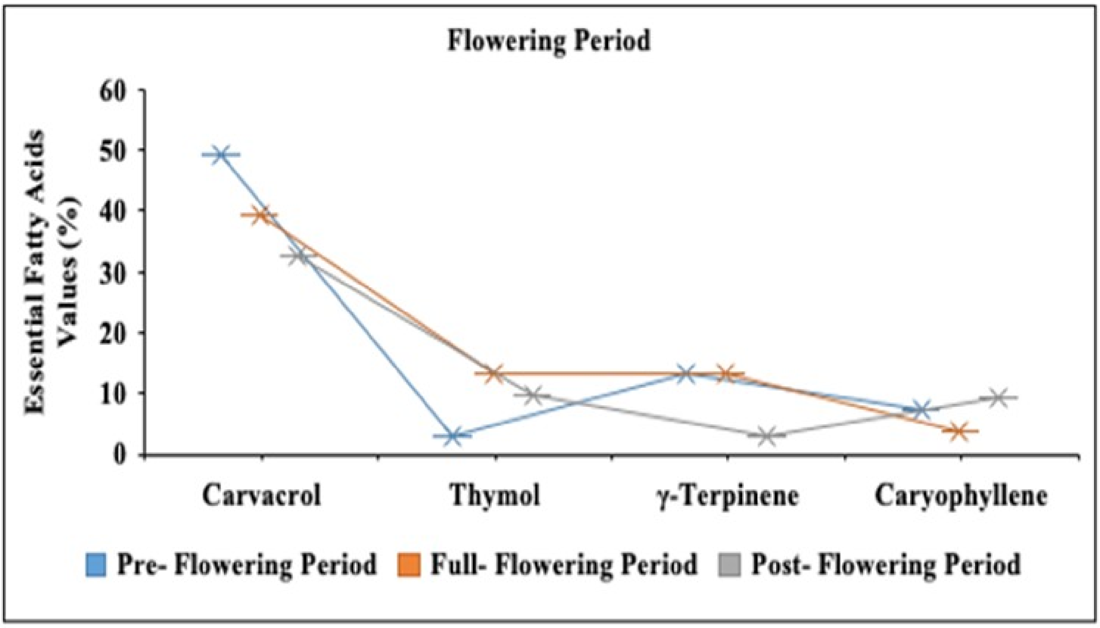

Figure 1. The most important fatty acid parameters of *Satureja spicigare* species from different growth periods

Table 1. Essential oil components of different growth periods of the *Satureja spicigera* species that grow naturally in the Samsun-Salıpazarı

Components	RI	Flowering Period			Method of identification
		Pre- Flowering Period	Full- Flowering Period	Post-Flowering Period	
α-Thujene	928	0,31	1,07	-	GC, MS, RI
1-Octen-3-ol	959	0,35	0,88	-	GC, MS, RI
β-Myrcene	979	0,65	1,23	-	GC, MS, RI
α-Phellandrene	997	-	0,30	-	GC, MS, RI
δ-3-Carene	1003	1,42	-	-	GC, MS, RI
α-Terpinene	1016	-	2,34	-	GC, MS, RI
p-Cymene	1027	4,59	10,58	4,17	GC, MS, RI
(Z)-β-Ocimene	1034	1,69	1,03	-	GC, MS, RI
γ-Terpinene	1047	13,15	13,33	3,11	GC, MS, RI
Trans-sabinene hydrate	1054	0,23	0,42	-	MS, RI
α-Terpinolene	1078	-	0,15	-	GC, MS, RI
Borneol	1148	1,11	0,59	-	MS, RI
Terpinen-4-ol	1161	0,35	0,52	0,49	GC, MS, RI
Isothymol methyl ether	1231	3,65	4,16	11,98	GC, MS, RI
Thymol	1266	3,04	13,21	9,62	GC, MS, RI
Carvacrol	1278	49,41	39,16	32,77	GC, MS, RI
(-)-.β.-Bourbonene	1408	0,15	0,10	1,12	MS, RI
Caryophyllene	1424	7,25	3,94	9,25	GC, MS, RI
β -Copaene	1428	0,17	0,10	0,49	GC, MS, RI
α-Amorphene	1431	-	0,57	1,47	GC, MS, RI
α-Copaene	1437	1,31	-	-	GC, MS, RI
Aromandendrene	1439	0,66	0,36	0,53	GC, MS, RI
α-Humulene	1456	0,46	0,25	0,78	GC, MS, RI
δ-Muurolene	1482	0,80	0,34	0,80	GC, MS, RI
Germacrene D	1490	0,71	0,17	1,33	GC, MS, RI
δ-.Selinene	1498	-	-	0,36	MS, RI
β.-Bisabolene	1500	2,51	0,54	1,05	GC, MS, RI
Viridiflorene	1505	0,59	0,27	0,72	GC, MS, RI
γ-Cadinen	1522	tr	tr	-	GC, MS, RI
Spathulenol	1569	0,38	0,68	3,70	GC, MS, RI
Caryophyllene oxide	1576	0,98	1,26	5,76	GC, MS, RI
.tau.-Cadinol	1628	0,16	0,10	-	GC, MS, RI
α.-Cadinol	1641	0,14	tr	0,89	GC, MS, RI
α-Bisabolol	1694	tr	-	-	GC, MS, RI
m-Camphorene	2352	-	-	0,44	GC, MS, RI
(E)-β-Farnesene	2352	-	0,16	-	MS, RI
Total		96,22	97,81	90,83	
Ratio of essential oil		0.67	1.17	0.12	

GC: co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (Adams, 2007); RI: identification based on comparison of retention index with those of published data (Adams, 2007); tr: traces (less than 0.01%). a Retention index relative to n-alkanes on SGE-BPX5 capillary column

Plants can produce secondary metabolites qualitatively and quantitatively depending on the geographical region and growing period. Thymol and carvacrol are the most important components that give the thyme features to plants. These components are used in foods, cosmetic products, and drugs for the treatment of diseases, and the most important factors affecting their quantity and quality are the collection period and geographic, climatic, and genetic features. In this study, carvacrol was found to be the component that determined the thyme feature in the *Satureja spicigera* species grown naturally in the Black Sea region. Carvacrol component has a much higher antioxidant effect than various synthetic antioxidants. It is also known to have several characteristics such as antibacterial, antifungal, antimutagenic and antitumor effects. Therefore, the result of this study can shed light on the features of the *Satureja spicigera* species, which were collected from different geographic regions in different periods.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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