VOLATILE OIL COMPOSITION OF *TEUCRIUM* SPECIES OF NATURAL AND CULTIVATED ORIGIN IN THE LAKE DISTRICT OF TURKEY

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Abstract. The genus *Teucrium* belongs to the *Lamiaceae* family. *Lamiaceae* is one of the important plant groups because most members of this group are used as medicinal and aromatic plants. In this study, the leaves and flowers of *Teucrium chamaedrys subsp. chamaedrys* and *Teucrium polium*, which were taken from both their natural habitat and cultivars, were analysed by SPME and GC-MS in two vegetation periods. The amount and the composition of volatile oil of cultivated plants were compared with plants from natural areas. Caryophyllene (12.20-23.74%) was the most abundant component in all samples of *Teucrium chamaedrys* except in samples from natural habitats of the first vegetation period. Moreover, 2-hexen-1-al (12.79-13.98%), germacrene-D (11.99-19.60%) and α-pinene (3.80-11.68%) were the dominant components of the *T. chamaedrys,* while in *Teucrium polium* samples, β-myrcene (19.52-25.28%) was the common component in both sampling area of the first vegetation period, limonene (30.87-31.79%) had the highest amount in the second vegetation period. Besides, α-thujone (3.13-18.87%), α-pinene (0.94-17.93%) and germacrene-D (4.31-15.63%) were detected as major components in samples from both natural habitats and cultivars of *T. polium*.
Keywords: *Lamiaceae, SPME, GC-MS, Myrcene, Pinene, Germacrene*

Introduction

Volatile oils can be obtained from plants or herbal drugs, and they have distinctive odor, taste besides color, appearance and have volatile compounds at room temperature. It is known that volatile oil contains a large amount of terpenes and a small amount of alcohols, aldehydes, esters and phenolics (Edris, 2007). There are many ways to reveal the volatile oil components of plants. Although distillation and extraction are quite well-known methods, Solid Phase Micro Extraction (SPME) is used as it is new and easily applicable. It is suitable for both combining the sample preparation, extraction and concentration sections in a single step and for providing significant gains in processing time and cost. To identify volatile organic compounds in the samples SPME can be combined with Gas Chromatography-Flame Ionization Detector (GC-FID) or Gas Chromatography-Mass Spectrometry (GC-MS) (Vas and Vekey, 2004). There are many studies about volatile oils (Tumen et al., 2010; Fakir et al., 2014; Dönmez and Salman, 2017), chemical composition of bush, shrubs or especially medicinal and aromatic plants are gaining interest because of the extension of the area of usage (Bahramikia and Yazdanparast, 2012; Özcan et al., 2013).

Volatile oils can be used in many areas and volatile oil of medicinal and aromatic plants has gained an important role from the aspect of science and economics. Using plants as a treatment of some illness is common all over the world since ancient times. Moreover, volatile oils are used to prepare primitive medicine and drugs by folk. In recent years, the use of volatile oils has also increased with the increase of interest in aromatherapy and phytotherapy, which is a branch of alternative medicine (Rangahau, 2001).

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The *Lamiaceae* family grows almost everywhere in the Mediterranean climate regardless of the plant type and height. *Lamiaceae* family has 400 genus and 3200 species around the world, and it is represented by 45 genus and more than 546 species in Turkey. Most of the members of the *Lamiaceae* family *Lavandula sp., Mellissa sp., Mentha sp., Origanum sp., Thymus sp., Salvia sp.*), due to their ornamental leaves and flowers, are rich in volatile oils. It has a great importance in some industries such as landscape, medicine, pharmacy, food, cosmetics and perfumery and there are many studies on these plants (Başer, 2008; Kargıoglu et al., 2008; Kahraman et al., 2009; Dönmez, 2016; Wesolowska and Jadczak, 2019; Alsaraf et al., 2021; Virchea et al., 2021). *Teucrium chamaedrys* subsp. *chamaedrys* and *Teucrium polium* are species which belong to the *Lamiaceae* family. Although there are studies on the medicinal and aromatic properties of these species (Katayoun et al. 2005; Bağcı et al., 2010; Belmakki et al., 2013; Özcan et al., 2013; Raei et al., 2013), the changes in the composition and amount of volatile oil after cultivation studies are not known. For this reason, these two taxa were evaluated in the study.

The genus *Teucrium* consists of about 100 species and it can be seen in temperate regions of Asia, Europe and North Africa (Mabberley, 1997; Bukhari et al., 2015). The species belonging to this genus have been used for the treatment of various diseases since ancient times, due to the various active substances they contain. It is known that some species of this genus, which are among the medicinal and aromatic plants, are used in the treatment of diabetes in different countries (Baytop, 1991; Dönmez, 2019; Dönmez and Önal, 2019).

Teucrium chamaedrys L. subsp. *chamaedrys* L. can be found up to 1800 m. from sea level in the European-Siberian region. It can also be seen in the western part of the Mediterranean, Agean, Marmara and Central Anatolian part of Turkey in forest clearings, steep cliffs, slopes and barren pastures (Tekin, 2007). It is a perennial woody plant which has 20-30 cm diameter and 15-30 cm length. Leaves have 1.4 × 1.8 cm in rectangle aspect, flowerbeds are 0.5 × 1.2 cm and they have pink color. Virgo flowers are 12-20 cm long and the flowering period is 2 months (June-July). It is also known that the vegetation period is 6 months (March-September).

Teucrium polium is widely distributed in the Mediterranean countries and in the Middle East. It grows naturally in all regions in Turkey up to 2050 m from sea level and can be found in bushes and maquies, steppes, fields, rocks and arid slopes (Davis, 1982; Tekin, 2007), *Teucrium polium* has 8-15 cm length and 10-40 cm diameter. Moreover, the leaves are 0.15 × 0.75 cm and rectangular, the flowers are 0.4 × 0.7 cm and whitish, Virgo flowers are 5-12 cm long. The structure of leaves and body are more effective in the visual characteristics of the plant compared to its flower. The vegetation duration is 6 months (March-August) and the flowering period is 4 months (April-July). (Elmasri et al., 2016).

In this study, it was aimed to determine the composition and the amount of volatile oil and the yield of the same plant species in both natural habitat and their cultivars after the cultivation of *Teucrium chamaedrys* subsp. *chamaedrys* and *Teucrium polium*.

Materials and methods

Materials

Teucrium chamaedrys subsp. *chamaedrys* and *Teucrium polium*, belonging to *Lamiaceae* family, were used for determining volatile oil yield and composition. In the

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vegetation period of 2015, field studies were carried out and the areas where the plants grow naturally were determined. *Teucrium chamaedrys* subsp. *chamaedrys* was taken from Aziziye, Burdur and *Teucrium polium* was removed from another region of Aziziye, Burdur. During the flowering period (July), the plants were collected with their roots and planted in Isparta Süleyman Demirel University (SDU) Botanical Garden, named as cultivation area. Environmental characteristics of both areas can be seen in *Table 1*. Experimental plots in the cultivation area were established in SDU Botanical Garden in accordance with the “Randomized Complete Block Design” with 3 replications and 25 plants for each replication at a depth of 30 cm with a distance of 20 cm between plants (*Fig. 1*). The plants were irrigated every other day between June and August. Survival rate of *Teucrium chamaedrys* subsp. *chamaedrys* and *Teucrium polium* was 96% and 72%, respectively, at the end of the 2016 and 2017 vegetation period. In 2016 and 2017, during the flowering period (July) of the plants, approximately 10 samples were taken simultaneously from both the natural and cultivation area and stored at -24 °C until volatile oil analysis.

Table 1. *Details and environmental characteristics of sampling areas*

		Longitude (E)	Latitude (N)	Altitude (m)	Relative humidity (%)	Min. temp. (°C)	Max. temp. (°C)	Average annual temp. (°C)	Average annual rainfall (mm)
N.H.	T. c.	30° 15’ 25”	37° 25’ 30”	1600	55	-15.0	41.0	13.3	429
	T. p.	30° 14’ 31”	37° 24’ 40	1200					
C.A.		30°31’ 39	37°50’ 46”	1025	61	-21.0	38.7	12.3	508.3

N.H.: Natural Habitat (Burdur-Aziziye), C.A.: Cultivation area (Isparta-SDU Botanical Garden), T.c.: *Teucrium chamaedrys* subsp. *chamaedrys*, T.p.: *Teucrium polium*

Figure 1. *A view of T. chamaedrys subsp. chamaedrys and (a) and T. Polium (b)*

Volatile oil yield

In order to determine volatile oil yield, hydrodistillation with Clevenger apparatus (European type Clevenger apparatus, Ildam Cam Ltd. Ankara-Turkey) was used. The material is directly immersed in water, and it had direct contact with hot water and heat. For determining volatile oil yield of each species, 100 g ground samples were submitted to hydrodistillation for 5 h using a Clevenger apparatus. Sampling and hydrodistillation with Clevenger apparatus can be seen in *Figure* 2. Volatile oil yield was calculated as ml/100 g samples (Tümen et al., 2010).

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Figure 2. *Sampling and hydrodistillation*

SPME and GC-MS analyses

The leaves and flower samples that were collected from both natural habitat and cultivars were put into paper packages and transferred to the laboratory in the same day to avoid exposure to sunlight and samples were subjected to solid phase microextraction (SPME). 2 g of samples were placed into a 10 ml vial. After incubation for 30 min at 60 °C, SPME fibre was pushed through the headspace of a sample vial to absorb the volatiles and then inserted directly into the injection port of the GC-MS (Shimadzu 2010 Plus GC-MS with the capillary column, Restek Rxi®-5Sil MS 30 m × 0.25 mm, 0.25 µm) at a temperature of 250 °C for desorption (5 min) of the adsorbed volatile compounds. The constituents were identified using retention times of standard substances by aligning mass spectra with the data given in the Wiley, NIST Tutor, FFNSC library.

Results and discussion

Analyses for getting volatile oil and determining volatile oil yield of both *Teucrium* species was performed in two vegetation periods (2016 and 2017) on the samples gathered from their natural habitat and cultivars at the same time.

Volatile oil yield of both *Teucrium* species was calculated after hydrodistillation (*Table 2*). It was found that volatile oil yield of *Teucrium chamaedrys* subsp. *chamaedrys* was found very close in both samples during two vegetation periods. It was determined as 0.20 ml/100 g in natural habitat plants and 0.30 ml/100 g in cultivars in the first vegetation period (2016). It was 0.20 and 0.35 ml/100 g, respectively, in the second vegetation period (2017).

Table 2. *Volatile oil yield of natural habitat and cultivars of Teucrium species*

Volatile oil yield (ml/100 g)	First vegetation period		Second vegetation period	
	Natural habitat	**Cultivar**	**Natural habitat**	**Cultivar**
Teucrium chamaedrys	0.20	0.30	0.20	0.35
Teucrium polium	0.80	0.90	0.85	0.90

Volatile oil yield of *Teucrium polium* was determined higher than that of the *T. chamaedrys*. In the first vegetation period, it was found 0.80 ml/100 g in natural habitat plants and 0.90 ml/100 g in cultivars for *T. polium*. It had also similar results for the

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second vegetation period. Natural habitat plants had 0.85 ml/100 g and cultivars had 0.90 ml/100 g volatile oil yield in the second vegetation period for *Teucrium polium*.

The volatile components in the leaves and flowers of *Teucrium* species collected from the sampling plots were identified through gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). The amount and the composition of volatile oil of *Teucrium chamaedrys* L. subsp. *chamaedrys* L. can be seen in *Table 3*. 41 components in the samples from both natural habitat and cultivars of the first vegetation period were determined. However, 44 and 39 components, respectively, were identified in the second vegetation period. Although limonene (13.49%) and 2-hexen-1-al (12.79%) were determined as the dominant components in the samples from natural habitat, caryophyllene (19.82%) and germacrene-D (14.49%) had the highest concentration in the samples from cultivars of the first vegetation period. In addition, caryophyllene, the most abundant component, was found as the major component in the samples from both natural habitat (23.03%) and cultivars (23.74%) in the second vegetation period, like in the samples collected in Corsica and Sardinia studied by Muselli et al. (2009). Germacrene-D was the other component having the highest amount in both samples in the second vegetation period as 18.14% and 19.60% respectively. In the studies by Bağcı et al. (2010), Katayoun et al. (2005) and Maccioni et al. (2021) that germacrene-D was found the dominant component. It was previously reported that germacrene-D was also the most abundant component in some *Lamiaceae* species (Dönmez, 2019).

Table 3. *The amount and the composition of T. chamaedrys subsp. chamaedrys volatile compounds (%)*

Compounds	1st vegetation period		2nd vegetation period	
	Amount (%)		Amount (%)	
	Natural habitat	Cultivars	Natural habitat	Cultivars
Acetaldehyde	0.16	-	-	-
Ethanol	-	0.20	0.02	0.08
2-Butenal	0.16	0.30	-	-
3-Methylbutanal	0.28	0.23	-	-
2- Methylbutanal	-	0.23	-	-
2-Propanone	-	-	0.14	0.20
2-Pentanone	0.67	-	-	-
2-ethyl-Furan	0.64	0.42	-	-
cis-3-Methylcyclohexanol	-	-	0.37	-
n-Hexanal	1.00	1.49	0.61	-
2-Hexen-1-al	12.79	13.98	0.13	0.17
3-Hexene-1-ol	1.91	0.66	0.09	0.10
2-Hexen-1-ol	0.30	0.28	-	-
n-Hexanol	0.83	0.74	-	-
2,4-Hexadienal	0.65	0.70	-	-
α-Thujene	-	-	0.09	0.10
α-pinene	7.68	3.80	10.42	11.68
Benzaldehyde	0.50	0.87	0.54	-
Sabinene	0.16	-	0.21	0.24
β-pinene	4.04	-	-	-
1-Octen-3-one	0.32	-	-	-

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Bicycloheptane	-	2.25	5.52	6.34
1-Octen-3-ol	8.74	6.57	0.31	0.36
β-Myrcene	6.98	5.53	0.64	-
Myrcene	-	-	1.21	1.66
2,4-Heptadienal	0.99	0.86	-	-
Ethyl-hexanol	0.32	0.23	-	-
3-Octanone	-	-	0.13	0.26
p-cymene	-	0.19	-	-
Me-Cymol	-	-	0.51	0.73
Limonene	13.49	6.77	16.81	17.72
Benzene acetaldehyde	0.17	0.28	-	-
Benzene	0.16	-	-	-
Benzoic acid	2.29	2.11	-	-
Ocimene	-	0.30	-	-
β- ocimene	0.25	-	0.13	0.26
1,4-Cyclohexadiene	-	-	0.10	0.12
α-terpinolene	0.19	-	-	-
1,6-Octadien-3-ol	0.83	0.61	0.09	0.10
Nonanal	0.16	0.21	-	-
2,4-Octadienal	-	0.24	-	-
l-linalool	-	-	0.19	0.14
α-copaene	0.37	1.08	0.58	0.63
α-Cubebene	0.19	1.04	0.19	0.24
β-bourbonene	2.89	2.51	3.89	4.31
Bicycloelemene	-	-	0.01	0.09
α-Bergamotene	-	-	1.01	0.68
β-cubebene	0.34	1.49	1.64	0.96
α-panasinsen	-	-	1.01	1.37
Caryophyllene	12.20	19.82	23.03	23.74
Caryophyllene oxide	0.82	0.31	0.24	0.30
Germacrene-D	11.99	14.49	18.14	19.60
bicyclogermacrene	0.87	1.12	1.96	1.39
β-Farnesene	-	-	1.32	1.65
Farnesene	-	-	0.09	0.13
Epi-bicyclosesquiphellandrene	0.56	0.30	0.42	0.33
β-Sesquiphellandrene	-	-	0.93	0.07
Cadina-1,4-diene	-	-	0.68	-
γ-Cadinene	0.69	1.33	0.81	0.64
δ-cadinene	-	1.28	-	-
β-selinene	-	-	0.61	0.09
α-Muurolene	0.34	0.37	0.41	0.35
β-Bisabolene	-	0.25	0.26	0.12
α-humulene	1.74	4.24	2.41	2.82
Alloaromadendrene	0.33	0.34	-	-
Camphene	-	-	0.13	0.08
Geranyl nitrile	-	-	0.09	0.07

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Volatile components determined by SPME and GC-MS of *Tecrium polium* can be seen in *Table 4*. While 48 components were determined in the natural habitat of *T. polium*, the amount and the composition of the 46 components from cultivars of *T. polium* were identified in the first vegetation period. β-myrcene was found as the highest component in both natural habitat (19.52%) and cultivars (25.28%). α-thujene and Germacrene-D can also be evaluated as major components of *T. polium*. α-thujene was determined as 18.87% in the samples from natural habitat and Germacrene-D was 15.63% in the samples from cultivars of first vegetation period. In the second vegetation period, 53 components in the samples from natural habitat and 45 components from cultivars of *T. polium* were detected. Limonene was the most abundant component of the second vegetation period and it was determined as 30.87% in the samples from natural habitat and 31.79% in the samples from cultivars. In addition, α-pinene was also dominant component of the second vegetation period with 15.68% in the samples from natural habitat and 17.93% in the samples from cultivars of *T. polium*. In most of the studies on the amount and composition of *T. polium*, β-myrcene, Germacrene-D and α-pinene were found as the components with the highest concentration (Belmakki et al., 2013; Özcan et al., 2013; Raei et al., 2013) like in the present study, however, α-thujone and limonene can also be considered as the significant components. On the contrary, the study of Saleh et al. (2020), 6-epi-shyobunol, t-muurolol, germacrene-D, Delta-cadinene and aromadendrene were found as dominant components in the samples collected from Egypt.

Table 4. *The amount and the composition of T. polium volatile compounds (%)*

Compounds	1st vegetation period		2nd vegetation period	
	Amount (%)		Amount (%)	
	Natural habitat	Cultivars	Natural habitat	Cultivars
Acetaldehyde	-	0.15	-	-
1,1-Ethylacetate	-	-	0.12	0.17
2-methylfuran	-	-	0.01	0.04
Allylbromide	-	-	0.11	0.08
Ethanol	0.25	0.80	-	-
2-Butenal	0.90	0.76	-	-
2-ethyl-Furan	0.26	-	-	-
Valeraldehyde	-	0.17	-	-
cis-3-Methylcyclohexanol	-	0.20	-	-
n-Hexanal	0.44	0.51	0.35	-
2-Hexen-1-al	5.62	4.88	0.2	0.24
3-Hexene-1-ol	0.39	0.56	0.1	0.11
n-Hexanol	0.25	0.26	-	-
2,4-Hexadienal	0.22	-	0.61	-
α-Thujene	18.87	11.43	3.13	3.80
α-pinene	0.94	-	15.68	17.93
2,6,6-Trimethylbicyclohept-2-ene	0.28	0.54	0.11	0.15
Bicyclohex-2-ene,	-	0.16	0.09	0.11
Camphene	-	-	0.2	0.21
Benzaldehyde	0.40	0.81	-	-
Sabinene	9.89	5.68	1.79	2.09
1-Octen-3-one	-	-	0.31	0.43

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Bicycloheptane,	1.16	0.65	4.98	5.87
1-Octen-3-ol	1.17	2.27	0.64	0.33
β-Myrcene	19.52	25.28	3.87	4.58
2,4-Heptadienal,	0.18	0.39	-	-
3-Octanol	0.24	-	-	-
l-Phellandrene	0.17	-	-	-
ethyl-Hexanol	-	0.71	-	-
2,4-Heptadienal,	0.29	0.55	-	-
α-terpinene	1.00	-	0.11	017
p-cymene	2.30	-	-	-
Cyclopentanecarboxyaldehyde	-	1.83	-	-
Cymol	-	-	3.04	2.92
Me-cymol	-	2.65	-	-
Limonene	3.18	4.48	30.87	31.79
Trans-Limonene oxide	-	-	0.42	0.14
Verbenol	-	-	0.21	0.27
4-terpineol	-	-	0.09	0.12
p-allylanisole	-	-	0.4	0.41
Berbenone	-	-	0.22	0.27
Eucalyptol	-	-	0.13	0.19
Ocimene	0.76	0.63	0.08	0.11
β- ocimene	3.52	3.84	0.71	0.73
Cyclopropane	0.18	-	-	-
Spirohexan-5-on	-	0.25	-	-
1,4-Cyclohexadiene	1.07	0.64	0.75	0.81
α-terpinolene	0.33	0.31	-	-
1,6-Octadien-3-ol	-	-	0.42	0.29
Benzene	0.42	-	-	0.55
Tridecane	-	-	0.51	0.24
l-linalool	0.17	-	0.40	0.43
α-thujone	-	-	0.18	0.16
p-Mentha-1,5,8-triene	0.53	0.49	0.96	-
2,4,6-octatriene	0.59	-	0.81	-
3-Cyclohexen-1-ol	0.48	-	0.23	-
α-copaene	0.88	-	0.15	-
Benzoic acid	-	0.65	-	-
α-Cubebene	-	0.20	-	-
β-bourbonene	0.31	1.22	0.61	0.72
β-elemene	0.30	-	0.18	0.22
Germacrene-B	1.81	-	1.19	1.21
α-Bergamotene	0.35	-	0.14	0.17
β-cubebene	-	0.21	-	-
Germacrene-D	4.31	15.63	10.87	11.33
α-gurjunene	-	-	0.09	0.11
1-Cyclopentacyclopropan benzene	-	0.42	-	-
β-Farnesene	9.23	1.98	4.94	5.26

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Epi-bicyclosesquiphellandrene	0.36	0.51	0.28	0.32
Naphthalene	-	0.66	-	0.19
Cadina-1,4-diene	0.29	-	0.87	-
β-selinene	-	0.17	0.15	-
α-cubebene	-	0.72	-	-
bicyclogermacrene	0.54	2.13	1.29	1.39
α-bisabolene	0.60	-	0.55	-
α-muurolene	-	0.37	0.11	0.17
β-Bisabolene	1.29	0.30	-	-
γ-Cadinene	3.14	0.97	0.81	0.91
δ-cadinene	0.19	1.47	0.51	0.63
α-humulene	0.42	-	0.69	-
β-eudesmol	-	0.51	-	-

Conclusions

T. chamaedrys subsp. chamaedrys and *T. polium* were cultivated in 2015. The amount and the composition of the volatile components were identified in 2016 and 2017 by using leaves and flowers of these plant species from both natural habitat and cultivars. It was found that they contained the same components and the amount of components were close to each other in both sampling areas. As a result of the study, it is understood that both *Teucrium* taxa can adapt to the culture area without changing their biochemical properties. This is a particularly important result for these two plant species belonging to the *Lamiaceae* family, which are known to be used as medicinal and aromatic plants. In this direction, it is thought that these plants can be evaluated in industrial scale and can also be used in different industries such as pharmaceuticals and cosmetics due to their chemical composition. While it is evaluated in industrial uses, it will be possible to grow these plants instead of collecting them from nature. Therefore, the biodiversity in the natural habitat will be protected and it will be possible to provide the required plant material sufficiently.

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