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

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Abstract. Leaves and flowers of *Ajuga chamaepitys* subsp. *chia* var. *chia* and *Ajuga orientalis*, which were taken from both their natural habitat and cultivars, were analyzed by SPME and GC-MS. The amount and the composition of volatile oil of cultivated plants were compared with natural area plants. β-pinene was detected as major component in *A. chamaepitys* in both natural habitat and cultivars. It was seen that its amount was between 17.86-25.21%. Moreover, germacrene-D was determined in significant amount (15.40-21.13%) in both samples of *A. chamaepitys*. 2-hexen-1-al was the most abundant component in the natural area (30.89%) and cultivated plants (23.12%) in the first vegetation period of *A. orientalis* but a decrease of almost 80% was determined in the second vegetation period. The amount of 1-octen-3-ol was found as one of the dominant components in *A. orientalis* and no significant difference was observed in both natural habitat and cultivars, during the vegetation periods. In addition, the amount of limonene in *A. orientalis* volatile oil increased in the second vegetation period compared to the first.
Keywords: *Lamiaceae, SPME, GC-MS, pinene, germacrene, Turkey*

Introduction

Volatile oil mainly consists of volatile components. It is fragrant, naturally occurring, colorless or light yellow and it is obtained from plants, leaves, fruits, bark and root parts, which are liquid and easily crystallizable (Ceylan, 1983). It is known that volatile oil contains a large amount of terpenes and a small amount of alcohols, aldehydes, esters and phenolics (Linskens and Jackson, 1997). There are many ways to reveal the volatile oil components of plants. Although distillation and extraction are quite well-known methods, SPME (Solid Phase Micro Extraction) 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. SPME is used to identify volatile organic compounds in the samples by combining with GC or GC-MS (Vas and Vekey, 2004).

Research on the volatile oil components of medicinal and aromatic plants has gained a significant place both scientifically and economically. In recent years, the use of volatile oil has also increased with the increase of interest in aromatherapy and phytotherapy, which is a branch of the alternative medicine (Rangahau, 2001).

Ajuga chamaepitys subsp. *chia* var. *chia* and *Ajuga orientalis* are species which belong to the *Lamiaceae* family. The *Lamiaceae* family grows almost everywhere in the Mediterranean climate without considering 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. Among the species belonging to the *Lamiaceae* family, *Thymus* and *Origanum* have an important position in food industry, *Pogestamon* and *Lavandula* are considered in the perfume industry, and particularly the volatile oil

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of the species of *Ajuga* and *Teucrium* is used for therapeutic purposes (Watson and Dallwitz, 1978; Clive and Stace, 1980; Davis, 1982; Baytop, 1991; Ozkan, 2007).

Ajuga chamaepitys subsp. *chia* var. *chia* can grow up to 2000 m altitudes in Turkey, Crimea, Greece, Palestine, Iran and Northern Iraq (Davis, 1982). It is a herbaceous plant with a 20 cm length, yellow flowering and pleasant fragrant perennial suffrutescens (Baytop, 1999). The length of the shoots is 5-30 cm. The whole plant is covered with white feathers and the flowers are yellow and the flowering time is between April and July (Akcan et al., 2006; Jakovljević et al., 2015; Dönmez et al., 2017).

Ajuga orientalis extends from Crimea to Sicily, West Syria, Cyprus, Caucasus and North West Iran. It can grow up to 2500 m from sea level in Mediterranean, Agean, Black Sea and Central Anatolia Regions in Turkey (Tekin, 2007). It is a perennial herbaceous plant with violet-blue and cream-colored flowers that can be sized up to 140 cm (Yazgın, 2010). The leaves are crossed on the trunk and rich in the lower part of the trunk. The flowering time is between April and May (Dönmez et al., 2017).

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 of the plants and their cultivars after the cultivation of *A. chamaepitys* subsp. *chia* var. *chia* and *A. orientalis*.

Material and method

Material

A. chamaepitys subsp. *chia* var. *chia* and *A. orientalis*, which belong to *Lamiaceae* family, were used for determining volatile oil yield and components. In the vegetation period of 2015, *A. chamaepitys* subsp. *chia* var. *chia* was taken from Aşağıgökdere, Isparta, where the altitude was 324 m (37º32´42´´ N, 30º46´42´´ E) and *A. orientalis* was removed from Kemer, Antalya, from an altitude of 413 m. (36º36´26´´ N, 30º27´53´´ E), with their roots. They were cultivated at Süleyman Demirel University (SDU), Botanical Garden by sowing. Plants, found in natural habitat and cultivars can be seen in *Figure 1*. In the vegetation period of 2016 and 2017, leaves and flowers of these plants, which were taken both from their natural habitat and cultivars at SDU, were used in the analyses of volatile oil production. The study area is located in the transition zone between the Mediterranean and terrestrial climate. According to data of Isparta Meteorological Station, the average annual temperature in the region was 12ºC. While the maximum temperature was 38.7ºC in July, and the minimum was -21ºC in January. The mean annual precipitation amount was 508.3 mm.

Volatile Oil Yield

In order to determine volatile oil yield, hydrodistillation with Clevenger apparatus was used. The material is directly immersed in water and it has 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. Volatile oil yield was calculated as ml/100 g samples.

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

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(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 x 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.

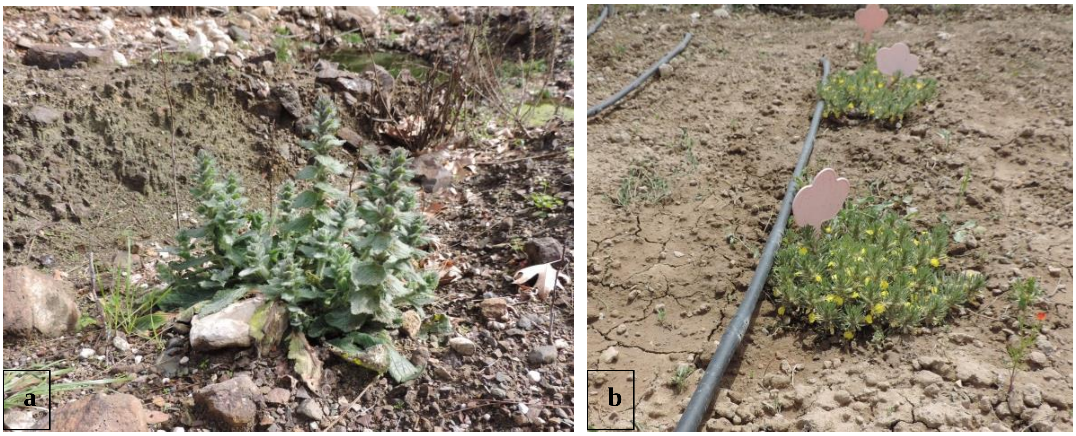

Figure 1. *A view from both natural habitat (a) of A. orientalis and cultivation (b) of A. chamaepitys subsp. chia var. chia*

Results and discussion

Analyses for getting volatile oil and determining volatile oil yield of both *Ajuga* 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 *Ajuga* species was determined after hydrodistillation. It was found that volatile oil yield of *A. chamaepitys* subsp. *chia* var. *chia* was determined very close in both samples during two vegetation periods. It was determined as 0.10 ml/100 g in natural area plants and 0.15 ml/100 g in field area plants in the first vegetation period. Moreover, it was 0.10 ml/100 g in both samples in the second vegetation period.

The volatile components in the leaves and flowers of *Ajuga* 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 *A. chamaepitys* subsp. *chia* var. *chia* can be seen in *Table 1*. 41 components in the samples from natural habitat and 39 components in the samples from cultivars of the first vegetation period were determined. However, 50 and 49 components, respectively, were identified in the second vegetation period. β-pinene was determined as the dominant component in all samples in both vegetation period of *A. chamaepitys* subsp. *chia* var. *chia*. While its amount was 19.15% in natural habitat and 17.86% in the samples from cultivars in the first vegetation period, it was found 22.25% in in natural habitat and 25.21 % in the samples from cultivars in the second vegetation period. Germacrene-D was the second component having highest amount. It was seen that its amount increased in the second vegetation period compared to the first vegetation period.

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Table 1. *The amount and the composition of A. chamaepitys subsp. chia var. chia volatile compounds (%)*

Compounds	1st Vegetation Period		2nd Vegetation Period	
	Amount (%)		Amount (%)	
	Natural Area	Field Area	Natural Area	Field Area
2-ethyl-Furan	0.22	0.43	-	-
Me-acetate	-	-	0.46	0.44
Linalyl acetate	-	-	0.08	0.04
Acetic acid	-	-	0.09	0.13
Me-propyl ketone	-	-	0.04	0.09
Me-heptyl ketone	-	-	0.09	0.12
n-Hexanal	0.48	0.78	-	-
2-Hexen-1-al	4.09	3.39	-	0.08
3-Hexene-1-ol	0.22	0.32	0.45	0.11
2-Hexen-1-ol	-	-	-	0.10
n-Hexanol	-	-	-	0.07
1,4-Cyclohexadiene	-	-	0.54	0.28
2,4-Hexadienal	0.81	0.51	-	-
α-Thujene	0.60	1.93	0.29	0.37
α-pinene	2.37	2.31	2.98	3.45
β-phellandrene	0.31	-	-	-
Benzaldehyde	-	0.20	0.02	0.03
Sabinene	-	0.64	0.29	0.34
β-pinene	19.15	17.86	22.25	25.21
1-Octen-3-ol	4.75	11.69	0.32	0.29
β-Myrcene	10.53	13.33	-	-
Myrcene	-	-	5.15	5.55
Ethyl-hexanol	0.19	1.00	1.52	-
l-Phellandrene	0.37	0.95	-	-
2,4-Heptadienal,	0.37	0.61	-	-
p-cymene	-	1.16	0.98	0.33
Me-cymol	0.14	-	1.23	-
Limonene	4.64	4.36	5.97	6.42
Trans-limonene oxide	-	-	0.41	0.04
Ocimene	0.25	0.23	-	-
β- ocimene	1.83	1.00	1.10	1.37
α-terpinolene	0.22	-	0.05	0.07
1,6-Octadien-3-ol	-	0.75	1.98	-
Nonanal	-	0.26	-	-
Dodecane	-	-	0.12	0.08
Butanoate	-	-	0.02	0.02
l-linalool	0.96	-	0.45	0.56
p-Mentha-1,5,8-triene	0.27	0.27	-	-
2-Pinen-3-one	-	0.22	-	-
2,4,6-octatriene	0.23	-	-	-
Benzoic acid	0.31	-	-	-
Tridecane	0.20	-	0.07	0.07
α-Cubebene	0.53	0.76	0.60	0.69
Copaene	3.71	8.10	-	-
α-Copaene	-	-	10.25	11.56
β-bourbonene	3.10	5.14	4.96	5.03
α-Gurjunene	0.12	-	0.78	0.80
1,2,4,5-tetramethyl-6-methylene-spiroheptane	0.14	-	-	-
β-caryophyllene	-	3.01	1.03	-
α-Amorphene	0.33	-	0.62	-
α-Chamigrene	0.26	-	-	-
p-Allylanisole	-	-	0.02	0.03
Cyclosativene	-	-	0.09	0.08
β-cubebene	-	0.95	0.52	0.15
α-Himachalene	-	0.34	-	-
Caryophyllene	12.17	-	5.82	5.99
Germacrene-D	19.04	15.40	19.63	21.13
β-Farnesene	-	0.48	-	-
Epi-bicyclo sesquiphellandrene	0.69	0.44	-	0.47
bicyclogermacrene	2.57	0.55	0.98	1.08

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Compounds	1st Vegetation Period		2nd Vegetation Period	
	Amount (%)		Amount (%)	
	Natural Area	Field Area	Natural Area	Field Area
α-Guaiene	-	-	0.03	0.04
β-elemene	-	-	0.02	0.02
Torreyol	-	-	0.09	0.07
Eucalyptol	-	-	0.21	0.05
Solanone	-	-	0.03	0.03
α-farnesene	0.50	-	0.63	-
Farnesene	-	-	-	0.19
α-Muurolene	-	0.45	0.22	0.38
Caryophyllene oxide	0.39	0.22	-	-
γ-Cadinene	0.70	0.96	3.12	4.75
1,4-Cadinadiene	-	-	-	0.55
trans-Pinocarveol	-	-	0.14	0.09
δ-cadinene	1.17	1.27	0.48	0.74
α-Patchoulene	-	0.20	-	-
α-humulene	0.84	-	0.34	0.41
Alloaromadendrene	0.22	0.31	0.49	-

The amount of germacrene-D was 19.04% in the samples from natural habitat and 15.40% in the samples from cultivars in the first vegetation period. However, it was detected 19.63% and 21.13%, respectively, in the second vegetation period.

Although β-myrcene was determined only in the first vegetation period in both sampling areas, myrcene was seen only in the second vegetation period samples. Besides, caryophyllene was detected only in natural habitat samples with the amount of 12.17% in the first vegetation period. In the second vegetation period, the amount of caryophyllene decreased almost 50% compared to the first vegetation period samples. It was found 5.82% in the samples from natural habitat whereas it was 5.99% in the samples from cultivars.

Volatile oil of *Ajuga* species were determined by several researches. Azizan et al (2002) determined volatile oil components of *A. chamaepitys* subsp. *chia* var. *chia* by hydrodistillation. Totally 25 components were identified and α-pinene (16.1%), β-pinene (34.38%) and germacrene-D (5.6%) were found as the dominant components. Saijadi and Ghanhadi (2004) found germacrene-D (24.2%), β-cubebene (18.3%), β-caryophyllene (16.9%) and α-cubebene (5.3%) as the highest in *A. chamaepitys* subsp. *chia* var. *chia* volatile oil. It was another study done by Delazar et al (2012) in which α-pinene (23.66%), β-pinene (9.33%), β-phellandrene (8.70%) and germacrene-D (7.92%) were the dominant components of Iranian *A. chamaepitys* subsp. *chia* var. *chia* volatile oil.

Volatile oil yield of *A. orientalis* was 0.10 ml/100 g in the samples from natural habitat and 0.05 ml/100 g in the samples from cultivars in the first vegetation period. In the second vegetation period, volatile oil yield of natural habitat samples was 0.15 ml/100 g while it was the same as the former in the samples from cultivars. The amount and the composition of *A. orientalis* volatile oil determined by SPME and GC-MS can be seen in *Table 2*. In both samples of the first vegetation period, 44 components were identified. However, the identified components were 40 in the samples from natural habitat and 53 in the samples from cultivars in the second vegetation period.

While 2-hexen-1-al had the maximum amount in the first vegetation period in the samples from natural habitat, 1-octen-3-ol was determined as the highest amount component in the samples from cultivars.

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Table 2. *The amount and the composition of A. orientalis volatile compounds (%)*

Compounds	1st Vegetation Period		2nd Vegetation Period	
	Amount (%)		Amount (%)	
	Natural Area	Field Area	Natural Area	Field Area
Acetaldehyde	0.29	0.22	1.51	1.31
Borane-methyl sulfide complex	0.55	0.33	1.97	1.22
Amyl ethyl ketone	-	-	1.53	1.03
2-Butenal	1.32	0.90	0.41	0.26
3-Methylbutanal	0.48	0.65	0.47	0.61
2-Methylbutanal	0.33	0.61	0.47	0.52
4-methylpentanenitrile	0.50	0.50	-	0.33
2-ethyl-Furan	1.14	1.01	-	0.89
2-pentanal	0.54	0.25	-	0.33
2-Penten-1-ol	0.70	0.98	-	0.41
n-Hexanal	3.85	4.22	1.42	1.26
2-Hexen-1-al	30.89	23.12	6.19	7.23
3-Hexene-1-ol	1.42	3.43	-	-
2-Hexen-1-ol	0.87	1.90	-	-
n-Hexanol	2.24	3.54	1.10	0.91
Styrene	-	-	0.94	0.86
4-hepten-1-al	0.29	1.12	1.00	1.23
Heptanal	0.26	0.42	-	0.33
2,4-Hexadienal	0.83	0.99	-	0.67
α-pinene	0.98	1.12	8.29	7.46
Phenylmenthanal	-	-	1.28	0.98
2-heptanal	0.44	0.81	0.40	0.31
Benzaldehyde	1.26	1.22	-	0.83
Sabinene	0.26	0.83	0.33	0.22
1-hexene	0.97	0.56	-	-
β-pinene	-	-	0.65	0.58
Vinyl amyl ketone	-	-	0.52	0.49
1-Octen-3-ol	26.30	28.34	28.16	26.79
β-Myrcene	2.58	3.01	4.84	3.86
2,4-Heptadienal,	3.21	4.56	1.28	1.03
2,2-Pentenylfuran	0.39	0.44	-	-
Ethyl-hexanol	0.85	1.01	-	-
n-octanal	0.57	1.67	-	-
δ3-carene	1.14	1.85	3.42	3.01
n-octan-3-ol	-	-	1.71	1.77
1-hexyl acetate	-	-	1.03	1.01
Hexyl-Ethanoate	2.00	1.12	-	0.98
Cymol	-	-	0.73	0.61
Limonene	3.64	2.56	12.36	11.69
Trans-limonene oxide	-	-	-	1.03
Hyacinthin	-	-	0.32	0.21
2-octenal	0.50	1.32	-	-
3,5-octadien-2-one	0.93	1.22	0.69	0.41
α-terpinolene	0.30	0.10	2.51	1.89
Nonanal	2.76	0.99	0.27	0.64
Dodecane	0.30	0.10	0.59	0.24
n-Decanal	0.48	0.55	0.26	0.63
Benzene	0.75	0.62	-	-
n-Octyl acetate	0.70	0.81	-	0.83
hexyl 2-methylbutanoate	0.51	0.23	-	0.44
l-linalool	0.45	0.12	0.68	0.43
Hexyl butanoate	-	-	0.47	0.44
p-allylanisole	-	-	0.23	0.23
n-octyl 2-methyl butyrate	0.35	0.33	-	0.22
3-Buten-2-one	0.43	0.11	-	0.31
2-Butenoic acid	0.45	0.21	2.81	2.63
Pentanoic acid	-	-	1.09	0.93
Tridecane	-	-	0.64	0.66
Tetradecane	-	-	1.37	1.29
Valencene	-	-	1.45	1.53
α-farnesene	-	-	3.76	3.11

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Moreover, 1-octen-3-ol was also dominant component of the second vegetation period samples with 28.16% in natural habitat and 26.79% in cultivars. Volatile oil composition and antimicrobial activity of *Ajuga orientalis* was presented by Yaldiz (2012). It was seen that germacrene-D, cubebene and caryophyllene were the major components of *A. orientalis* volatile oil.

Conclusions

A. chamaepitys subsp. *chia* var. *chia* and *Ajuga orientalis*, which grow naturally in Lake District in Turkey, were cultivated in 2015. Volatile oil analyses were carried out in 2016 and 2017 by using leaves and flowers of these plant species from both natural habitat and cultivars. It was seen that there were same components and the amount of components were close to each other in both sampling areas. It was concluded that these plants were cultivated without losing their chemical structure. 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 produced in industrial sense and can also be used in different industries such as pharmaceuticals and cosmetics due to their chemical composition.

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