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Volatile components of *Stachys cretica* subsp. *anatolica* Rech. F., Ann. in Koçtepe province of Isparta, Turkey

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Abstract
Turkey is an important gene center in terms of the Lamiaceae family plants, which take the first place among the plant species traded on the world market and have an important role in alternative medicine applications. *Stachys* is one of the important genus of Lamiaceae and represented by approximately 200 taxa. In this study which was conducted in 2017 and 2018, above ground parts of *Stachys cretica* subsp. *anatolica* in flowering period of plant were collected from Koçtepe province of Isparta. Volatile components were determined by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). Collected samples were placed in paper packages and transported to the laboratory on the same day and dried at room temperature (25°C), after they were subjected to solid phase microextraction (SPME). 2 g of samples were placed into a 10 mL vial and was heated to 60°C for 30 minutes. SPME apparatus for adsorbing volatiles vials passed into the upper cavity and then rested Rx-5Sil MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) used in Shimadzu 2010 Plus GC-MS and injected directly into the device. That was connected to mass selector detector which is operated in hand mode (70 eV). Helium with a flow rate of 1.61 mL per minute was used as carrier gas. Injection and detection temperatures were set at 250°C for desorption (5 min) of the adsorbed volatile compounds for analysis.
As result, 58 volatile components were determined and (E)-2-hexenal (12.58%), germacrene-D (34.56%) and also β-caryophyllene (21.04%) were found as main components. *Stachys* species are becoming increasingly important due to the aromatic compounds and essential oils they contain, as well as being the plants that are used by the public in the treatment of diseases and which are medicinal properties.

Keywords: *Stachys cretica* subsp. *anatolica*, SPME, GC-MS, volatile oil, Isparta

INTRODUCTION

The presence of three phytogeographic regions in Turkey as, Europe-Siberia in north, Mediterranean in Western and Southern and also Iran-Turan phytogeographic region in the interior, eastern and south-eastern Anatolia, leads to a rich plant cover [1]. Flora of Turkey has approximately 11.466 plant taxa. By the way, the whole European continent has about 12.000.

Approximately 300.000 flowering or seeded plant species currently registered in the world, about 20.000 are suitable for medical purposes. It is known that around 4000 herbal drugs (medicines) are used intensively and especially for the economic purposes of nearly 500 [2].

According to the World Health Organization (WHO) surveys, the number of medicinal plants used for treatment is about 20.000 [3]. Primer and secondary metabolites which are natural products produced by plants, constitute the most basic products of the industry directly and indirectly [4].

Medicinal and aromatic plants, especially those rich in volatile oil content, have an important precaution. Essential oils (essences, etheric oils) and their aromatic extracts are widely used by the fragrance and taste industries as a starting material for the synthesis of perfume, food additives, cleaning products, cosmetics and medicines, as a source of aroma chemicals. It is observed that there is a large increase in the demand for essential oils for use in aromatherapy applications, which have been particularly prominent in recent years [5].

Turkey is an important gene center in terms of the Lamiaceae family plants, which take the first place among the plant species traded on the world market and have an important role in alternative medicine applications. This cosmopolitan family is usually represented by approximately 200 genera and 3000 species, with fragrant one or perennial ovaries, rarely trees or trees. In Turkey, members of Lamiaceae of 45 genera and more than 546 species are important in pharmacology and perfumery industry because they contain volatile and aromatic oils [6, 7].

Stachys is one of the important genus of Lamiaceae and represented by approximately 200 taxa. This genus is represented by 121 taxa and endemism rate as 43.4% at the species level. *Stachys* species are used by the public for the treatment of diseases due to their medical feature [8, 9]. In this study, volatile components of leaf and flower in flowerinf period of *Stachys cretica* subsp. *anatolica* were determined by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME).

MATERIALS AND METHODS

In this study which was conducted in 2017 and 2018, above ground parts of *Stachys cretica* subsp. *anatolica* in flowering period of plant were collected from Koçtepe province of Isparta (Figure 1).

Figure 1. *Stachys cretica* subsp. *anatolica*

Volatile components were determined by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). Collected samples were placed in paper packages and transported to the laboratory on the same day and dried at room temperature (25°C), after they were subjected to solid phase microextraction (SPME). 2 g of samples were placed into a 10 mL vial and heated to 60° C for 30 minutes. SPME apparatus for adsorbing volatiles vials passed into the upper cavity and then rested Rx-5Sil MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) used in Shimadzu 2010 Plus GC-MS and injected directly into the device. That was connected to mass selector detector which is operated in hand mode (70 eV). Helium with a flow rate of 1.61 mL per minute was used as carrier gas. Injection and detection temperatures were set at 250°C for desorption (5 min) of the adsorbed volatile compounds for analysis. Wiley, NIST Tutor and FFNSC libraries were used to identify volatile components.

RESULTS AND DISCUSSION

For *Stachys cretica* subsp. *anatolica*, 58 volatile components were determined and (E)-2-hexenal (12.58%), germacrene-D (34.56%) and also β-caryophyllene (21.04%) were found as main components (Table 1.).

Table 1. Volatile oil components of *Stachys cretica* subsp. *anatolica*

	Rt	COMPONENTS	%		Rt	COMPONENTS	%
1	1.370	Isobutanal	0,30	**30**	10.968	(E,E)-2,4-Heptadienal	0,68
2	1.517	3-Methyl-2-butanone	0,28	**31**	11.458	Cymene	0,47
3	1.559	2-methylfuran	0,12	**32**	11.649	Limonene	1,20
4	1.887	2-Butenal	0,22	**33**	12.005	3-Octen-2-one	0,37
5	1.929	3-Methylbutanal	0,38	**34**	12.134	Benzeneacetaldehyde	0,44
6	2.009	2-Methylbutanal	0,39	**35**	12.763	(E)-2-Octenal	0,42
7	2.325	Pentanal	0,22	**36**	13.205	3,5-Octadien-2-one	0,52
8	2.357	2-ethyl-Furan	0,54	**37**	14.417	Linalool	0,25
9	2.892	(E)-3-Penten-2-one	0,34	**38**	14.590	n-Nonanal	1,20
10	3.181	(E)-2-Pentenal	0,10	**39**	15.916	3-Methyl-2-butenyl pentanoate	0,67
11	4.101	n-Hexanal	1,58	**40**	17.824	Methyl salicylate	0,70
12	5.408	Ethyl 2-methylbutyrate	0,19	**41**	18.452	n-Decanal	0,52
13	5.514	(E)-2-Hexenal	12,58	**42**	19.578	hexyl 2-methylbutanoate	0,31
14	5.589	3-Hexen-1-ol	0,26	**43**	22.573	1-Undecene	0,58
15	5.906	(E)-2-Hexenol	0,46	**44**	23.506	.α.-Cubebene	0,48
16	6.028	n-Hexanol	0,31	**45**	24.479	.α.-Copaene	1,34
17	6.612	Heptan-2-one	0,38	**46**	24.744	.β.-Bourbonene	1,24
18	7.025	Heptanal	0,89	**47**	24.949	.β. -Elemene	1,09
19	7.294	Sorbaldehyde	1,02	**48**	26.076	β-Caryophyllene	21,04
20	8.069	α-Pinene	1,22	**49**	26.458	Octyl 3-methylbutanoate	0,79
21	8.482	Propenylbenzene	0,14	**50**	27.108	Farnesene	0,65
22	8.944	2-Heptenal	0,45	**51**	27.971	Germacrene-D	34,56
23	9.043	Benzaldehyde	1,54	**52**	28.089	2-Phenylethyl 2-methylbutanoate	0,65
24	9.647	2-.β.-Pinene	0,30	**53**	28.202	.β.-Selinene	0,46
25	9.873	Vinyl amyl ketone	0,35	**54**	28.305	α-Copaene	0,30
26	9.909	Vinyl amyl carbinol	1,17	**55**	28.438	Bicyclogermacrene	0,46
27	10.023	6-Methyl-5-hepten-2-one	0,25	**56**	28.548	α-Muurolene	0,39
28	10.208	2-Pentylfuran	0,28	**57**	29.176	δ-Cadinene	0,32
29	10.705	n-Octanal	0,45	**58**	40.239	Curcumene	1,19

The total phenolic contents and the essential oil compositions of the *Stachys cretica* L. subsp. *mersinaea* (Boiss.) Rech. that is endemic to Turkey, were studied by Özkan et al. (2005) [10]. α-curcumene (34.10%) was found as main component for *S. cretica* in that study. Results differ from our results.

In another study, the essential oil from the aerial parts of *Stachys cretica* L. subsp. *smyrnaea* Rech. fil. (Lamiaceae) that is also endemic to Turkey were determined by Öztürk et al. (2009) [11]. Thirty-four of 37 components, represented 99.7% of the total oil, were identified. The main components were found as β-caryophyllene (51.0%), germacrene-D (32.8%), a-humulene (3.1%), delta-cadinene (2.1%) and delta-elemene (2.1%).

Germacrene-D and β-caryophyllene components were identified as the main components in both studies. The results for the other components vary according to our study. The other main component identified in our study was (E) -2-hexenal.

Goren et al. (2011) [12] were found 24 components of S*tachys* cretica L. subsp. *cassia* (Boiss.) Rech.f. The main components were determined as germacrene-D (27.8%), δ-elemene (14.9%) and β-caryophyllene (8.9%). They also determined a total 24 components of *S. cretica* L. subsp. *garana* (Boiss.) Rech.f. and of them α-cadinol (16.4%), verbenol (11.4%) and dodecanoic acid (8.9%) were main components. In same study, for *S. cretica* L. subsp. *lesbiaca* (Boiss.) Rech.f., 27 components were determined and the main components were found as germacrene-D (13.9%), β-caryophyllene (12.5%) and α-cadinol (7.4%). 23 components of the essential oil of *S. cretica* L. subsp. *symrnaea* (Boiss.) Rech.f. were identified. Germacrene-D (38.9%), β-caryophyllene (14.8%) and caryophyllene oxide (8.7%) were the main components. 26 components were determined for *S. cretica* L. subsp. *kutahyensis* Akçiçek. The main components were germacrene-D (28.1%), τ-muurolol (9.3%) and cubenol (8.8%).

Germacrene-D and β-caryophyllene components were identified as main components similar in our study. The results for the other components vary according to our study. The other component identified in our study was (E) -2-hexenal.

62 volatile components were found for *Stachys cretica* subsp. *anatolica* in another study that was conducted by us in Seydişehir region of Konya in 2015 and 2016 [13]. (E)-2-Hexenal (%8.50), Benzaldehyde (%46.34) and β-caryophyllene (%11.23) were determined as main components. For studies, (E) -2-hexenal and β-caryophyllene components were found as major components.

CONCLUSIONS

As result, 58 volatile components were determined and (E)-2-hexenal (12.58%), germacrene-D (34.56%) and also β-caryophyllene (21.04%) were found as main components. *Stachys* species are becoming increasingly important due to the aromatic compounds and essential oils they contain, as well as being the plants that are used by the public in the treatment of diseases and which are medicinal properties.

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