CHEMICAL CONSTITUENTS OF THE VOLATILE OILS OF *STACHYS CRETICA* SUBSP. *ANATOLICA* AND *STACHYS LAVANDULIFOLIA* FROM SEYDISEHIR, TURKEY

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Abstract. This study was conducted to determine volatile oil components for leaf and flowers of *Stachys cretica* subsp. *anatolica* Rech. and *Stachys lavandulifolia* Vahl. in different reaping periods, such as pre-flowering, flowering, and post-flowering by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). To achieve this aim, leaf and flower samples were collected from Tınaztepe Cavern province of Seydisehir, in 2015 and 2016. A total of 62 components were determined for *Stachys cretica* subsp. *anatolica*, with benzaldehyde (46.34%), β-caryophyllene (11.23%) and (E)-2-hexenal (8.50%) being the main components. Additionally, 55 volatile components were found for *S. lavandulifolia,* and the main components were β-phellandrene, myrcene, and α-pinene at 27.71%, 11.56% and 11.20% respectively.
Keywords: *Stachys, SPME, (E)-2-Hexenal, Benzaldehyde, β-phellandrene, Turkey*

Introduction

Turkey is situated at the intersection of different climate zones due to its geographical location. Therefore, it is rich in plant species and diversity. Turkey is also an important germplasm for the plants in the Lamiaceae family, which is the number one plant species traded across the world that has an important place in alternative medicine (Anonymous, 1996; Arslan et al., 2000; Ozkan, 2007). The members of the Lamiaceae family represented by 45 genera and more than 546 species in Turkey are vital for the pharmacology and perfumery industry thanks to their volatile and aromatic oil content (Secmen et al., 2000).

The economic value of the Lamiaceae family members is very high, as they contain the aromatic compounds and essential oils. Some taxa in the *Pogestemon* and *Lavandula* genera are used in the perfumery industry while *Thymus*, *Mentha* and *Origanum* species are used in the food industry (Watson and Dallwitz, 1978; Clive and Stace, 1980; Baytop, 1991; Ozkan, 2007). Furthermore, several species in the family (*Stachys*, *Sideritis*, *Salvia*, *Lamium*, *Ajuga*, *Teucrium*, *Thymus*, *Phlomis* etc.) are known to be used for therapeutic purpose (Baytop, 1984, 1991; Ozkan, 2007).

The genus *Stachys* from the Lamiaceae family, which was the research material, in this study, comprises nearly 200 taxa. The members of *Stachys* L. are found almost everywhere in the world except Australia and New Zealand. The Balkans, South and East Anatolia, Caucasians, Northwest Iran and north of Iraq are the regions where these species grow intensively (Bhattacharjee, 1974; Davis, 1988). They are represented by 121 taxa in Turkey with an endemism rate of 43.4%.

Stachys species are medicinal plants used by people for the treatment of diseases. *Stachys annua* is used to heal insomnia and menstrual irregularities; *S. sylvatica* is used as antispasmodic, diuretic, tonic and astringent agent, *S. officinalis* is used as tonic and

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for the treatment of insomnia, headache and cough, while *S. palustris* is used externally to heal wounds (Steinmetz, 1954; Garnier et al., 1961).

Stachys cretica is perennial with basal sterile rosettes. Flowering stems are 25-100 cm, erect, they can be branched or unbranched, usually densely or sparsely adpressed greyish tomentose, rarely patent-pilose with glandular hairs being less frequent than eglandular hairs. Basal leaves are oblong-spathulate, 4-10 x 1-3.5 cm, obscurely crenulate to faintly crenate, apex obtuse sometimes acute, attenuate or rarely rounded to subcordate at base, petiole 2-8 cm. Cauline leaves are like basal but gradually become smaller above, shortly petiolate to subsessile or sessile. Floral leaves are oblong-lanceolate to lanceolate, 1-6 x 0.5-1.2 cm, the same lenght or longer compared to verticillasters. Additionally, verticillasters usually remote throughout, rarely approximate above, 10-16-flowered. Brachteoles ovate-lanceolate to lanceolate, 5-16 mm, herbaceous, not spinescent. Pedicels 1-2.5 mm. Calyx sub-bilabiate, subcampanulate to tubular, 12-16 mm, densely tomentose, teeth subequal, ovate to lanceolate, 1/3-1 x tube; mucro 1-3 mm. Corolla rose-pink, 18-20mm, tube subexserted. Nutlets obovoid, 2 x 1.5 mm, smooth (Davis, 1982).

S. lavandulifolia is suffrutescent perennial with basal rosettes of sterile shoots. Flowering stems numerous, 10-30 cm, sparsely to densely pilose, sometimes absent, rarely white-tomentose with unequal stellate hairs. Basal leaves subsessile to sessile, oblong-lanceolate to oblanceolate, 2-6 x 0.4-1.5 cm, entire to faintiy serrate, attenuate at base, cauline similar. Floral leaves oblong to ovate, 1.2-2 x 0.25-0.8 cm, shorter than verticillasters. Verticillasters (at least lower ones) usually remote, (2-)4-6-flowered. Brachteoles few, linear, 2-3 mm, hirsute. Pedicels 1.5-2 mm. Calyx ± regular, subcampanulate, patently pilose, tip softly spinescent. Corolla purple to mauve, 13-15 mm, tube subincluded. Nutlets obovoid, 2.5-3 x 2-2.5 mm (Davis, 1982).

Stachys species are becoming increasingly important due to the aromatic compounds and essential oils they contain. In this study, we analyzed the volatile compounds in the leaves and flowers of *Stachys cretica* subsp. *anatolica* and *S. lavadulifolia* species at three different vegetation stages, pre-flowering, flowering and post-flowering.

Materials and Methods

The material of the study conducted in 2015 and 2016 comprised of *Stachys cretica* subsp. *anatolica* and *S. lavandulifolia* samples collected during pre-flowering, flowering and post-flowering stages from two different sampling sites in Konya-Seydisehir, Tınaztepe Cavern province that is situated N 37° 14', E 31° 55', and 1525 m elevation (*Figure 1*). Samples of *S. cretica* subsp. *anatolica* were collected in April, May, and June of both 2015 and 2016 at pre-flowering, flowering, and post-flowering stages. Additionally, *S. lavandulifolia* samples were collected in May, June, and July of both years.

The leaves and flower samples that were collected were put into paper packages and transferred to the laboratory the same day to avoid exposure to sunlight. After the collected plant materials were dried at room temperature (25°C), and flower and leaf 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 x 0.25 mm, 0.25 μm) at a temperature of 250°C for

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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. *Sampling area for Stachys materials*

Results

The volatile compounds in the leaves and flowers of *Stachys cretica* subsp. *anatolica* and *S. lavandulifolia* species were collected at pre-flowering, flowering, and post-flowering stages were analyzed by SPME (solid phase microextraction) method. With SPME method, 62 different volatile compounds were identified in *Stachys cretica* and 55 in *S. lavandulifolia*. These results are shown in *Table 1*. (E)-2-Hexenal, Benzaldehyde, and β-caryophyllene *Stachys cretica* were identified as the main compounds of *Stachys cretica*. Specifically, it contained 7.82% (E)-2-Hexenal, 40.54% Benzaldehyde, and 8.36% β-caryophyllene at the pre-flowering stage; 8.50% (E)-2-hexena, 46.34% Benzaldehyde and 11.23% β-caryophyllene at the flowering stage; and 6.62% (E)-2-Hexenal, 41.80% Benzaldehyde and 10.93% β-caryophyllene at the post-flowering stage. Additionally, α-pinene, myrcene and β-phellandrene were found to be the main compounds of *Stachys lavandulifolia*. Specifically, these flowers contained 10.70% α-pinene, 10.98% myrcene and 26.12% β-phellandrene at the pre-flowering stage; 11.20% α-pinene, 11.56% myrcene and 27.71% β-phellandrene at the flowering stage; and 10.90% α-pinene, 10.25% myrcene and 26.06% β-phellandrene at the post-flowering stage (*Table 1*).

Table 1. *Chemical Constituents of the Volatile Oils of Stachys cretica subps. anatolica and Stachys lavadulifolia in different reaping periods*

			Stachys cretica			*Stachys lavandulifolia*		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
1.	1.370	Isobutanal	0.33	-	-	0.03	0.06	0.07
2.	1.440	2-Methylpropenal	-	-	-	-	0.03	0.02
3.	1.517	3-Methyl-2-butanone	0.16	-	-	-	-	-
4.	1.531	2-Ethylbutanal	-	-	-	0.05	0.03	0.04

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			Stachys cretica			***Stachys lavandulifolia***		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
5.	1.559	2-methylfuran	0.20	-	-	-	0.01	-
6.	1.638	3-methylfuran	-	-	-	0.01	0.01	-
7.	1.887	2-Butenal	1.22	0.89	1.38	0.15	0.22	0.20
8.	1.929	3-Methylbutanal	0.44	0.19	-	-	-	-
9.	2.009	2-Methylbutanal	0.37	-	-	0.11	0.10	0.09
10.	2.235	1-Ethylcyclopropanol	-	-	-	0.02	0.04	0.01
11.	2.325	Pentanal	0.69	0.70	0.77	0.25	0.22	0.18
12.	2.357	2-ethyl-Furan	0.54	-	-	0.23	0.23	0.20
13.	2.892	(E)-3-Penten-2-one	0.30	0.22	0.34	0.02	0.05	0.04
14.	3.032	3-Methylenepentane	-	-	-	-	0.01	0.08
15.	3.181	(E)-2-Pentenal	0.22	-	-	0.05	0.08	0.03
16.	4.101	n-Hexanal	3.98	1.02	1.47	0.68	0.74	0.71
17.	5.408	Ethyl 2-methylbutyrate	-	0.19	-	-	-	-
18.	5.514	(E)-2-Hexenal	7.86	8.50	6.62	4.24	3.45	4.82
19.	5.589	cis-3-Hexene-1-ol	0.44	0.33	0.27	0.68	0.34	0.29
20.	5.906	(E)-2 -Hexenol	0.40	0.27	-	-	-	-
21.	6.028	n-Hexanol	1.31	0.46	0.29	0.06	0.06	-
22.	6.612	Heptan-2-one	0.35	-	-	-	-	-
23.	7.025	Heptanal	1.29	0.70	0.67	-	-	-
24.	7.294	Sorbaldehyde	-	0.31	0.37	0.05	0.09	0.07
25.	7.837	α-thujene	-	-	-	1.98	1.06	1.92
26.	8.069	α-pinene	2.12	1.09	1.22	10.70	11.20	10.90
27.	8.482	Propenylbenzene	3.14	1.64	2.02	2.98	2.07	2.02
28.	8.944	cis-2-Heptenal	0.47	0.22	-	0.11	0.13	0.08
29.	9.043	Benzaldehyde	40.54	46.34	41.80	0.05	0.10	0.04
30.	9.647	2-β-pinene	1.60	0.85	1.05	5.56	5.02	5.87
31.	9.767	Sabinene	-	-	-	4.88	4.11	4.12
32.	9.873	Vinyl amyl ketone	-	-	0.24	0.65	0.70	0.72
33.	9.909	Vinyl amyl carbinol	1.70	1.52	1.17	-	-	-
34.	10.023	6-Methyl-5-hepten-2-one	0.95	0.79	0.70	-	-	-
35.	10.208	2-Pentylfuran	1.30	0.78	0.76	-	-	-
36.	10.202	Myrcene	-	-	-	10.98	11.56	10.25
37.	10.705	n-Octanal	1.15	0.97	0.78	-	-	-
38.	10.744	α-phellandrene	-	-	-	3.36	2.43	3.20
39.	10.968	(E,E)-2,4-Heptadienal	-	0.46	0.58	-	-	-
40.	11.352	p-Dichlorobenzene	-	-	-	0.09	0.18	0.12
41.	11.491	α-terpinene	-	-	-	0.85	1.07	1.99
42.	11.458	Cymene	0.46	-	-	-	-	-
43.	11.649	dl-Limonene	2.95	1.57	1.20	0.15	0.15	0.12
44.	12.005	3-Octen-2-one	0.68	0.79	0.89	-	-	-
45.	12.134	Benzeneacetaldehyde	-	0.39	0.40	-	-	-
46.	12.139	β-phellandrene	-	-	-	26.12	27.71	26.06
47.	12.331	Cis-Ocimene	-	-	-	4.71	4.98	4.52
48.	12.370	trans-β-Ocimene	-	-	-	0.54	0.75	0.66
49.	12.763	(E)-2-Octenal	0.36	0.45	0.43	-	-	-
50.	13.065	γ-terpinene	-	-	-	0.18	0.23	0.11
51.	13.205	3,5-Octadien-2-one	-	0.31	0.43	-	-	-

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			Stachys cretica			*Stachys lavandulifolia*		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
52.	14.417	Linalool	-	0.39	-	-	-	-
53.	14.590	n-Nonanal	1.90	1.26	1.82	-	-	-
54.	15.510	.+/-.-4-Acetyl-1-methylcyclohexene	0.25	-	-	-	-	-
55.	15.841	2,4,6-Octatriene, 3,4-dimethyl-	-	-	-	0.57	0.73	0.69
56.	16.311	2,4,6-Octatriene,2,6-dimethyl-, (E,Z)-	-	-	-	0.42	0.60	0.62
57.	15.916	3-Methyl-2-butenyl pentanoate	1.19	0.74	0.64	-	-	-
58.	17.824	Methyl salicylate	0.30	0.71	0.74	0.15	0.22	0.21
59.	18.452	n-Decanal	0.48	0.53	0.49	-	-	-
60.	19.457	Pentylallyl butyrate	-	0,24	-	-	-	-
61.	19.578	hexyl 2-methylbutanoate	0.80	0.81	0.81	-	-	-
62.	22.573	1-Undecene, 1-ethoxy-	0.55	0.66	0.57	0.25	0.22	0.20
63.	23.315	Bicycloelemene	-	-	-	0.55	0.62	0.58
64.	23.450	n-Octyl isobutyrate	-	-	0.64	-	-	-
65.	23.506	α-cubebene	0.80	1.14	0.65	-	-	-
66.	24.479	α-copaene	0.84	1.41	1.66	0.07	0.10	0.12
67.	24.744	β-bourbonene	1.04	1.61	1.09	0.28	0.49	0.33
68.	24.949	β-elemene	0.59	-	1.44	0.19	0.26	0.27
69.	25.264	β-cubebene	-	-	-	0.09	0.12	0.10
70.	25.431	6,10-Dimethylundecan-2-one	-	0.34	-	-	-	-
71.	26.076	trans-Caryophyllene	8.36	11.23	10.93	-	-	-
72.	26.283	α-amorphene	-	-	-	0.31	0.23	0.14
73.	26.458	Octyl 3-methylbutanoate	1.32	1.36	2.69	-	-	-
74.	26.803	α-bergamotene	-	-	-	0.42	0.48	0.36
75.	26.970	(+)-Aromadendrene	-	-	-	0.12	0.18	0.19
76.	27.108	(E)-β-farnesene	2.03	2.42	4.84	-	-	-
77.	27.359	Epi-bicyclosesquiphellandrene	-	-	-	0.25	0.24	0.17
78.	27.501	β-sesquiphellandrene	-	-	-	0.76	0.98	0.90
79.	27.670	Neoalloocimene	-	-	-	0.18	0.20	0.13
80.	27.971	Germacrene-D	0.52	0.82	0.59	6.84	6.37	6.32
81.	28.089	2-Phenylethyl 2-methylbutanoate	-	0.78	0.55	-	-	-
82.	28.202	β-selinene	-	-	0.45	-	-	-
83.	28.438	Bicyclogermacrene	0.72	0.94	0.98	5.52	5.96	5.80
84.	28.548	α-muurolene	-	0.36	0.44	-	-	-
85.	28.998	γ-cadinene	-	-	-	0.19	0.21	0.12
86.	29.176	δ-cadinene	-	0.30	0.27	-	-	-
87.	29.645	α-panasinsen	-	-	-	2.10	2.28	2.84
88.	30.954	Spathulenol	-	-	-	0.22	0.29	0.36
89.	31.105	Caryophyllene oxide	-	-	0.32	-	-	-
90.	40.239	Curcumene	0.79	-	1.54	-	-	-

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Discussion and Conclusions

Stachys cretica subsp. *anatolica* was found to have 62 different volatile compounds, with (E)-2-Hexenal (8.50%), Benzaldehyde (46.34%), and β-caryophyllene (11.23%) being its main compounds.

Ozkan et al. (2005) found that the main component of *Stachys cretica* subsp. *mersinaea* was α-curcumene (34.10%). In another study, Ozturk et al. (2009), identified 37 components of *Stachys cretica* subsp. *smyrnaea* using GC-MS analyses, with β-caryophyllene (51.0%), germacrene-D (32.8%), a-humulene (3.1%), Δ-cadinene (2.1%), and Δ-elemene (2.1%) being its main components. β-caryophyllene was found to be the main compound in both studies. The results for the other compounds was different from our findings. The other main compounds identified in our study were (E)-2-Hexenal and Benzaldehyde.

Goren et al. (2011) identified 24 main components of two *Stachys cretica* subsp. *cassia* and *S. cretica* subsp. *garana*, and 27, 26 and, 23 components of *S. cretica* subsp. *lesbiaca*, *S. cretica* subsp. *kutahyensis* and *S. cretica* subsp. *symrnaea* respectively. As results, germacrene-D (27.8%), δ-elemene (14.9%), and β-caryophyllene (8.9%) were identified for *Stachys cretica* subsp. *cassia*, however α-cadinol (16.4%), verbenol (11.4%) and dodecanoic acid (8.9%) were found for *S. cretica* subsp. *garana*. Additionally, germacrene-D, β-caryophyllene, and α-cadinol were found to be the main components of *S. cretica* subsp. *lesbiaca* at 13.9%, 12.5%, and 7.4% respectively. In the same study, they found germacrene-D (38.9%), β-caryophyllene (14.8%), and caryophyllene oxide (8.7%) to be the main components of *S. cretica* subsp. *symrnaea* and germacrene-D (28.1%), τ-muurolol (9.3%) and cubenol (8.8%) to be the main components of *S. cretica* subsp. *kutahyensis*. β- caryophyllene in both studies. The results for the other compounds differed from our findings. Goren et al. (2011) identified germacrene-D as the other dominant compound. The other main compounds identified in our study were (E)-2-Hexenal and Benzaldehyde.

Through SPME analysis, *Stachys lavandulifolia* was found to have 55 different volatile compounds, with α-pinene (11.20%), myrcene (11.56%), and β-phellandrene (27.71%) being its main compounds.

In another study conducted in Iran by Feizbaksh et al. (2003), 55 components of *Stachys lavandulifolia,* which is endemic to Iran, were identified, and α-pinene (20.1%), β-pinene (12.1%), and spathulenol (7.2%) were found to be its main components. Moreover, α-pinene was found to be the main compound in both analyses. Their results for the other compounds differed from our findings. Feizbaksh et al. (2003) found that the other dominant compounds were β-pinene and spathulenol while the other main compounds identified in our study were myrcene and β-phellandrene.

The studies of Javidnia et al. (2004) found that germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%), and Z-β-ocimene (5.8%) are the main components of *Stachys lavandulifolia*, however, Morteza-Semnani et al. (2005) identified 4-hydroxy-4-methyl-2-pentanone (9.3%), α-pinene (7.9%), and hexadecanoic acid (5.2%). In both studies, α-pinene was found to be the main compound. The result for the other compounds differed from our findings. Morteza-Semnani et al. (2005) found that the other dominant compounds were 4-hydroxy-4-methyl-2-pentanone and hexadecanoic acid. The other main compounds identified in our study were Myrcene and β-phellandrene.

Meshkatalsadat et al. (2007) reported that the main components of *Stachys lavandulifolia* are α-pinene (25.66%), myrcene (17.33 %), β-phellandrene (21.96 %),

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and β- caryophylene (14.3 %). In another study, Sajjadi ve Amiri (2007) determined the components as α-pinene (27.25, 25.66, 8.52%), myrcene (17.33, 9.33, 23.85%), and β-phellandrene (21.96, 37.49, 12.58%) for pre-flowering, flowering, and post-flowering periods of *S. lavandulifolia,* respectively. These studies supported our results.

Among 37 compounds of *Stachys lavandulifolia* Vahl. subsp. *lavandulifolia*, Iscan et al. (2012) found β-phellandrene (27%), α-pinene (18.5%), and germacrene-D (13%) to be the major components. In both studies, β-phellandrene and alpha-pinene were found to be the main components. In their study, germacrene-D was another dominant compound. The other main compounds identified in our study were myrcene and β-Phellandrene. Additionally, Pirbalouti et al. (2013) determined α-thujone (0.3%–32.3%), α-pinene (trace to 37.3%), myrcene (0.5%–15.9%), β-phellandrene (1.1%–37.9%), germacrene-D (0.4%–11.3%), Δ-cadinene (trace to 11.6%) and 1, 4-methano-1 H-indene (trace to 10.1%) to be the main components of *S. lavandulifolia*. These studies also supported our results.

In conclusion, *Stachys cretica* subsp. *anatolica* and *Stachys lavandulifolia*, the leaves and flowers of which were collected from the field, had the highest efficiency in terms of volatile compounds when they were collected during the flowering stage. The findings of this study inform the merchants and local people about the collection time to prevent random plant collection and potential economic losses.

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