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Comparison of volatile components of *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger due to reaping time

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Abstract: Volatile components of *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger collected in March, April, May and June 2013 were analyzed by gas chromatography mass spectroscopy (GC-MS) after headspace solid phase micro extraction (HS-SPME). Volatile components of the plant samples were compared due to reaping time. Results showed that 61 constituents were detected in the April and May samples, whereas 64 constituents were detected in the March and June samples. 99.27, 99.62, 99.60 and 99.30 % of total volatile constituents were identified in the March, April, May and June samples, respectively. *Thymus zygioides* offers more *p*-Cymene (24.30 and 32.71%) and thymol (17.39 and 10.16%) as main volatile components in the March and June samples, while it provides more γ-Terpinene (19.63 and 22.75%) and *p*-Cymene (18.32 and 19.17%) as major volatile components in the April and May samples. Monoterpenes (53.18, 60.44, 64.71 and 52.39%), sesquiterpenes (12.52, 15.71, 15.95 and 13.91%) and alcohols (22.49, 15.82, 13.07 and 19.25%) were distinctive component groups in the *Thymus zygioides* samples from March, April, May and June 2013.
Keywords: *Thymus zygioides*, Reaping time, Volatile components, HS-SPME-GC-MS

Thymus zygioides Griseb. var. *lycaonicus* (Celak.) Ronniger'in uçucu bileşenlerinin toplama zamanına göre karşılaştırılması

Özet: 2013 yılının mart, nisan, mayıs ve haziran aylarında toplanan *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger bitkisine ait örneklerin uçucu bileşenleri katı faz mikro ekstraksiyonu işleminden (HS-SPME) sonra gaz kromatografisi kütle spektroskipisi (GC-MS) ile analiz edilmiştir. Toplama zamanına göre bitkiye ait uçucu bileşenler karşılaştırılmıştır. Nisan ve mayıs aylarında toplanan örneklerde 61 bileşen belirlenirken, mart ve haziran aylarında toplanan örneklerde 64 bileşen saptanmıştır. Mart, nisan, mayıs ve haziran aylarında toplanan örneklerde sırasıyla uçucu bileşenlerin %99.27, 99.62, 99.60 ve 99.30'u tanımlanmıştır. *Thymus zygioides* bitkisinin mart ve haziran ayına ait örneklerinde uçucu bileşen olarak *p*-Cymene (%24.30 ve 32.71) and thymol (%17.39 ve 10.16) baskın iken, nisan ve mayıs aylarına ait örneklerde γ-Terpinene (%19.63 ve 22.75) ve *p*-Cymene (%18.32 ve 19.17) öne çıkmaktadır. Monoterpenler (%53.18, 60.44, 64.71 ve 52.39), sesqiterpenler (%12.52, 15.71, 15.95 ve 13.91) ve alkollerin (%22.49, 15.82, 13.07 ve 19.25) miktarı dört toplama dönemine ait örneklerde belirgin bir şekilde göze çarpmaktadır.
Anahtar kelimeler: *Thymus zygioides*, Toplama zamanı, Uçucu bileşenler, HS-SPME-GC-MS

1. Introduction

Thymus is one of the largest genera in Labiatae family, which is polymorphic with 60 taxa belonging to 39 species in Turkey and the endemism rate of *Thymus* is 45% (Baser, 2002). *Thymus* species are called as "Kekik" in Turkey and plant parts dried are used for various purposes such as tea mixture, flavor and medicine. Essential oil *of Thymus* obtained by traditional method is often utilized for medicinal therapy by local people, because of its valuable components (Baser, 2001). *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger growing in the Thrace region and west, central and south-west Anatolian regions of Turkey prefers sparse maquis, sandy and rock areas as growth place (Davis, 1982).

Solid phase micro extraction (SPME) is an analytical technique whereby a component is sorbed onto the surface of the coated silica fiber. This is followed by desorption of the components into a suitable chromatography instrument for the separation which is attached with an appropriate detector for quantification. SPME is generally performed with GC in the applications. In SPME-GC analysis, the fiber is inserted into the injector port and components are thermally desorbed from the coating for chromatographically detection (Malik et al., 2006).

Since its invention in 1989, SPME was often used in the studies (Belardi and Pawliszyn, 1989). In the early developmental period, SPME was majorly applied in environmental chemistry (Fattore et al., 1996; Abalos et al., 2002; Mousavi et. al., 2007). To analyze volatile and semi volatile components, headspace solid phase micro extraction (HS-SPME) was often used (Zhang and Pawliszyn, 1993). So far, HS-SPME was utilized to determine aromatics (James and Stack, 1996). The effect of high temperature and water addition were reported to use in analysis of low volatile compounds in the sample (Doong et al., 2000). Using a polar polyacrylate-coated extraction fiber, it was also possible to extract the polar analytes or specific trace

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components from HS (Bauer et al., 1997; Jelen et al., 1998; Luan et al., 2000; Pino et al., 2002).

In the present study, we revealed the effect of reaping time on the composition of volatile constituents from aerial parts of *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger plant samples were collected four times and compared using gas chromatographic analysis by mass selective detection after headspace solid phase micro extraction.

2. Material and method

2.1. Plant material

The flowering aerial parts of *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger were gathered from Atabey-Isparta in mediterranean region of Turkey about 1000 m in the middle of March, April, May and June 2013. The aerial parts collected were dried in dark place at the room temperature.

2.2. HS-SPME-GC-MS analysis

The used solid phase micro extraction (SPME) apparatus had a fiber coated with a 75 μm-thick layer of Carboxen/Polydimethylsiloxane (CAR/PDMS). 2.5 g of hand-picked material were used in each experiment. The sample was placed in a 10 mL vial which was sealed with a silicone septum and a crimp cap and incubated for 30 min at 60 °C. SPME fiber was pushed through the headspace of a sample vial to adsorbed the volatiles and then introduced immediately into the injection port of the Shimadzu 2010 Plus GC-MS equipped with a Restek Rx-5Sil MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) coupled to a mass selective detector of the same company operated in EI mode (70 eV). Carrier gas was helium with a flow rate at 1.61 mL/min. The injection and detection temperatures were 250 °C. The temperature of column was kept at 40 °C for 2 min, afterwards increased to 250 °C with a 4 °C/min rate and then programmed to 230 °C and kept constant for 5 min.

2.3. Identification of components

Linear Retention Indices (LRIs) of the volatile components were calculated using a series of the standards of C_7-C_{30} saturated *n*-alkanes that were analyzed at the same chromatographic conditions as described above for GC-MS. The volatile compounds were identified by comparison of their mass spectra with the Wiley, NIST Tutor and FFNSC library.

3. Results and discussion

Composition of volatile constituents from *T. zygioides* collected at different times (March, April, May and June 2013) are given in Table 1. Results indicated that 61 constituents were determined in the April and May samples, whereas 64 constituents were detected in the March and June samples. 99.27, 99.62, 99.60 and 99.30 % of total volatile constituents were identified in the March, April, May and June samples, respectively. Distinctive compounds of samples from March, April, May and June were p-Cymene (24.30, 18.32, 19.17 and 32.71 %), Thymol (17.39, 12.01, 9.18 and 10.16 %) and γ-Terpinene (13.23, 19.63, 22.75 and 6.90 %).

In the literature, there were various studies on the constituents of volatile oil of *T. zygioides* (Mericli and Tanker, 1986; Baser et al., 1999; Zamfirache et al., 2010). Mericli and Tanker (1986) determined thymol (24.6%) and linalool (12.2%) as main constituents of the volatile oil from *T. zygioides*. Baser et al. (1999) obtained linalool (33.7%) and (E)-nerolidole (12.5%) as major constituents of the volatile oil from *T. zygioides* studied. Zamfirache et al. (2010) found camphene (18.12%), α-pinene (9.45%) and germacrene D (7.67%) as most abundant components of the volatile oil from *T. zygioides*.

p-Cymene was the major volatile component in the March and June samples and the second main volatile component in the April and May samples of *T. zygioides*. *p*-Cymene is an important and worthful intermediate in the flavor, fragrance, herbicide and pharmaceutics industry. It can be transformed to *p*-cresol or 4-isopropylbenzaldehyde under different oxidation conditions. It is also used for synthesis of non-nitrate musk such as tonalide. However, *p*-Cymene is utilized as solvent, heat transfer agent and masking odor for industrial products as well (Derfer and Derfer, 1978; Du et al., 2005; Martin-Luengo et al., 2008).

Thymol was the second main volatile component in the March and June samples and the third major volatile component in the April and May samples of *T. zygioides*. Thymol is a volatile compound extracted mainly from thyme, which shows anti-inflammatory, immunomodulatory, antioxidant, antibacterial and antifungal properties. It is used as antimicrobial agent in polymer film due to its effectiveness and physical properties appropriate for blown film process (Petchwattana and Naknaen, 2015). Rota et al. (2008) indicated that thymol is a productive antimicrobial agent for preserving the food spoilage and increasing the shelf-life.

γ-Terpinene was the dominant volatile compound in the April and May samples and the third main volatile compound in March and June the samples. γ-Terpinene represents antimicrobial properties against various human pathogens. The antioxidant, anti-inflammatory and anti-proliferative activities of volatile substance γ-terpinene are investigated (Anonymous, 2016).

Table 1. The volatile constituents of *Thymus zygioides* collected at different times

No	Rt	LRI	Constituent	T1 (%)	T2 (%)	T3 (%)	T4 (%)
1	1.850	<700	3-Methyl butanal	0.15	0.19	-	-
2	1.966	<700	2-methyl-Butyraldehyde	0.31	0.16	0.04	-
3	2.152	<700	Ethyl vinyl ketone	0.13	0.07	-	-
4	2.304	<700	Pentanal	0.17	0.15	0.06	0.19
5	3.121	751	Pent-2(E)-enal	-	-	-	0.06
6	4.029	801	Hexanal	0.38	0.24	0.1	0.39
7	5.400	850	Hex-2(E)-enal	2.37	1.04	0.58	1
8	6.407	885	Heptan-3-one	0.15	0.08	0.04	0.16
9	7.743	927	α-Thujene	0.85	1.87	2.45	1.08
10	7.970	933	α-Pinene	0.89	1.64	2.11	1.77
11	8.530	953	Camphene	1.68	1.54	2.08	2.76
12	8.845	956	Hept-2(E)-enal	0.11	0.08	-	-
13	8.936	964	Benzaldehyde	0.12	0.09	-	-
14	9.402	972	Sabinene	-	-	0.08	0.13
15	9.535	978	β-Pinene	0.4	0.44	0.59	0.5
16	9.805	981	Octen-3-ol	1.8	1.38	1.01	1.89
17	9.974	986	Octan-3-one	3.33	2.12	1.46	6.26
18	10.130	991	Myrcene	6.67	9.79	6.86	3.01
19	10.418	999	ethyl-Hexanol	0.56	0.35	0.29	1.16
20	10.647	1007	α-Phellandrene	0.54	0.62	0.57	0.45
21	10.742	1009	δ-3- Carene	0.12	0.18	0.2	0.08
22	10.896	1013	2,4-trans-Heptandienal	0.77	0.52	0.22	0.42
23	11.102	1018	α-Terpinene	2.47	3.77	3.92	1.08
24	11.512	1025	p-Cymene	24.3	18.32	19.17	32.71
25	11.606	1030	Limonene	1.71	2.27	2.2	1.87
26	11.683	1032	Eucalyptol	1.34	2.28	2.43	3.34
27	12.040	1045	Phenylacetaldehyde	-	0.07	-	-
28	12.274	1046	β-Ocimene	0.32	0.37	1.73	0.05
29	12.744	1058	γ-Terpinene	13.23	19.63	22.75	6.9
30	13.219	1076	Capryl alcohol	-	-	-	0.08
31	13.582	1079	Oct-3(Z)-enol	0.22	0.09	0.06	0.22
32	13.688	1086	Terpinolene	0.2	0.39	0.4	0.21
33	13.744	1088	Nonan-3-one	0.08	-	-	0.18
34	14.285	1101	Linalool	0.15	0.15	0.22	0.57
35	14.465	1107	Pelargonaldehyde	0.17	0.07	-	0.3
36	15.940	1149	Camphor	0.61	0.21	0.27	0.69
37	16.955	1165	Isoborneol	0.69	0.39	0.8	2.63
38	17.288	1180	Terpinen-4-ol	0.49	0.27	0.37	0.63
39	17.562	1187	Hex-3(Z)-enyl butyrate	0.11	-	0.13	-
40	17.842	1198	α-Terpineol	0.09	0.09	0.12	0.29
41	17.923	1207	(Z)-Carvone	0.08	-	0.09	0.12
42	18.321	1208	Capraldehyde	-	-	-	0.1
43	19.243	1231	2-methyl-Butanoate	-	-	0.08	-
44	19.448	1238	3-hexenyl-Isovalerate	-	-	0.17	0.07
45	19.555	1247	Cuminaldehyde	0.07	-	-	0.09
46	21.131	1285	Bornyl acetate	0.18	-	-	0.12
47	21.510	1300	Thymol	17.39	12.01	9.18	10.16
48	21.711	1317	Carvacrol	0.76	0.57	0.5	0.69
49	23.363	1346	α-Cubebene	0.08	-	0.08	0.05
50	24.335	1375	α-Copaene	0.23	0.39	0.51	0.16
51	24.596	1382	β-Bourbonene	0.1	0.13	0.21	0.37
52	25.286	1400	Tetradecane	0.08	-	-	0.11
53	25.874	1418	β-Caryophyllene	3.44	6.66	7.14	5.45
54	26.137	1423	β-Cedrene	-	0.1	0.21	0.23
55	26.283	1432	α-trans-Bergamotene	0.13	0.13	0.13	0.09
56	26.422	1438	Aromadendrene	0.92	0.83	0.61	0.08
57	26.966	1454	α-Humulene	0.19	0.33	0.42	0.27
58	27.114	1458	Alloaromadendrene	0.11	0.11	0.05	0.07
59	27.631	1482	α-Amorphene	0.25	0.4	0.61	0.25
60	27.825	1485	Germacrene D	0.16	0.16	0.2	0.32
61	28.049	1487	β-Selinene	0.08	0.08	0.08	0.11
62	28.151	1491	Viridiflorene	0.38	0.33	0.32	-
63	28.287	1495	β-Chamigrene	0.15	0.1	0.12	0.14
64	28.396	1497	α-Muurolene	0.14	0.14	0.17	0.13
65	28.564	1500	Pentadecane	0.14	0.09	0.09	0.07
66	28.763	1508	β-Bisabolene	2.83	2.66	2.67	3.55
67	28.849	1512	γ-Cadinene	0.21	0.25	0.37	0.25
68	29.043	1518	δ-Cadinene	0.45	0.46	0.71	0.38
69	29.198	1523	β-Sesquiphellandrene	-	0.05	0.07	0.06
70	29.735	1557	Germacrene B	2.67	2.4	1.27	1.95
71	30.955	1576	Spathulenol	-	0.05	0.07	0.64
72	33.467	1680	Myristic alcohol	0.14	0.08	0.05	0.08
73	34.574	1700	Heptadecane	0.1	0.08	0.04	-
74	40.028	1900	Nonadecane	0.13	0.11	0.07	0.08
			Total:	99.27	99.62	99.60	99.30

Rt: Retention times on Restek Rx-5Sil MS column, LRI: Linear retention indices on Restek Rx-5Sil MS column, T1: Sample of *Thymus zygioides* collected on 15th March of 2013, T2: Sample of *Thymus zygioides* collected on 15th April of 2013, T3: Sample of *Thymus zygioides* collected on 15th May of 2013, T4: Sample of *Thymus zygioides* collected on 15th June of 2013.

Table 2. Classification of main groups of determined volatile constituents of *Thymus zygioides* collected at different times

Main Groups	T1 (%)	T2 (%)	T3 (%)	T4 (%)
Monoterpenes	53.18	60.44	64.71	52.39
Sesquiterpenes	12.52	15.71	15.95	13.91
Alcohols	22.49	15.82	13.07	19.25
Aldehydes	4.62	2.61	1	2.55
Ketones	4.38	2.48	1.86	7.41
Terpene oxides	1.34	2.28	2.43	3.34
Alkanes	0.45	0.28	0.2	0.26
Esters	0.29	-	0.38	0.19
Total	99.27	99.62	99.60	99.30

T1: Sample of *Thymus zygioides* collected on 15^{th} March of 2013, T2: Sample of *Thymus zygioides* collected on 15^{th} April of 2013, T3: Sample of *Thymus zygioides* collected on 15^{th} May of 2013, T4: Sample of *Thymus zygioides* collected on 15^{th} June of 2013.

Main groups of determined volatile constituents of *T. zygioides* collected at different times were classified in Table 2. The distinctive components including monoterpenes were identified as 53.18, 60.44, 64.71 and 52.39% in the samples from March, April, May and June, respectively. Alcohols determined totally 22.49, 15.82, 13.07 and 19.25% in the samples collected in March, April, May and June. Sesquiterpenes with their contents being 12.52, 15.71, 15.95 and 13.91% were detected in the samples from March, April, May and June. Other components consist of aldehydes, ketones, terpene oxides, alkanes and esters were found totally 11.08, 7.65, 5.87 and 13.75% in samples of *T. zygioides* collected in March, April, May and June.

4. Conclusion

The findings of the study showed that different contents and main groups of determined components in the samples of *Thymus zygioides* Griseb. var. *lycaonicus* (Celak.) Ronniger collected in March, April, May and June 2013. *Thymus zygioides* offers more *p*-Cymene, thymol as main volatile components in the March and June samples, whereas it provides more γ-Terpinene and *p*-Cymene as major volatile components in the April and May samples. Monoterpenes, sesquiterpenes and alcohols are the dominant component groups in the plant samples collected in March, April, May and June 2013.

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