VOLATILE COMPONENT COMPOSITION OF *BALLOTA NIGRA* SUBSP. *ANATOLICA* AT DIFFERENT VEGETATION PERIODS IN ÇAMLICA PROVINCE OF KÜTAHYA CITY, TURKEY

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Abstract. The volatile component composition of leaves and flowers for *Ballota nigra* subsp. *anatolica* P.H. Davis that were collected between 2015 and 2016 from Çamlıca provice of Kütahya city during three different vegetation periods as pre-bloom, bloom and post flowering were determined by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). 59 different volatile oil components were determined of *Ballota nigra* subsp. *anatolica*. As main components, Hexenal (17.60%), germacrene D (6.70%) and β-caryophyllene (8.80%) were found during pre-blooming period, also Hexenal (21.16%), germacrene D (7.77%) and β-caryophyllene (10.03%) in blooming period and Hexenal (15.88%), germacrene D (7.60%) and β-caryophyllene (9.48%) in post-flowering period.
Keywords: *SPME, black horehound, volatile component, Lamiaceae*

Introduction

The research on the essential oil content of medicinal plants is quite important both in scientific and economic aspects. The increase in microorganism resistance against all known antibiotics has made it necessary to investigate plant sources and their effects on cells. There are also essences, most of which constitute essential oils in medicines as well as some substances such as cellulose, pectin, sugar and it is known that these substances have an important pharmacological effect (Kilic, 2005)

Lamiaceae family, one of the greatest families that can be grown almost anywhere without discrimination of habitat type and height, is the most spread especially in the Mediterranean Basin (Watson and Dallwitz, 1978). This family has 200 genus and approximately 3200 species and family members represented with 45 genus and more than 546 species in Turkey are important in pharmacology and perfumery industry because they contain essential and aromatic oil (Davis, 1982, Baytop, 1991). Lamiaceae has a rate of endemism of 44.2% in Turkey (Baser, 1993).

The genus *Ballota* L., which is a member of the Lamiaceae family that constitutes the material of this study, has about 35 taxa around the world (Patzak, 1958). It is represented with 12 species and 8 subspecies in Turkey (Guner, 2012). *Ballota* L. is a well-known genus in Europe due to its spasmolytic and sedative effects (Garnier et al., 1961). It is used in folk medicine for cough, asthma, headache, nausea, hemorrhoids, wound and burn treatment (Baytop, 1984).

The Black horehound (*Ballota nigra*) bearing only simple hairs. Stems erect, 50-100 cm, simple or branched above. Cauline leaves ovate-orbicular to ovate, 25-70 x 20-50 mm, acute, crenate dentate, truncate or rounded at base, distinctly petiolate. Inflorescence long, lax below. Floral leaves 1-2 x dense verticillasters. Bracteoles subulate, usually shorter than calyx tube. Calyx 7-10 mm, tubular obconical, dilated above into usually 5, very short to attenuate teeth that are porrect to patent or recurved,

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mucronate or aristate. Corolla purple or pink, 9-14 mm; upper lip concave, emarginate or erose (Davis, 1982; Anonymous, 2016).

In this study, volatile components of leaf and flower of *Ballota nigra* subsp. *anatolica* P.H. Davis, that belong to three different vegetation periods including prebloom, bloom and post-flowering were determined by SPME (solid-based microextraction method).

Materials and Methods

The materials of this study which was conducted in 2015 and 2016 are leaves and flower samples of *Ballota nigra* subsp. *anatolica* P.H. Davis that were collected in 3 different periods as pre-flowering term, flowering term and post-flowering from Çamlıca province (39° 43´ N, 29° 91´ E) of Kütahya city which is situated in the inner part of western Turkey.

The leaves and flower samples that were collected were put into paper packages and transferred to the laboratory in the same day without kept waited and exposed to sunlight. After the plant materials collected were dried at room temperature (25°C), 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 adsorbed 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 for analysis. Identification of constituents was carried out with the help of retention times of standard substances by composition of mass spectra with the data given in the Wiley, NIST Tutor, FFNSC libraries.

Results

59 different volatile components were specified by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME) for *Ballota nigra* subsp. *anatolica*. The results are given in *Table 1*. Hexenal, germacrene D and β-caryophyllene were specified as the main components of *Ballota nigra* subsp. *anatolica*. Hexenal (17.60%), germacrene D (6.70%), and β-caryophyllene (8.80%) in the prebloom period; Hexenal (21.16%), germacrene D (7.77%) and β-caryophyllene (10.03%) in the bloom period, Hexenal (15.88%), germacrene D (7.60%), β-caryophyllene (9.48%) in post flowering period were identified (*Table 1*). It was observed that there was an increase in the proportion of volatile components during the bloom period.

Table 1. *Volatile components of Ballota nigra subsp. anatolica P.H. Davis according to different vegetation periods*

	Retention Time	Components	Pre-bloom (%)	Bloom (%)	Post-flowering (%)
1	1.401	Isobutanal	0.17	0.70	-
2	1.438	2-Methylpropenal	-	0.87	-
3	1.549	2-Ethylbutanal	1.52	1.14	1.20
4	1.581	2-Methylfuran	1.10	0.96	1.30
5	1.635	3-Methylfuran	0.93	0.48	0.70

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	Retention Time	Components	Pre-bloom (%)	Bloom (%)	Post-flowering (%)
6	1.910	2-Butenal	1.17	1.39	0.64
7	1.949	3-Methylbutanal	1.41	1.24	0.55
8	2.031	2-Methylbutanal	1.33	1.38	0.25
9	2.246	1-Ethylcyclopropanol	1.70	1.30	0.75
10	2.372	Pentanal	6.16	6.93	6.32
11	2.970	(E)-3-Penten-2-one	1.30	0.85	1.11
12	3.025	3-Methylenepentane	0.27	0.33	0.20
13	3.260	(E)-2-Pentenal	1.57	0.90	0.59
14	3.544	2-methyl-1-Penten-3-one	0.55	0.40	-
15	3.584	Pentanol	-	0.38	0.32
16	3.642	2-Penten-1-ol	0.44	0.26	0.25
17	3.870	3-Methyl-2-butenal	0.45	0.25	0.55
18	4.037	2-Acetylfuran	0.55	0.22	0.25
19	4.225	Hexanal	17.60	21.16	15.88
20	5.692	(E)-2-Hexenal	3.06	3.00	3.20
21	5.855	cis-3-Hexene-1-ol	5.65	6.83	5.52
22	6.184	trans-2-Hexen-1-ol	0.70	0.42	0.44
23	6.302	Hexanol	0.67	0.56	2.20
24	6.572	2,6-dimethyl-Pyridine	0.50	0.51	-
25	6.808	2-Heptanone	0.50	0.50	0.71
26	7.137	(Z)-4-Heptenal	-	0.20	0.40
27	7.218	Heptanal	0.39	0.53	0.20
28	7.548	2,4-Hexadienal	0.54	0.68	0.50
29	8.007	α-thujene	1.48	0.14	1.66
30	8.238	α-pinene	1.20	0.31	1.85
31	8.321	2,7- Dimethyloxepine	0.17	0.18	-
32	9.088	6-methyl-2-Heptanone	0.30	0.20	0.30
33	9.193	(Z)-2-Heptenal	1.50	1.30	1.25
34	9.353	Benzaldehyde	1.74	1.52	2.12
35	9.974	Cyclopentane, 1,2,4-trimethyl-, (1α)	0.71	0.64	0.33
36	10.281	1-octen-3-ol	0.80	0.20	1.34
37	10.446	2-pentyl-Furan	2.40	2.34	2.23
38	10.732	(E,E)-2,4-Heptadienal,	1.87	1.46	2.82
39	10.881	<ethyl->Hexanol	0.82	0.28	0.63
40	10.960	Octanal	0.90	0.73	0.51
41	11.297	(E,E)-2,4-Heptadienal	1.27	1.44	1.94
42	11.765	p-Cymene	1.53	1.67	1.49
43	11.913	Limonene	4.67	5.24	5.70
44	12.312	3-Octen-2-one	1.96	1.90	2.51
45	12.496	Bicyclo[6.1.0]nona-5,8-dien-4-one	1.14	1.04	1.13
46	12.794	2-ethyl-6-meth-1,5-heptadiene	0.30	0.25	0.23
47	13.076	2 Octenal	0.34	0.39	0.41
48	13.545	3,5-octadien-2-one	1.13	0.85	1.49
49	14.393	1-Undecene	2.45	2.73	2.65
50	14.886	Nonanal	1.39	1.07	1.59
51	18.263	Methyl salicylate	0.82	0.60	0.85
52	18.787	Decanal	0.30	0.19	0.30
53	21.967	3-hexadecene	0.40	0.25	0.60
54	24.845	Copaene	1.23	0.21	1.57
55	25.118	β-bourbonene	0.41	0.37	0.54
56	26.328	Germacrene D	6.70	7.77	7.60
57	26.076	β-caryophyllene	8.80	10.03	9.48

	Retention Time	Components	Pre-bloom (%)	Bloom (%)	Post-flowering (%)
58	28.390	Cyclopropanecarboxylic acid 2,2-dimeth	0.82	0.17	0.65
59	31.585	Caryophyllene oxide	0.22	0.16	0.20

Discussion and Conclusions

Bader et al. (2003) studied the volatile oil components of *Ballota nigra* ssp. *foetida*, *B. undulata* and *B. saxatilis*. β-caryophyllene (25.1%) and germacrene D (24.2%) were identified as the main components of *Ballota nigra* ssp. *foetida*. Morteza-Semnani et al. (2007) determined 42 components, specifying caryophyllene oxide (7.9%), epi-α-muurolol (6.6%), δ-cadinene (6.5%), and α-cadinol (6.3%) as the main components. The main components differ according to our study. Kazemizadeh et al. (2009) found the germacrene D (18.1%), nerolidol epoxyacetate (15.4%), sclareol oxide (12.1%), linalyl acetate (11.5%), and β-caryophyllene (10.5%) as the main components in *B. nigra* subsp. *anatolica*. Vukovic et al. (2009) determined 115 components in *Ballota nigra* in Serbia. They reported that β-caryophyllene, germacrene D, and α–humulene in the leaves and stalks, p-vinylguiacol (9.24%), borneol (7.51%), myrtenol (7.13%), trans-pinocarveol (5.22%), pinocarvone (4.37%), 2-methyl-3-phenylpropanal (4.32%), and p-cymen-8-ol (4.30%) in the roots as the main components.

Fraternale et al. (2009) identified 37 components in *Ballota nigra*. They found β-caryophyllene (20.0%), germacrene D (18.0%), and caryophyllene oxide (15.0%) as the main components. Fraternale and Ricci (2014) have specified β-caryophyllene (22.6% and 21.8%), caryophyllene oxide (18.0% and 20.5%) and germacrene D (16.5 and 13.1%) as the main components in *Ballota nigra* L. ssp. *foetida*. In our study, germacrene D (7.77%) and β-Caryophyllene were determined as the main components, sharing similarities to other works. Unlike other studies, hexenal component were identified.

Jamzad et al. (2013) reported 12 different components including β-pinene (39.0%) and α-pinene (34.5%). However, 59 different components were found, specifying Hexenal (21.1%), germacrene D (7.77%) and β-caryophyllene (10.03%) as the main components. The main components in our work differ from those of Jamzad et al. (2013).

As conclusion; *Ballota* taxa are consumed in South Anatolian regions against cough, asthma, headache, nausea and hemorrhoids, and they are used externally in wound and burn treatment. Studies should be increased for people to consume and use consciously. It is considered that local people will be informed, random collection of plants and economic losses to be incurred due to false information will be prevented, and collection of plants will be performed more consciously. Further studies related to volatile components and possibilities of their usage within the scope of antibacterial, antimicrobial and deterrent etc. should be investigated.

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