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Research article

The Effect of Different Levels of Altitutes on Composition and Content of Essential Oils of *Ziziphora clinopodioides* in Southern of Turkey

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Abstract. The genus *Ziziphora* L. belongs to the *Lamiaceae* family and consists of four species (*Ziziphora clinopodioides, Ziziphora capitata, Ziziphora persica* and *Ziziphora tenuior*). *Z. clinopodioides* is an edible medicinal plant that its leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavor. The plant known locally as 'Kır nanesi' is used in the preparation of an aromatic tea for gastrointestinal disorders and as a carminative, antiseptic and wound healing material in Turkey. In this study, essential oil content and compositions of three *Ziziphora clinopodioides* plant were collected from Ermenek (Southern part of Turkey). The altitudes of collected places of plants were varied between 908 m and 1286 m. from sea level. The essential oil content of *Z. clinopodioides* plants was hydro-distilled by Clevenger apparatus, and they were determined between 0.12 and 0.50%. The highest essential oil content (0.50%) was obtained from 908 m. The composition of essential oils was analyzed by GC/MS.

Türkiye'nin Güneyinde Farklı Yükseklik Düzeylerinin *Ziziphora clinopodioides'in* Uçucu Yağ Bileşimine ve İçeriğine Etkisi

Anahtar kelimeler:
Ziziphora clinopodioides, Ermenek, essential oil

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Özet. *Ziziphora* cinsi *Lamiaceae* familyasına aittir ve 4 türden oluşur (*Ziziphora clinopodioides, Ziziphora capitata, Ziziphora persica* ve *Ziziphora tenuior*). *Z. clinopodioides* yenilebilir bir tıbbi bitkidir ve yaprakları, çiçekleri ve sapları sık sık yabani sebze veya gıdalara aroma ve lezzet vermek için kullanılır. Türkiye'de Kır nanesi olarak bilinen bitki mide ve bağırsak bozukluklarında, gaz giderici, antiseptik ve yara iyileştirici olarak aromatik çay hazırlanmasında kullanılır. Bu çalışmada Ermenkten toplanan üç *Ziziphora* bitkisinin uçucu yağ içeriği ve bileşimi belirlenmiştir. Bitkilerin toplandığı yerlerin yüksekliği 908 m ile 1286 m arasında değişmektedir. *Z. clinopodioides* bitkisinin uçucu yağ içeriği Clevenger hidro-distilasyon cihazında damıtma ile %0.12 - 0.50 arasında olduğu belirlenmiştir. En yüksek uçucu yağ içeriği %0.50 ile 908 m yükseklikten elde edilmiştir. Uçucu yağ bileşimi GC/MS ile analiz edilmiştir.

1. INTRODUCTION

Ziziphora belongs to Lamiaceae is represented in Turkey by 6 taxa belonging to five species (Baser 2002). These species are strongly aromatic and annual or perennial herbs. They are distributed in West Anatolia, Mediterranean Region, Central and East Anatolia in Turkey (Edmondson *et al*., 1988; Kaya *et al*., 2012). *Ziziphora clinopodioides* with the Turkish name "Kır Nanesi" is The plant known locally as 'Kır nanesi' is used in the preparation of an aromatic tea for gastrointestinal disorders and as an carminative, antiseptic and wound healing material in Turkey (Baytop 1996). In additional, *Z. clinopodioides* oils exhibited the strongest antifungal activity (Khosravi *et al*., 2011).

A literature survey showed that the oil of *Ziziphora* species has been found to be rich in pulegone. The main constituents found in the oil of *Z. vychodceviana* and *Z. persica* collected from Kazakhstan were pulegone (57.5-66%) and isomenthone (5.1-15.7%) (Dembistikii *et al*., 1995). The analysis of the volatile components in the essential oil of *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis (Lamiacea) by GLC, demonstrated the presence of at least 34 compounds; 28 of them were identified. The identified components represent about 82.26% of the oil. Major components found are pulegone (46.7%) and isomenthone (19.2%). (Sezik and Tümen 1990). The major constituent found in the oil of *Z. tenuior* L. has been reported to be pulegone (87.1%) (Sezik *et al*., 1991). The essential oil of Turkish endemic *Z. taurica* subsp. *clenioides* was found to contain pulegone (81.9%), limonene (4.5%) and piperitenone (2.3%) (Shahla 2012). Baser (2002) reported that it was found that *Z. persica* contains mainly thymol (31%) in the volatile fraction, whereas the essential oils of *Z. taurica* and *Z. clinopodioides* show comparatively high levels of pulegone (over 65 and 22% respectively.

In this study, the essential oil of *Ziziphora clinopodioides* collected at different altitudes and chemical composition of its oil were determined.

2. MATERIAL AND METHODS

2.1. Plant

Ziziphora clinopodioides is a perennial plant with stemmy bushes, green or green-gray with the height of 10-15 cm, with different stems at the wooden stem, leafy, standing flowered stems, sometimes creeping, leading into capitulum, oval, pointed, circular, wide and log leaves with hair or full of hair, leaves of flowering steam are like the others parts but smaller and wider, the flower is light or dark purple, without peduncle, gathered in masses and mass capitulum and end compact, ball shape or oval, wide and long, calyx with the length of 6-4(-3) mm, thing cylinder, hairy or thin on each other or short toothed, linear-pointed with near apex and erected petal pipe from calyx (Beikmohammadi 2011). Plant materials from *Ziziphora clinopodioides* were collected from three different altitudes (908, 1282 and 1286 m) during its flowering stage in July, 2012 from Ermenek (Southern part of Turkey). The plants were identified at Department of Agronomy of Agricultural Faculty of Çukurova University.

2.2. Essential Oil Isolation

Air- dried plant material (25 g) was hydro distilled for 3 h using a Clevenger type apparatus. The oil was kept in sealed vial at 4 °C until analysis.

2.3. GC–MS Analysis

Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL min^{-1}; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 °C, MS Ionization source temperature was 220 °C, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1 µl. Oven temperature was programmed in the range of 50 to 220 °C at 3 °C min^{-1}. The structure of each compound was identified by comparison with their mass spectrum (Wiley9). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

3. RESULT AND DISCUSSION

The essential oil content of *Z. clinopodioides* plants was hydro-distilled by Clevenger apparatus, and they were determined between 0.12% and 0.50%. The highest essential oil content (0.50 %) was obtained from 908 m. Essential rate of at the altitude 1282 m of collected *Ziziphora clinopodioides* was obtained as 0.35% while from 1286 m collected plant essential oil content was found as 0.12%. The identified components and their percentages are listed according to their Retention Time (RT) Table 1. For the altitude 908 m of collected *Ziziphora clinopodioides* thirty-nine components were identified representing 97.55% of total essential oil. The major constituents of the oil were (+)-α-terpineol (33.33%), Camphor (10.86%), 1,8 Cineole (8.62%), Camphene (6.99%), α-pinene (-)- (6.55%) and (S)-cis-Verbenol (4.60%). The altitude of second collected place was 1282 m. The major constituents of the essential oil was Borneol (24.42%), followed by α-pinene, (-)- (17.65%), Camphene (8.86%), Camphor (8.57%) and Cis-Verbenol (4.67%). Isoborneol (21.22%) was determined as the main components of the naturally growing at 1286 m from sea level. Camphor (12.44%), α-pinene, (-)- (10.82%), Camphene (10.04%), caryophyllene oxide (7.41%), Verbenol (5.6%), Linalool (4.90%) and Dl-limonene (4.22 %) were recorded as other principle essential oil components. According to results, α-pinene, (-)- and camphor existed for all collected places with high levels.

Major component of collected plant varied according to altitudes, (+)-α-terpineol was at 908 m while borneol and Isoborneol were 1282m and 1286 m respectively. Last to collected places were much closed, and also main components of them were similar. Because Isoborneol is exo-isomer of borneol, through an oxidation-reduction reaction sequence, Borneol is converted to isoborneol (url-2013). They were not main component of the essential oils listed in previous works. Pulegone is the major component species of Ziziphora in the published literatures (Sezik *et al*., 1991; Meral *et al*., 2002; Baser 2002; Shahla 2012). A comparison of the findings of the current work with those of other studies shows a remarkable discrepancy that could be due to the part of the plant under analysis, the stage of plant development, the time of harvesting or picking, differences in climatic and ecological conditions, and the distinctive distillation methods applied in the studies (Ascı *et al*., 2010; Ipek *et al*., 2012).

Table 1. Constituents of the essential oils from collected different altitudes of *Ziziphora clinopodioides* (%).
Çizelge 1. Farklı yüksekliklerden toplanan Ziziphora clinopodioides'in uçucu yağ bileşimleri (%).

RT	Compound	908 m	1282 m	1286 m
2.78	Benzene	2.34	3.73	1.98
3.28	Tetradecane	-	0.45	0.23
3.46	Δ-4-Carene	0.25	0.36	0.38
3.66	α-Pinene, (-)-	6.55	**17.65**	**10.82**
4.37	Camphene	6.99	**8.86**	10.04
5.16	2- Pinene, (-)-	-	-	0.69
5.17	β-Pinene	0.72	0.97	-
5.47	Sabinene	-	0.48	0.27
5.47	α-Phellandrene	1.37	-	-
5.56	2,4 (10) Thujadine	-	0.25	0.23
5.68	Benzene, ethyl-	0.19	-	-
6.02	o-Xylene	0.57	-	0.51
6.03	p- Xylene	-	0.98	
6.52	(-)-α-Pinene	-	-	0.29
6.91	Ocimene	-	0.22	0.39

Table 1. Continued
Çizelge 1. Devamı

6.92	α-Terpinolene	0.35	-	-
7.17	2,3-Dehydro-1,8 Cineole	0.85	-	-
7.44	dl-Limonene	1.59	2.98	4.22
7.63	1,8-Cineole	**8.62**	3.32	0.83
7.70	4(10)-Thujene	-	-	0.21
8.87	γ-Terpinene	0.61	0.43	0.88
9.70	p-Cymene	1.29	1.71	1.53
10.07	cis-Ocimene	0.18	-	0.25
10.55	Dodecane	-	0.16	-
14.07	Oxamyl	-	0.17	-
15.71	Linalool Oxide (2)	0.38	0.23	0.36
16.17	Limonen-6-ol, pivalate	-	0.27	0.19
16.17	1 Octen 3 Ol	0.32	-	-
16.28	Camphenilone	0.20	-	0.19
16.58	Trans- Sabinene Hydrate	1.63	2.04	2.23
17.60	α-Campholene Aldehyde	-	0.73	0.46
17.60	α-Pinene oxide	0.29	0.94	0.84
18.12	Chrysanthenone	0.52	0.94	0.84
18.35	Camphor	**10.86**	8.57	**12.44**
18.64	9,12,15-Octadecatrienoic acid, methyl ester	-	0.22	-
19.53	P-menthane	0.23	-	0.24
19.72	Cis-Sabinenehydrate	0.90	-	0.70
19.91	Linalool	2.67	2.10	4.90
20.38	Pinocarvone	0.29	0.36	0.37
20.68	Isobornyl ester	0.27	0.54	0.35
20.88	Endobornyl Acetate	3.40	1.06	0.50
21.36	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	-	0.16	-
21.37	trans-Caryophyllene	0.18	-	0.55
21.79	Terpinen-4-ol	1.57	1.02	1.59
22.64	(1R)-(-)-Myrtenal	0.32	0.43	0.38
23.67	Isopinocarveol	-	-	0.37
23.67	trans-Pinocarveol	0.41	-	-
23.76	Verbenol	-	3.33	5.60
23.75	(S)-cis-Verbenol	4.60	4.67	-
24.39	Santolina Alcohol	0.19	-	0.63
25.35	(+)-α-Terpineol	**33.33**	-	-

Table 1. Continued
Çizelge 1. Devamı

25.36	Isoborneol	-	-	**21.22**
25.37	Borneol	-	**24.42**	-
26.44	5,8,10-Undecatrien-3-Ol	0.36	-	-
26.55	D-Carvone	0.24	0.35	0.63
28.45	9,12-Octadecadienoyl chloride, (Z,Z)-	-	0.39	-
28.69	Myrtenol	-	0.26	0.21
30.28	trans-Carveol	-	0.39	0.50
32.83	Icosapent	-	-	0.63
34.54	cis-Z-α-Bisabolene epoxide	-	-	0.46
34.83	(-)-Caryophyllene oxide	1.40	0.98	7.41
38.24	Ethyl 9,9-diformylnona-2,4,6,8 tetraenoate	-	0.16	-
42.58	Carvacrol	0.52	-	0.55
	Total	97.55	97.28	99.09
	Number of component	39	40	45

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