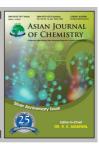
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# Essential Oils of Three Ziziphora L. Taxa from Turkey and Their Chemotaxonomy

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The essential oil aerial parts of *Ziziphora clinopodioides* Lam., *Z. persica* Bunge and *Z. tenuior* L. were investigated by GC and GC-MS. The yield of oils are *ca.* 0.30, 0.35 and 0.40 mL/100 g, respectively. Thirty seven, fourty five and thirty six compounds were identified representing 90.20, 93.12 and 92.69 % of the oil, respectively. Pulegone (20.18 %), piperitone (14.28 %) and limonene (10.66 %) in *Z. clinopodioides*, pulegone (33.27 %), β-pinene (5.75 %) and piperitone (5.68 %) in *Z. persica*, pulegone (30.00 %) and 1,8-cineole (9.65 %) were identified as major components in *Z. tenuior*. The chemical distribution of the essential oil compounds in the genus pattern were discussed in means of chemotaxonomy and natural products. In conclusion, pulegone and piperitenone derivatives are characteristic and represent excellent chemotaxonomical markers for *Ziziphora* taxa.

Key Words: Ziziphora, Lamiaceae, Essential oil, Chemotaxonomy.

#### INTRODUCTION

Turkey is situated at the junction of three important phytogeographic regions, namely Mediterranean, Irano-Turanian and Euro-Siberian with three different climates. Therefore its flora is highly used with medicinal purposes, is rich and diverse with over 10,000 vascular plant taxa and 32 % of endemism¹. The genus *Ziziphora* L. (Lamiaceae) is represented in Turkey by six taxa belongs to five species (*Z. clinopodioides* Lam., *Z. capitata* L., *Z. persica* Bunge., *Z. tenuior* L. and *Z. taurica* Bieb.) that widespread all over Turkey.

Z. taurica has two subsp. (subsp. taurica Bieb. and subsp. cleonioides (Boiss.) Davis<sup>2</sup>. For centuries, indigenous plants have been used in herbal medicine for curing various diseases and there is a popularity and scientific interest to screen essential oils and extracts of plants used medicinally in all over the world<sup>3</sup>. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries<sup>4</sup>. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases<sup>5</sup>. In Turkish folk medicine, Ziziphora taxa have been used as infusion for various purposes such as sedative, stomach ache and carminative among others. They are also used to treat various ailments such as antiseptic and wound healing<sup>6,7</sup>. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against microbial infections<sup>8,9</sup>.

At the Medicinal and Aromatic Plant and Drug Research Centre (TBAM), extensive research has been carried out into studying the chemical composition essential oils of the aromatic plants of Turkey. So far, essential oils of over 484 taxa belonging to 94 genera in 23 families have been investigated. Some genera in Lamiaceae family, such as Acinos Mill., Lavandula L., Melissa L., Origanum L., Satureja L., Sideritis L., Thymbra L., Salvia L., Thymus L., Mentha L., Nepeta L. and Ziziphora L., all the existing taxa have been studied for essential oils. From these, essential oil composition of Nepeta nuda L. subsp. nuda was investigated by Kilic et al. 10. Z. clinopodioides is an edible medicinal plant, which is widely distributed in the Anatolia. The leaves, flowers and stem of the plant are frequently used as wild vegetable or additive in foods to over aroma in Turkey. It is found in particular in central and eastern parts of Turkey. The plant known locally as 'Kirnanesi' is used in the preparation of an aromatic tea for gastrointestinal disorders and as an aperitive, carminative, antiseptic and wound healing material in Turkey<sup>11</sup>. Besides, in the Eastern part of Turkey, it is added in a special cheese, namely 'herby cheese'. To make herby cheese, sheep milk is wrist altered immediately after milking and then coagulated with calf rennet at the milking temperature. After cutting the coagulum, whey is removed and previously prepared herbs are added into the curd. About 25 kinds of herbs can be used to make herby cheese, e.g., Falcaria vulgaris Bernh., Allium

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L., *Thymus* L., *Z. clinopodioides*, *Anthriscus nemorosa* (M. Bieb.) Spreng., *etc.*<sup>12</sup>. *Ziziphora persica* also is an edible medicinal plant, which is widely distributed in the Anatolia and leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavour.

This paper reports the chemical composition of the essential oil of three *Ziziphora* taxa which were collected in the Eastern Anatolian region of Turkey. The aim of the present study is to provide chemical data that might be helpful in potential usefulness, to summarize the available information in order to facilitate and guide future researches and to examine potential chemotaxonomic significance of these species.

#### **EXPERIMENTAL**

Three *Ziziphora* taxa were collected from their natural habitats, in an island which behind the Atatürk dam wall, from Adiyaman/Turkey, on June 2011 at an altidude of 1100-1200 m. Voucher specimens of *Z. clinopodioides*, *Z. persica* and *Z. tenuior* (FUH-10285, 10286 and 10287) were kept at the Firat University Herbarium (Plant Products and Biotechnology Research Laboratory, PPRL).

**Isolation of the essential oil:** Air-dried aerial parts of the plant materials were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h.

Gas chromatographic (GC) analysis: The essential oil was analyzed using HP 6890 GC equipped with and FID detector and an HP-5 MS column (30 m  $\times$  0.25 mm i.d., film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas chromatography/mass spectrometry (GC-MS): The oils were analyzed by GC-FID-MS, using a Hewlett Packard system. HP-Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Research Laboratory (BUBAL) in Firat University. HP-5 MS column (30 m  $\times$  0.25 mm i.d., film tickness (0.25 µm) was used with helium as the carrier gas. Injector temperature was 250 °C, split flow was 1 mL/min. The GC oven temperature was kept at 70 °C for 2 min and programmed to 150 °C at a rate of 10 °C/min and then kept constant at 150 °C for 15 min to 240 °C at a rate of 5 °C/min, alkanes were used as reference points in the calculation of relative retention indices (RRI). Mass spectra were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST). The identified constituents of the essential oils of Ziziphora taxa are listed in Table-1 and the major components of some Ziziphora taxa in literature are listed in Table-2.

## RESULTS AND DISCUSSION

Water-distilled essential oil from aerial parts of *Z. clinopodioides*, *Z. persica* and *Z. tenuior* were investigated by GC and GC-MS. Thirty seven, fourty five and thirty six compounds representing 90.20, 93.12 and 92.69 % of the oil were identified, respectively. Pulegone (20.18 %), piperitone (14.28 %) and limonene (10.66 %) were identified the major components of *Z. clinopodioides. pulegone* (33.27 %),  $\beta$ -pinene (5.75 %)

and piperitone (5.68 %) were identified the major components of *Z. persica* and pulegone (30.0 %) and 1,8-cineole (9.65 %) were identified the major components of *Z. tenuior* (Table-1). Ozturk and Ercisli<sup>13</sup> reported that, most representative compounds of the essential oil were monoterpene hydrocarbons and among them the main constituents were pulegone (79.33 %), limonene (6.78 %) and piperitenone (4.20 %) in *Z. persica*. In present study 45 compounds were detected representing 93.12 % of the oil and the major compounds were pulegone (33.27 %),  $\beta$ -pinene (5.75 %) and piperitone (5.68), however limonene (3.95 %) were found minor in *Z. persica* (Table-1).

Pulegone (20.18, 33.27 and 30.0 %) was found as the major compound in the essential oils of Z. clinopodioides, Z. persica and Z. tenuior, respectively (Table-1). This compound was also detected as the main compound in the essential oil of Z. persica (79.33 %)<sup>13</sup>, Z. taurica subsp. cleonioides (81.86 %)14 and Z. clinopodioides (31.86 %) from Turkey13, Z. clinopodioides Lam. subsp. rigida (Boiss) Rech.f. from Iran (45.8 %)<sup>15</sup> and Z. clinopodioides Lam. subsp. bungeana (Juz.) Rech. f from Iran (65.2 %)<sup>16</sup>. It is conspicuous that, pulegone was not detected as major component of Z. taurica subsp. taurica<sup>17</sup>. The main compounds of Z. taurica subsp. cleonioides were pulegone (81.86 %) and limonene (4.48 %)<sup>14</sup>. Like this study, pulegone (20.18 %) and limonene (10.66 %) were found as major constituents in Z. clinopodioides (Table-1). Z. taurica. subsp. taurica contained caryophyllene oxide (26.16 %), βcaryophyllene (24.80 %) and germacrene-D (7.92 %) and Z. taurica. subsp. cleonioides contained pulegone (69.24 %), piperitenone (6.47 %) and limonene (3.59 %)<sup>17</sup>. Unlike these studies, caryophyllene oxide (3.58, 2.08 and 3.05 %) and βcaryophyllene (4.91, 2.11 and 4.08 %) were detected less percentages in Z. clinopodioides, Z. persica, Z. tenuior, respectively (Table-1). Z. clinopodioides subsp. rigida predominant portion of the oil with pulegone (45.8 %), piperitenone (17.4 %), p-menth-3-en-8-ol (12.5 %) and thymol  $(8.0 \%)^{15}$ . Also, according to Sonboli et al. (2005), predominant fraction of the Z. clinopodioides subsp. bungeana oil with pulegone (65.2 %), isomenthone (11.9 %), 1,8-cineole (7.8 %) and piperitenone (6.5 %) as the main constituents 16. Like these results, pulegone (20.18, 33.27 and 41.05 %) were found main constituents of Z. clinopodioides, Z. persica and Z. tenuior, respectively (Table-1). The studies undertaken on Z. clinopodiodies which was collected during two years from Tajikistan<sup>18</sup>, pulegone (72.8 and 35.0 %) was the main components first and second year, respectively. Like this study, pulegone (20.18, 33.27 and 30.0 %) have been reported major component in Z. clinopodioides, Z. persica and Z. tenuior, respectively (Table-1).

Ziziphora L., Origanum L. and Nepeta L. genus are belongs to the Lamiaceae family. Camphor (23.5 %), 1,8-cineole (21.0 %), borneol (18.77 %) and camphene (6.50 %) were determined main compounds of Nepeta nuda subsp.  $nuda^{10}$ . Different vegetation periods of Origanum vulgare subsp. gracile, thymol (23.1 %), γ-terpinene (10.04 %) in unflowered, α-terpinolene (28.5 %), thymol (18.60 %) in flowered and thymol (28.70 %), p-cymene (16.80 %) were determined seeded period of the main constituents<sup>19</sup>. In the literature there are some reports on the chemical constitutions of Z. clinopodioides growing in the former USSR and west

TABLE-1 CHEMICAL PROFILES OF <i>Ziziphora</i> taxa											
No	Compounds	RRI	Z. clinopodioides (%)	Z. persica (%)	Z. tenuior (%)						
1	α-Pinene	1023	2.82	3.20	2.45						
2	Camphene	1034	0.76	0.28	_						
3	Sabinene	1052	1.73	0.42	1.80						
4	β-Pinene	1056	3.90	5.75	1.05						
5	β-Mrycene	1063	1.15	1.70	1.20						
6	Benzene	1068	_	0.09	0.15						
7	3-Octanal	1070	0.90	_	0.65						
8	Limonene	1097	10.66	3.95	3.88						
9	1,8-Cineole	1095	4.13	4.19	9.65						
10	cis-Ocimene	1100	0.48	0.11	-						
11	γ-Terpinene	1115	0.09	0.25	0.23						
12	α-Terpinolene	1138	0.03	-	-						
13	Linalool	1145	0.41	0.20	-						
14	trans-Pinocarveol	1178	-	0.36	0.42						
15	Verbenol	1183	-	0.26	-						
16	Camphor	1184	0.30	0.28	0.90						
17	Cyclohexanone	1190	1.67	0.23	2.35						
18	Pinocarvone	1193	0.35	0.16	0.54						
19	Borneol	1200	0.61	0.14	-						
20	3-Cyclohexan-1-ol	1208	0.39	-	1.45						
21	α-Terpineol	1215	-	1.65	1.85						
22	Menthol	1217	-	3.81	-						
23	α-Terpinolene	1230	0.03	0.51	0.55						
24	Decanal	1221	-	0.57	-						
25	Pulegone	1240	20.18	33.27	30.00						
26	Piperitone	1250	14.28	5.68	2.75						
27	2-Cyclohexen-1-one	1254	-	3.94	0.25						
28	Methyl acetate	1257	0.12	-	3.45						
29	cis-Piperitone oxide	1262	4.37	1.12	3.87						
30	Bornyl acetate	1282	0.13	0.4	-						
31	α-Cubebene	1286	0.16	0.47	0.20						
32	Thymol	1297	4.02	3.01	3.05						
33	α-Bourbenene	1365	0.19	0.52	-						
34	α-Cubebene	1369	0.05	0.34	-						
35	Cyclohexane	1371	-	0.75	1.25						
36	β-Caryophyllene	1393	4.91	2.11	4.08						
37	<i>trans</i> -β-Farnesene	1415	_	0.35	_						
38	α-Humulene	1418	0.31	_	0.40						
39	Aromadendrene	1421	_	0.43	1.54						
40	Dodecanal	1425	-	1.55	-						
41	Germacrene D	1432	4.07	3.51	2.01						
42	Bicyclogermacrene	1443	0.63	1.89	2.05						
43	Naphtalene	1456	0.06	0.32	0.87						
44	δ-Cadinene	1459	1.46	2.32	1.65						
45	Spathulenol	1495	0.73	0.25	_						
46	Caryophyllene oxide	1498	3.58	2.08	3.05						
47	Muurolene	1523	_	0.36	0.55						
48	Bicyclosesquiphellandrene	1532	0.43	-	-						
49	α-Cadinol	1539	-	0.05	1.80						
50	Aromadendrene	1558	-	0.12	0.45						
51	Azulene	1565	-	0.17	-						
52	α-Farnesene	1575	0.11	-	0.30						
_	-	Total	90.20	93.12	92.69						
RRI, relative reter	ntion index.										

part of Turkey. According to these studies, major components of the essential oil were pulegone (13.2-21.9 %), isomenthone (2-10.8 %), menthone (4.6-5.44 %), limonene (1.8-8.19 %) and 1,8-cineole (2.3-14.5 %), respectively<sup>20,21</sup>. In our study, pulegone (20.18 %) and limonene (10.66 %) were identified the major components of *Z. clinopodioides*. Pulegone (33.27 %)

and piperitone (5.68 %) were identified the major components of *Z. persica* and pulegone (30 %) and 1,8-cineole (9.65 %) were identified the major components of *Z. tenuior* (Table-1). Germacrene D (4.07, 3.51 and 2.01 %) was the minor components of *Z. clinopodioides*, *Z. persica* and *Z. tenuior*, respectively (Table-1). Whereas germacrene D, was not determined in *Z.* 

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TABLE-2 MAJOR COMPONENTS OF SOME <i>Ziziphora</i> L. TAXA											
Ziziphora taxa.	Pulegone	Piperitenone	Limonene	1,8- Cineole	β-Pinene	β- Caryophyllene	Caryophyllene oxide	Ref. No.			
Z. persica	79.33	4.20	6.78	-	1.88	-	-	13			
Z. taurica subsp. cleonioides	81.86	2.30	4.48	0.21	0.88	_	_	14			
Z. clinopodioides	31.86	4.18	10.48	12.21	6.88	_	_	24			
Z. clinopodioides subsp. rigida	45.8	17.4	_	2.7	0.6	_	_	15			
Z. clinopodioides subsp. bungeana	65.2	6.5	_	8.7	_	_	_	16			
Z. taurica subsp. taurica	-	-	-	_	_	24.80	26.16	17			
Z. taurica subsp. cleonioides	69.24	6.47	3.59	_	_	_	_	17			
Z. clinopodioides	44.5	_	_	4.1	_	_	_	22			

persica<sup>13</sup>, Z. taurica subsp. cleonioides<sup>14</sup> and Z. clinopodioides<sup>22</sup> essential oils. In literature, composition of the essential oils Ziziphora taxa have showed some variations. When all results of Ziziphora taxa from literature were compared with our results, composition of the essential oils showed different qualitative and quantitative oil profiles (Table-2). These differences could be due to the local, genetical and seasonal factors<sup>23</sup>. An examination of Table-2 revealed definite chemotaxonomic similarities and differences among the Ziziphora taxa. All Ziziphora taxa, contained high percentage of pulegone, piperitenone and limonene, it is noteworhy that these compounds were not present in Z. taurica subsp. taurica only. On the other hand, our samples and the other samples have similar composition and resamblance dominated by the presence of pulegone, piperitenone and limonene. Work described in this paper showed that in respect to the major components Ziziphora taxa were chemically similar except Z. taurica subsp. taurica (Table-2).

#### Conclusion

This paper reports the chemical composition and comments the pattern of distribution of the essential oil compounds of three Ziziphora species collected from eastern Anatolian region in Adiyaman. Furthermore, this study demonstrates the occurrence of pulegone/Piperitone/limonene chemotype in Z. clinopodioides, pulegone/β-pinene/piperitone chemotype in Z. persica and pulegone/1,8-cineole chemotype in Z. tenuior (Table-1). Besides, some Ziziphora taxa have different types of essential oils, like pulegone/limonene/piperitenone chemotype in Z. persica<sup>13</sup>, pulegone/limonene/piperitenone chemotype in Z. taurica subsp. cleonioides14 and pulegone/ 1,8-cineole/limonene chemotype in Z. clinopodiodies<sup>13</sup>. According to the chemotype results, some variations can be seen in Ziziphora taxa. So, the changes in the essential oil composition of Ziziphora taxa, might have arisen from several divergences as; climatical, seasonal, geographical and geological. Furthermore pulegone and piperitenone derivatives are characteristic and represent excellent chemotaxonomical markers for Ziziphora taxa.

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