

## The Essential Oil Composition of *Ziziphora clinopodioides* Lam. (Lamiaceae) From Turkey.

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### Abstract

This study was designed to examine the chemical composition of the essential oils of *Ziziphora clinopodioides* (Lamiaceae) from Turkey. *Z. clinopodioides* belongs to the Lam. family, that are widespread all over Turkey. *Ziziphora clinopodioides* is an edible medicinal plant, which is widely distributed in the Turkey. The leaves, flowers and stem of the plant are frequently used as wild vegetables or additives in food to offer aroma and flavour. *Z. clinopodioides* Lam. harvested in the Eastern part of Turkey, were analyzed by GC and GC-MS and 28 components were identified. Pulegone (32.6%), *neo*-menthol (11.1%), *iso*-pulegone (10.1%), *iso*-menthone (8.9%), 2-cyclohexen-1-one (7.5%) were the main components of the *Z. clinopodioides*. The result of analysis of essential oils are presented in Table 1. Overall, twenty eight compounds which accounted for 94.1% in *Z. clinopodioides*.

**Keywords:** *Ziziphora clinopodioides*, Lamiaceae, GC-MS, Essential oil.

## *Ziziphora clinopodioides* Lam. ' in Uçucu Yağ Kompozisyonu (Türkiye)

### Özet

Bu çalışma, Türkiye' de yetişen *Ziziphora clinopodioides*' in (Lamiaceae) uçucu yağlarının kimyasal bileşimini incelemek üzere tasarlandı. Lamiaceae familyası altında bulunan *Ziziphora clinopodioides*, Türkiye' nin her yerinde yaygındır. Türkiye' de geniş bir yayılışa sahip olan *Ziziphora clinopodioides* yenilebilir tıbbi bir bitkidir. Bitkinin yaprakları, çiçekleri ve gövdesi yiyeceklere aroma ve tat katmak için yabancı sebze ya da katkı maddesi olarak sıkça kullanılır. Türkiye' nin doğu bölgelerinden toplanan *Ziziphora clinopodioides* GC ve GC-MS tarafından analiz edildi ve 28 bileşen tespit edildi. *Ziziphora clinopodioides*' in ana bileşenleri Pulegone (32.6%), *neo*-menthol (11.1%), *iso*-pulegone (10.1%), *iso*-menthone (8.9%), 2-cyclohexen-1-one (7.5%)' dir. Uçucu yağların analizinden elde edilen sonuçlar Tablo 1' de sunulmuştur. Genel olarak *Ziziphora clinopodioides* içerisinde 94.1% için toplam 28 bileşen hesaplanmıştır.

### 1.Introduction

The plant family Lamiaceae Martinov (Labiatae Adans., the mint family) has a world-wide distribution and comprises more than 7200 species across approximately 240 genera which are classified in seven subfamilies [1]. The family Lamiaceae (Labiatae) is represented in Turkey by 46 genera and 571 species of which 44.2% are endemic. The essential oils of Lamiaceae taxa growing in Turkey have been published previously [2]. Labiatae family is one of the few plant's families includes numerous genera species that

uses as culinary vegetative, herbal medicine and also as great ornamental interest, extensively in planning of parks, gardens and the urban green areas [3].

Genus *Ziziphora* include annual and perennial herbs, strongly aromatic species [4]. *Ziziphora clinopodioides* belongs to the Lamiaceae family, that are also widespread all over Iran. The leaves, flowers and stems of the plant are frequently used as wild vegetables or additives in food to offer aroma and flavour [5]. *Ziziphora clinopodioides* is an edible medicinal plant, which is widely distributed in the Anatolia. It is found in particular

in Central and Eastern parts. The plant known locally as 'Kır nanesi' is used in the preparation of an aromatic tea for gastrointestinal disorders and as an aperitive, carminative, antiseptic and wound healing material in Turkey [6].

The chemical composition of a *Ziziphora* herb includes an essential oil, tanning substances, ascorbic acid and flavonoids. It is just the present of the essential oil that is responsible for its pharmacological action. The main components of *Ziziphora* essential oils are reported as  $\alpha$ -pinene,  $\beta$ -pinene, limonene, menthone, isomenthone, pulegone and tyhmol [7].

Plants produce a large variety of chemicals, some of which are essential to themselves, while others apparently have no direct function on plants. Plant volatiles are usually complex mixtures, comprising several hundred compounds [8-10]. In spite of the differences, there is a structural similarity among plant volatiles, which include mainly terpenes (homo-, mono- and sesqui-), aromatic compounds and green leaf volatiles [11].

The aim of our study are to investigate the chemical composition of the volatile oils isolated from the aerial parts of *Ziziphora clinopodioides* from Turkey; and also to determine the distribution of the essential oil components in *Ziziphora* genus.

## 2. Materials and Methods

### 2.1. Plant Source

*Ziziphora clinopodioides* was collected from natural habitats in Elazığ, Bağcı-1890. Voucher specimens are kept at the Firat University Herbarium (FUH).

### 2.2. Isolation of the essential oils

Air-dried aerial parts of the plant materials (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to yield.

### 2.3. 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 x 0.25 mm *i.d.*, film thickness 0.25  $\mu$ m) 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.

### 2.4. Gas Chromatography / Mass Spectrometry (GC-MS) Analysis

The oils were analyzed by GC-MS, using a Hewlett Packard system. HP- Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Res. Lab. (BUBAL) in Firat University. HP-5 MS column (30 m x 0.25 mm

*i.d.*, film thickness 0.25  $\mu$ m) 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). MS 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 are listed in Table 1.

## 3. Result and Discussion

The essential oil yields of *Ziziphora clinopodioides* was found as 0.7 % v/w. The result of analysis of essential oils are presented in Table 1. Overall, twenty eight compounds which accounted for 94.1% in *Ziziphora clinopodioides*. The oils were complex mixtures of monoterpenes and sesquiterpenes. Totally, twenty eight components were identified in essential oils in the study. The pulegone (32.6%), *neo*-menthol (11.1%), *iso*-pulegone (10.1%) and *iso*-menthone (8.9%) were the major compounds of *Ziziphora clinopodioides* (Table 1). Regarding main component of the oils of different *Ziziphora* species, *Ziziphora clinopodioides* with containing pulegone as major constituent for other species were chemically different.

**Table 1.** Essential oil constituents of *Ziziphora clinopodioides* L.

NO	Compounds	RRI	%(percentage)
1	$\alpha$ -Pinene	1021	0.4
2	Camphene	1034	0.2
3	Sabinene	1051	0.5
4	$\beta$ -Pinene	1051	1.1
5	Myrcene	1063	0.1
6	Etil amyl carbinol	1069	0.1
7	Limonene	1094	0.3
8	1,8-cineole	1098	4.8
9	<i>Trans</i> -isolimonene	1125	0.2
10	<i>iso</i> -menthone	1189	<b>8.9</b>
11	<i>I</i> -menthone	1195	5.1
12	<i>neo</i> -menthol	1201	<b>11.1</b>
13	<i>iso</i> -pulegone	1204	<b>10.1</b>
14	3-cyclohexen	1205	0.3
15	Cyclohexanol	1214	0.7
16	Shiso furan	1235	0.6
17	Pulegone	1250	<b>32.6</b>
18	3-cyclohexen-1-one	1259	5.9
19	Carveol	1277	0.9
20	Bicyclo [2.2.1] heptan-2-ol	1281	0.4
21	Bicyclo [4.1.0] heptane	1286	0.2
22	2-cyclohexen-1-one	1331	<b>7.5</b>
23	$\beta$ -bourbonene	1365	0.3
24	1,6,10 dodecatriene	1413	0.2
25	Germacrene D	1433	0.7
26	Bicyclogermacrene	1443	0.4
27	Spathulenol	1493	0.4
28	<i>iso</i> -spathulenol	1524	0.1
Total			<b>94.1</b>

The chemical composition of the essential oils obtained from the aerial parts of *Z. clinopodioides* Lam. in different growth stages were analysed by GC and GC-MS, and 29 components were identified. Pulegone (30.1%), thymol (21.3%), p-mentha-3-en-8-ol (12.9%) and piperitenone (9.3%) were the main components in the pre-flowering stage. In the flowering stage, pulegone (44.6%), p-mentha-3-en-8-ol (10.5%), 1,8-cineoil (10.4%), piperitenone (8.7%) and thymol (6.7%) were identified as the main constituents and in the post-flowering stage, pulegone (41.3%), isomenthone (11.6%), p-mentha-3-en-8-ol (11%), p-mentha-3,8-diene (7.2%) and thymol (5.8%) were identified as the major components [12].

The volatile oil from aerial parts of *Ziziphora clinopodioides*, (+)-pulegone (31.86%), 1,8-cineole (12.21%), limonene (10.48%), menthol (9.13%),  $\beta$ -pinene (6.88%), menthone (6.73%), piperitenone (5.30%) and piperitone (4.18%) were found as the main constituents [13]. This study has not shown congruency with Ozturk's findings, except for pulegone, menthone major compounds. They are reported that it is possible to conclude that aerial parts of *Ziziphora* have a strong and broad spectrum of antibacterial activity against many foodborne bacteria.

The essential oil components of *Ziziphora clinopodioides* Lam. from Iran were isolated via hydrodistillation and analyzed by GC-MS, and were identified twenty seven components in the essential oil of the plant [14]. The main compounds consisted of pulegone (44.5%), terpineol (14.5%) methyl acetate (10.9%), isoneomenthol (7.1%) and 1, 8-cineole (4.1%) [14]. On the contrary, our analysis has not comprised the terpineol (14.5%) methyl acetate (10.9%) and 1, 8-cineole (4.1%) as major compounds in the essential oils of *Z. clinopodides*.

In the study of [15] Thorup et al. (1983) showed that at the two highest doses, pulegone induced atonia, decreased blood creatinine content, lowered terminal body weight and caused histopathological changes in the liver and in the white matter of cerebellum. A study from [16] Mucciarelli et al. (2001) reported that the possible interaction of pulegone and menthol with the mechanism of action of pulegone, menthone and menthol on mitochondrial respiration.

It is reported that the composition of the essential oils of some *Ziziphora* species (*Z. clinopodioides*, *Z. denticulata*, *Z. tenuior*, *Z. bungeana*, *Z. pedicellata* and *Z. persica*) grown under different ecological conditions in Russia were studied by GC. Pulegone and isomenthone were the major constituents of these oils [17]. In conclusion, pulegone was largely predominant in the oils of the *Ziziphora clinopodioides* in Turkey and world countries samples as mentioned above. The qualitative and quantitative essential oil contents of the this species showed that it has the pulegone / neo - menthole type essential oil.

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