

Chemical Constitutions and Antioxidant Activity of *Ziziphora clinopodioides* Lam Ecotypes from Turkey

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Abstract. The chemical composition of essential oil from eight ecotypes of *Ziziphora clinopodioides* grown in Coruh valley located in Northeastern Turkey was determined and its total phenolic content and antioxidant activities were evaluated. Seventeen components, representing 88.56-96.83% of the oil were identified by gas chromatography mass spectrometry. Differences for essential oil compositions and antioxidant activity were observed among ecotypes. The main components of all samples include (+)-pulegone, 1,8-cineole, limonene, menthol, β -pinene, menthone, piperitenone and piperitone. *Ziziphora clinopodioides* essential oil showed remarkable total phenolic content and antioxidant activity. The present study provides a theoretical basis for the potential application of essential oil from *Ziziphora clinopodioides* to be used as a natural resource of antioxidant agents in food industry. The results also support the traditional use of *Ziziphora clinopodioides* use in traditional medicine in Turkey.

Keywords: *In vitro* antioxidant screening, total phenolics, diversity, *Ziziphora clinopodioides*

1. Introduction

Having a number of natural habitats, ranging from Mediterranean, Aegean and Black Sea coasts to towering coastal and interior mountains, from deeply incised valleys to expansive steppes, from fertile alluvial plains to arid, rocky hill slopes, Turkey really deserves much more attention. Numerous community types and habitat mosaics, containing a rich mixture of plant and animal species many of which are endemic, crop up in this country. The flora of Turkey contains over 10 000 vascular plant taxa, a considerable number of which are used by humans for mostly traditional medicinal use [1].

Blue Mint Bush with the scientific name of *Ziziphora clinopodioides* belongs to the family Lamiaceae. This plant grows in central and eastern part of Turkey, Iran and Iraq. The leaves, flowers and stem of the plant are valuable medicine species in Northeastern part of Turkey that is used mostly in traditional food and medicine purposes. The plant known locally as 'Kirnesi' is used in the preparation of an aromatic tea for gastrointestinal disorders and as an aperitive, carminative, antiseptic and wound healing material in Turkey [2]. In particular, Eastern part of Turkey, it is added in a special cheese, namely 'herby cheese' [3].

The oxidative damages caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases. Several studies have shown that reactive oxygen species are involved in the etiology of many diseases, such as aging, cancer, atherosclerosis, coronary heart diseases, diabetes, asthma and rhinitis [4]. The body's non-enzymatic antioxidant defense system is made up of some antioxidants, such

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as vitamin C, vitamin E, vitamin K and glutathione. The exogenous antioxidants are mainly comprised of synthetic and natural antioxidants. However, there is widespread agreement that some synthetic antioxidants such as butyl hydroxyl anisole and butyl hydroxyl toluene need to be replaced with natural antioxidants because they were found to be toxic and carcinogenic in animal models [5]. Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin. The aim of the study is to determine chemical composition and antioxidant activity of eight *Ziziphora clinopodioides* ecotypes.

2. Materials and Methods

2.1. Plant Material

Fig. 1 shows natural distribution of *Z. clinopodioides* in Turkey. The plant (aerial parts) used for the present study was collected locally in Coruh valley in Turkey. Plant materials were further identified by senior taxonomists Meryem Sengul, in the Department of Biology, Ataturk University, Erzurum-Turkey. The plant samples were cleaned, shade dried and pulverized to powder in a mechanical grinder before essential oil distillation.

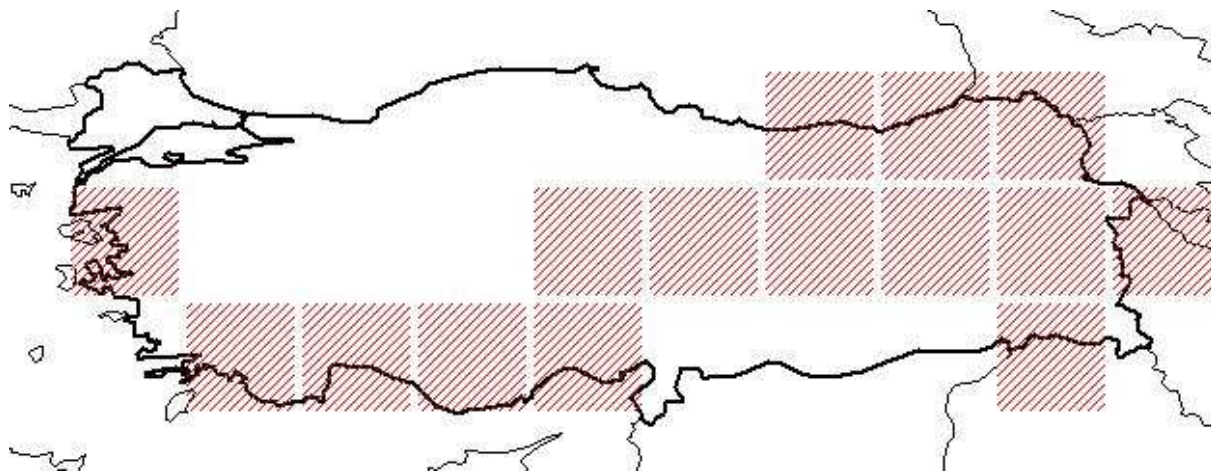


Fig. 1. Natural distribution of *Ziziphora clinopodioides* in Turkey

2.2. Essential Oil Distillation

Aerial parts of the plants were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (ILDAM Ltd., Ankara-Turkey) to produce essential oil. The isolated essential oils were dried over anhydrous sodium sulfate and stored in dark at 4 °C until analyzed.

2.3. GC-MS Analysis

GC-MS analysis of these extracts performed with GC clarus 500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite -1 fused silica capillary column (30 mm x 0.25 mm ID x 1 µm df, composed of 100% Dimethyl poly siloxane. For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/ min and an injection volume of 2 µl was employed (Split ratio of 10:1); Injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbo mass.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.4. Determination of Total Phenolic Content in the Extracts

Generally, measurement of color occurred by reaction between Folin-Ciocalteu's phenol reagent, and this method is a preferred method for the determination of the phenolic compounds present in plants, because the majority of plant antioxidants are polyphenols [6]. Total contents of the phenolic compounds in the extracts were determined by Folin-Ciocalteu's method as gallic acid equivalents (GAE) [7]. Briefly, 500 μ l of the EOs in methanol (2.5 mg/ml) was mixed with 2.25 ml distilled water and then 250 μ l of Folin-Ciocalteu reagent was added. The mixture was vortexed for 1 min and was allowed to react for 5 min. Then, 2 ml of Na₂CO₃ solution (7.5%) were added. After incubation at room temperature for 120 min, the absorbance of each mixture was measured at 760 nm. The same procedure was also used to the standard solution of gallic acid, and a standard curve was obtained. Total phenolic contents were expressed as mg of gallic acid equivalent per g of the essential oil. All tests carried out in triplicate.

2.5. DPPH Test for Antioxidant Activity

The capacity of the essential oils to donate a hydrogen atom or electron and scavenge DPPH radical was evaluated. Briefly, 50 μ l of the different concentrations (2.5, 5 and 10 μ l/ml) of essential oils in ethanol was mixed with 2 ml of ethanol solution of DPPH (24 μ g/ml). The mixture was incubated at room temperature for 60 min in the dark. Then, the absorbance was measured against a blank at 517 nm with a UV/Vis spectrophotometer. Radical scavenging activity (RSA) was calculated according to the following formula: $RSA (\%) = (A_{DPPH} - A_{EO} / A_{DPPH}) \times 100$. Where A_{DPPH} was the absorbance value of DPPH solution, and A_{EO} was the absorbance value of the test solution. All experiments were carried out in triplicate and results were reported as means \pm SD of triplicates.

3. Results and Discussion

3.1. Compounds of Essential Oil

Essential oil from aerial parts of *Ziziphora clinopodioides* was analyzed by GC-MS and resulted in the identification of 17 compounds representing 92.91 % of the oil (Table 1).

The main components of all samples include (+)-pulegone (40.13-51.13%), 1,8-cineole (6.18-9.34%), limonene (6.98-9.78%), menthol (6.57-8.83%), β -pinene (2.15-6.88%), menthone (4.45-5.47%), piperitenone (4.44-7.03%) and piperitone (2.98-4.56%) (Table 1). A previous study conducted on nine populations of the *Ziziphora clinopodioides* ssp. *rigida* from Lashgardar protected region in Iran indicated that 39 compounds were found in the oils, and a relatively high variation in their contents was found. They found that the main constituents of the essential oils were pulegone (0.7-44.5%), 1,8-cineole (2.1-26.0%), neomenthol (2.5-22.5%), 4-terpineol (0.0-9.9%), 1-terpineol (0.0-13.2%), neomenthyl acetate (0.0-7.1%) and piperitenone (0.0-5.4%) [8]. In another study which conducted in Iran, reported twenty six compounds in the oil of *Z. clinopodioides*, representing 97.6% of the total oil. They also founded that the main constituents of the essential oil were pulegone (34.4%), piperitenone (15.1%), 1-8- cineole (6.5%), neo-menthol (5.8%), menth- 2-en-1-ol (5.3%), menthol (5.2%), carvacrol (5.1%), and menthone (4.5%).

The literature survey revealed that pulegone has already been reported as the main compound of the essential oils of *Z. clinopodioides* [9]-[11]. Other *Ziziphora* species with high pulegone contents, such as *Z. taurica* ssp. *cleonioides* [12], *Z. tenuior*, *Z. taurica* ssp. *taurica* [13], and *Z. persica* [14], have also been reported. All these studies point out that the composition of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions [15].

3.2. Total Phenolic Content of *Ziziphora clinopodioides* Samples

The total phenolic content of the tested *Ziziphora clinopodioides* ecotypes ranged from 43.41 (AC4) to 55.71 mg GAE/100 g FW (AC5) (Table 2). The ecotype AC5 accumulated approximately 1.2 times higher total phenolic content in comparison with the mean value of total phenolic content (48.82 mg GAE per 100 g FW) of other investigated *Z. clinopodioides* ecotypes. Shuge et al. [16] studied on *Z. clinopodioides* Lam. and reported that the plant rich in in total flavonoids, total polyphenols and total free amino acids. They reported total phenolic content of *Z. clinopodioides* between 9.91-12.80 mg/g in different development stages. Aliakbarlu and Shameli [17] studied on essential oil of *Ziziphora* species and they found that the total phenolic contents of essential oils were the highest in *Z. clinopodioides* (114 mg GAE/g) while it was the

lowest in *Z. tenuior* (17 mg GAE/g). Gursoy et al. [18] found that total phenolic content of methanol extract of *Z. clinopodioides* was 129 µg/mg in Turkey.

3.3. Radical Scavenging Activity of *Ziziphora clinopodioides* Samples

The ability of *Ziziphora clinopodioides* extracts to scavenge the DPPH radical measured as IC_{50} varied significantly from 3.60 to 4.20 mg/ml. The ecotypes AC5, AC3, AC7, AC8 and AC2 showed high antioxidant activity with their lower IC_{50} value from 3.60 to 3.90 mg/ml. Aliakbarlu and Shameli [17] studied on essential oil of *Ziziphora* species and they found that the antioxidant activities of essential oils can be attributed to their phenolic contents. Our data suggests an inverse correlation between the amount of polyphenolic and the value of IC_{50} . This implies that polyphenolic compounds in plant might contribute to their radical scavenging activity. Our results demonstrate that *Ziziphora clinopodioides* ecotypes are good sources of dietary antioxidants as determined by the chemical DPPH radical scavenging assay. However, the ability of these ecotypes to protect cell components from oxidative damage remains to be investigated. No much literature has been found regarding the total polyphenolic content and antioxidant capacity of *Z. clinopodioides* Lam. extracts. The results obtained herein are in agreement to a certain degree with the traditional uses of *Z. clinopodioides* Lam. as a valuable source for antioxidant drugs.

4. Conclusion

The current study indicates that the essential oil components of wild populations of *Z. clinopodioides* vary with environmental conditions. Essential oils of *Z. clinopodioides* aerial parts were characterized by high levels of oxygenated monoterpenes, especially pulegone. This monoterpene ketone (pulegone) is a widespread component of the essential oils and is used as fragrance and flavour in the cosmetic, perfume, drug and food industries.

Table 1. Composition of the essential oil of *Ziziphora clinopodioides* ecotypes

COMPOUNDS	ECOTYPES							
	AC1	AC2	AC3	AC4	AC5	AC6	AC7	AC8
Monoterpenes								
Hydrocarbons								
α -Pinene	0.37	1.10	0.30	0.51	tr	tr	tr	ND
Camphene	0.51	tr	tr	0.24	0.51	0.31	ND	Tr
Sabinene	Tr	tr	0.09	0.13	tr	tr	ND	0.08
β -Pinene	6.88	5.20	2.15	3.98	2.26	2.87	3.10	3.02
β Myrcene	0.50	tr	ND	ND	tr	0.11	0.32	0.18
Limonene	9.08	9.78	8.54	6.98	8.62	8.32	7.11	7.98
γ - Terpinene	0.89	1.02	tr	0.55	0.36	0.72	tr	Tr
Oxides								
1,8-Cineole	9.34	8.16	6.98	5.67	7.43	6.18	8.11	9.13
Piperitenone	4.88	6.02	4.44	5.25	5.02	4.88	7.03	5.11
Piperitenone oxide	0.50	0.45	0.33	0.52	0.24	0.56	0.13	Tr
Alcohols								
Menthol	8.83	7.33	7.78	6.67	8.11	6.57	6.98	7.11
Ketones								
Isomenthone	0.23	tr	0.15	Tr	0.23	0.45	tr	Tr
Menthone	5.15	4.45	5.02	4.56	4.98	5.28	5.47	4.66
(+)-Pulegone	40.13	46.18	51.13	47.61	45.45	49.86	49.11	44.89
Piperitone	4.56	3.26	3.67	2.98	4.11	3.78	3.27	3.45
Phenol								
Thymol	4.14	3.56	2.86	2.49	3.33	3.67	2.55	2.47
Carvacrol	0.51	0.32	0.44	0.52	0.66	0.36	0.42	0.48
Total	96.50	96.83	93.88	88.66	91.31	93.92	93.60	88.56

RRI, relative retention indices calculated against n-alkanes. %, calculated from FID.

Table 2. Total phenolic and antioxidant capacity of *Ziziphora clinopodioides* ecotypes

Ecotypes	Total phenolic (mg GAE/100 g FW)	DPPH
		IC_{50} (mg/ml)
AC1	44.07±2.2	4.20±0.3
AC2	48.11±2.8	3.90±0.2
AC3	51.07±2.9	3.75±0.3
AC4	43.41±2.1	4.11±0.3
AC5	55.71±3.1	3.60±0.1
AC6	47.19±2.5	4.07±0.3
AC7	50.06±2.8	3.81±0.2

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