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Chemical composition of essential oils of *Ziziphora clinopodioides* Lam. (endemic Iranian herb) collected from different natural habitats

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Ziziphora clinopodioides Lam. is as an important medicinal and aromatic plant belonging to the Lamiaceae family. *Ziziphora* species are medicinal and aromatic plants that traditionally are used for the treatment of various diseases. In this study, essential oil of wild populations of *Z. clinopodioides* collected from various natural habitats in the alpine regions, Southwestern Iran was investigated. The hydro-distillated essential oils analyzed by GC and GC/MS. The essential oil yields ranged from 0.12 to 0.98 ml/100 gm dry plants. Results indicated that the main chemical compositions were pulegone (5.19 to 57.85%), limonene (0.26 to 12.79%), 1,8-cineole (0.00 to 27.4%), *p*-mentha-3-en-8-ol (2.03 to 15.15%), neo-menthol (0.36 to 7.56%), bornyl acetate (0.47 to 9.37%), and piperitenone (0.70 to 9.05%). There were significant differences among the essential oils from *Z. clinopodioides* populations for yield oil and the major constituents such as pulegone, 1,8-cineole, *p*-mentha-3-en-8-ol, bornyl acetate, and piperitenone. In conclusion, the main source of variability in chemical composition and oil yield of the studied populations of *Z. clinopodioides* seemed to be a difference in environmental conditions and chemotypes as plant populations collected from close geographical areas were classified in a cluster.

Keywords: *Ziziphora clinopodioides* Lam., Chemical composition, Essential oils

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The genus *Ziziphora* L. (Lamiaceae family) consists of four species, including *Z. clinopodioides* Lam., *Z. capitata* L., *Z. persica* Bunge., and *Z. tenuior* L. that widespread all over Iran[1]. *Z. clinopodioides* with the common Persian name "*Kakuti-e-kuhi* or *Taramoshk*" is an endemic species, which grows wild in Iran and also Afghanistan and Iraq. *Z. clinopodioides* is medicinal and aromatic plant that leaves, flowers, and stems of the herb are frequently used as wild vegetable or additive in foods to offer aroma and flavor[2]. In Iranian traditional medicine (*Unani*), *Ziziphora* species have been used as infusion, decoction and maceration for various purposes such as sedative, stomach tonic, heart disorders, common cold, inflammation, depression, diarrhea, expectorant, coughing, antiseptic, migraine, and fever, carminative and wound healing material[2,3,4,6,7]. Phytochemical investigations on the *Ziziphora* genus mainly focused on its essential oil composition[6,8,9]. The *Ziziphora* species are source of essential oils, flavonoids, caffeoyl derivatives, fatty acids and sterols[8,10,11,12]. Many literature surveys indicated that the oils of *Ziziphora* species have been found to be rich in pulegone [6,13,14,15]. A report by Ebrahimi *et al.* [24], the essential oil yield of the air-dried leaf and stem of *Z. clinopodioides* were 0.90 and 0.44% (w/w) oil, respectively. A previous report by Sonboli *et al.*[16] reported that pulegone (65.2%), isomenthone (11.9%), 1,8-cineole (7.8%) and piperitenone (6.5%) were the major components from the aerial parts of the essential oil of *Ziziphora clinopodioides* subsp. *bungeana* (Juz.) Rech. f. from Iran. In addition, Salehi *et al.*[17] indicated the major components of the essential oil of *Z. clinopodioides* subsp. *rigida* (Boiss.), were pulegone (45.8%), piperitenone (17.4%), *p*-menth-3-en-8-ol (12.5%) and thymol (8.0%).

The essential oil yield and chemical composition of essential oil in plants are related to genetic (species, subspecies, ecotype, cultivar, chemotype, etc.), environmental factors (climate, edaphic, elevation, and topography) and their interaction effects[18,19,20,21,22]. To our knowledge, no documented

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reports on diversity of chemical composition and oil yield of the essential oil of various populations of *Z. clinopodioides* in the alpine regions in Southwestern Iran (Chaharmahal va Bakhtiari and Isfahan provinces) are available. The main aim of this study was to determine the variation of chemical composition and oil yield of different populations of *Z. clinopodioides* collected from the alpine regions of Iran.

Methodology

Plants material

Samples of wild populations of *Z. clinopodioides* collected from Chaharmahal va Bakhtiari and Isfahan provinces, Southwestern Iran. Three replicates samples of each plant were gathered from five natural habitats. The aerial parts (100 gm) of the plants (10-15 cm above ground level) were harvested from 22 June to 8 July 2013. Selected geographic and characteristics of accessions differed (Table 1). Each sample was labeled and the location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver. Soil physical and chemical characteristics, including pH, electrical conductivity (EC), organic carbon (OC %) and soil texture were determined (Table 1). Climatic data of the locations were determined using data collected by the nearest meteorology station. Plant identify was confirmed by Mr. H A Shirmardi, and a representative voucher specimen (No. 1594) has been placed in the Herbarium of Research Center for Agricultural and Natural Resources, Chaharmahal va Bakhtiari Province, Shahrekord, Iran.

Essential oil extraction

The fresh aerial parts of *Z. clinopodioides* were dried inside for five days at room temperature (30 ± 5°C), and the ground to fine a powder using Moulinex food processor and passed through a 20 mesh sieve to remove large pieces of debris. The essential oil was extracted from 50 gm of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100°C for 3 hrs in a Clevenger-type apparatus, according to producers outlined British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4°C until analyzed.

Identification of the oil components

Chemical composition of the essential oils was determined by GC-FID and GC/MS. GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column (30.00 m x 0.25 mm, 0.25 μm film thicknesses). The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60°C and programmed to increase at 4°C/min to 280°C. The split ratio was 100:1. The injector temperature was set at 300°C. The purity of helium gas was 99.999% and 0.1 μL samples were injected manually in the split mode. GC/MS analysis was done on the mentioned Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV. Mass range was from m/z 50-550. Constituents were identified by comparison of their KI (Kovats index) relative to C5-C24 n-alkanes obtained on a nonpolar DB-5MS column by comparison of the KI, provided in the literature[23], by comparison of the mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology) and Willey (Chem Station data system). The percentage composition was computed from the GC peak areas without using any correction factors.

Table 1—Geographical and climate of natural habitats of *Z. clinopodioides* populations

Origin	Province	Altitude (masl)	Latitude	Longitude	P	T	*p*H	EC	OC	Sand	Silt	Clay
Saman	Ch va Bk	2240	485936	3587510	320.1	14	7.59	0.645	0.605	28	48	24
Farrokhshahr	Ch va Bk	2349	495270	3573889	291.2	12.9	7.63	0.874	0.761	40	48	12
Shirmard	Ch va Bk	2643	524253	3476493	718.5	14.4	7.39	0.682	1.326	30	38	32
Chadegan	Isfahan	2020	481698	3618851	295.5	11.4	7.53	0.573	0.351	74	20	6
Semirom	Isfahan	2707	553086	3477049	490.6	12.32	7.37	0.824	0.156	50	34	16

P: Annual precipitation (mm), T: Average temperature (°C), EC.: Electrical conductivity ($dS.m^{-1}$), OC.: Organic carbon (%); Sand, Silt and Clay in %.

Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10 to 15 yrs data.

Soil characteristics are based on average of samples taken from three farms in each region.

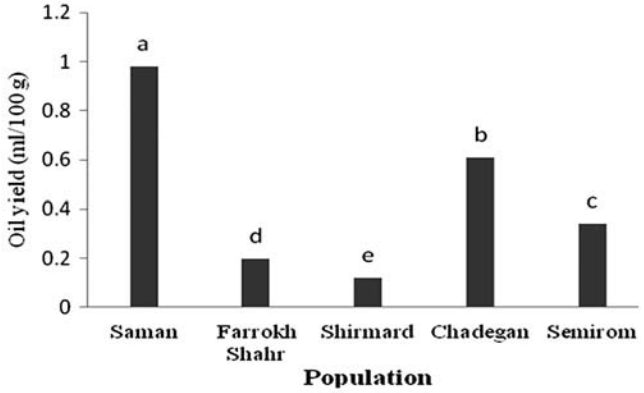

Fig. 1—The essential oil yield of various populations of *Z. clinopodioides*

Statistical analyses

The data were statistically analyzed using one-way ANOVA by the program SPSS (17.0). Means of the main constituents of the essential oils were compared by the Duncan's multiple range test at $p \leq 0.05$ level. Analytical data for Hierarchical Cluster Analysis (HCA) were treated by means of the SPSS (17) software.

Results

Statistical analysis indicated that there was significant difference ($p \leq 0.01$) among various populations for essential oil yield. The highest essential oil yield was obtained from the Saman population with 0.98 ml/100 gm dry matter. The lowest value of essential oil yield was obtained from the plants collected from the Shirmard with 0.12 ml/100 gm dry matter (Fig. 1).

According to GC and GC–MS analysis of the essential oils from various populations of *Z. clinopodioides*, 42 volatile components were identified, representing 95.91% of total oils (Table 2). The analysis of essential oils detected the major compounds, including pulegone (5.19 to 57.85%), limonene (0.26 to 12.79%), 1,8-cieneole (0.00 to 27.4%), *p*-mentha-3-en-8-ol (2.03 to 15.15%), neo-menthol (0.36 to 7.56%), terpinene-4-ol (0.00 to 6.14%), isomenthol (1.14 to 3.3%), bornyl acetate (0.47 to 9.37%) and piperitenone (0.70 to 9.05%). Statistical results indicated significant differences ($p \leq 0.01$) among the studied *Z. clinopodioides* populations for percentages of the main components (Table 2).

A hierarchical cluster analysis of the percentages of major compounds in the essential oil of *Z. clinopodioides* grouped into two distinctive clusters

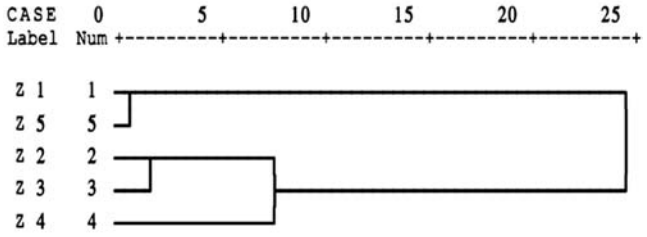

Fig. 2—Dendrogram obtained by hierarchical cluster analysis (HCA) using Ward method, based on the main compositions of the essential oils of *Z. clinopodioides*.

(Fig. 2). The first cluster formed by oils from two samples, including the Saman and Semirom populations of *Z. clinopodioides*. The second cluster was formed by the essential oil from the Farrokhshahr, Chadegan and Shirmard populations.

Discussion

Results of ethnobotany study of *Z. clinopodioides* by indigenous people western Iran indicated that the dried aerial parts of the herb have been frequently used as culinary, spice in foods, and are very popular as teapot herbs that sold in local markets and herbal stores in Iran. In addition, the dried herb is added to a yogurt and cheese, in particular in the eastern part of Iran. The clear, light yellow colored oil yields of studied populations of *Z. clinopodioides* ranged between ~ 0.1 to 1.0 ml/100 gm dry matter. Probably, this variation can be attributed to genetic (subspecies and/or ecotype), and the geographic origin of the plants. Similarly, the essential oil yields obtained from the aerial parts of different populations of *Z. tenuior* collected from Kerman provinces, Iran were from 0.61 to 0.75 ml/100 gm dry plants[6]. Verdian-Rizi[15] reported that essential oil yield of *Z. clinopodioides* was 0.9% (w/w) based on the dry weight of the plant. Results of other study by Salehi *et al.*[17] indicated the hydro-distillation essential oil from the aerial parts of *Z. clinopodioides* subsp. *rigida* was 1.0% (w/w). A comparison of our results with the previous reports suggests differences in the essential oil yield of the plant material could be attributed to genetic (genus, species, subspecies, and ecotype), the geographic origin of the plants, harvesting time, and extraction methods[20].

Results of our study indicated the essential oils obtained from different populations of *Z. clinopodioides* contained oxygenated monoterpenes, hydrocarbons monoterpenes, and sesquiterpenes. Oxygenated monoterpenes were also the main chemical

Table 2—Chemical compositions of the essential oils of Z. *clinopodioides* populations

Components	RI†	Semirom	Chadegan	Shirmard	Farrokh shahr	Saman	ANOVA
α-Thujene	927	0.00	0.28	0.65	0.30	0.00	
α-Pinene	934	0.00	1.94	3.06	1.03	0.43	
Camphene	948	0.00	0.84	1.92	0.88	0.21	
Sabinene	973	0.00	4.22	1.62	1.84	0.32	
β-Pinene	977	0.00	3.70	3.31	1.27	0.50	
Myrcene	991	0.00	1.48	0.53	0.57	0.14	
α-Terpinene	1016	0.00	1.05	2.52	1.27	0.00	
p-Cymene	1024	0.00	0.09	0.55	0.28	0.02	
Limonene	1028	0.25	12.79	1.77	5.92	0.81	$p > 0.05$
1,8-Cieneole	1032	0.70	27.4	0.00	7.97	2.21	$p \leq 0.01$
β-Ocimene <E>	1035	0.00	3.95	4.70	1.30	0.11	
β-Ocimene<z>	1045	0.00	0.00	1.86	0.00	0.03	
ɣ-Terpinene	1056	0.00	3.18	4.39	2.33	0.00	
Sabinene hydrate <E>	1065	0.00	1.32	2.42	3.94	0.21	
α-Terpinolene	1086	0.00	0.17	1.23	0.63	0.00	
Linalool	1099	0.00	0.47	0.01	0.09	0.00	
p-Mentha-3-en-8-ol	1149	14.99	2.49	2.03	4.18	15.14	$p \leq 0.01$
Menthone	1161	0.22	0.82	0.22	0.41	0.24	
Neo-Menthol	1165	2.59	2.86	0.36	7.56	3.77	$p > 0.05$
Borneol	1166	0.00	1.02	2.32	0.00	1.48	
Menthol	1180	0.00	0.27	0.00	0.29	0.22	
Neo-Iso Menthol	1184	0.00	0.00	0.00	0.19	0.41	
Terpinene-4-ol	1186	0.00	0.71	5.47	6.13	0.30	$p \leq 0.01$
α-Terpineol	1190	0.00	0.51	0.38	0.72	0.16	
Isomenthol	1197	3.3	1.98	2.81	3.26	1.14	$p > 0.05$
Pulegone	1235	57.85	5.19	7.18	17.53	56.27	$p \leq 0.01$
Piperitone	1250	0.72	0.84	0.41	2.69	1.09	
Bornyl acetate	1266	0.46	3.82	9.37	4.34	0.73	$p \leq 0.01$
Neomenthyl acetate	1272	0.00	0.00	0.56	1.13	0.00	
Geraniol	1281	0.00	0.37	0.56	1.36	0.00	
Thymol	1291	0.00	0.00	0.00	0.12	0.05	
Piperitenone	1320	8.31	0.69	1.04	2.39	9.05	$p \leq 0.01$
β-Bourbonene	1378	0.00	0.92	0.38	0.61	0.15	
β-Caryophyllene	1413	0.00	1.48	8.85	0.88	0.04	
β-Farnesene <z>	1446	0.00	0.19	0.73	0.24	0.00	
α-Humulene	1453	0.00	0.09	0.56	0.03	0.00	
Aromadendrene	1464	0.00	0.00	0.29	0.00	0.00	
Germacrene-D	1475	0.59	2.29	1.29	1.89	0.67	
Bicyclogermacrene	1489	0.00	1.32	6.2	0.67	0.00	
Spathulenol	1553	5.18	0.51	2.42	0.49	0.00	$p \leq 0.01$
Caryophyllene oxide	1557	3.78	0.68	4.32	0.42	0.00	
Veridiflorol	1582	0.00	0.00	3.43	0.00	0.00	
Total		98.94	91.93	91.72	90.17	95.9	

†Retention indices (RI) relative to C_5-C_{24} *n*-alkanes on HP-5MS capillary column.

group of the essential oil from the aerial parts of collected plants. Verdian-rizi[15] reported the major components of the essential oil from the aerial parts of *Z. clinopodioides* growing in Iran were pulegone (36.45%), piperitenone (19.12%), menth-2-en-1-ol (5.31%), carvacrol (5.10%), neomenthol (4.78), and menthone (4.46 %). Sharopov and Setzer[25] reported the main constituents of the essential oils *Z. clinopodioides* collected from Tajikistan were pulegone (72.8 and 35.0%), neomenthol (6.5 and 23.1%), menthone (6.2 and 13.3%), p-menth-3-en-8-ol (1.7 and 3.5%), piperitenone (2.6 and 1.1%) and piperitone (0.7 and 1.2%). The published reports on the chemical composition of the essential oil from other members of the *Ziziphora* indicate the major constituents were pulegone (71.2 to 85.3%), limonene (0.51 to 7.8%), thymol (1.0 to 4.3%), and menthone (0.01 to 3.7%) in *Ziziphora tenuior* [6]. In a study[9], (+)-pulegone (81.86%), limonene (4.48%) and piperitenone (2.30%) were the main constituents of essential oil of *Ziziphora taurica* subsp. *cleonioides*. A report by Aghajani *et al.*[26] indicated the main constituents of the essential oil from the aerial parts of *Z. capitata* were germacrene-D (31.1%) and (*Z*)- -ocimene (15.4%), followed by (*E*)- -ocimene (9.7%), limonene (7.8%), -caryophyllene (6.1%), hexadecanoic acid (5.9%), and bicyclogermacrene (5.2%).

Results of this study and other investigations indicate that pulegone is the major constituent in the essential oil from the aerial parts of various species of *Ziziphora*. (+)-Pulegone (C_{10} H_{16} O) is as a natural monoterpenes found in essential oils from plants of the Lamiaceae family. De Sousa *et al.*[27] reported that (+)–pulegone is a psychoactive compound and has the profile of an analgesic drug. In addition, (+)–pulegone is used in flavoring agents, perfumery, and aromatherapy[28].

Conclusion

The results of this study provide, data on ethnobotany study and the variation in chemical composition of essential oil of different populations *Z. clinopodioides* in the alpine regions in Southwestern Iran. The main constituents of these essential oils of *Z. clinopodioides*, include pulegone, limonene, 1,8-cieneole, *p*-mentha-3-en-8-ol, neo-menthol, terpinene-4-ol, isomenthol, bornyl acetate, and piperitenone. The main source of variability in chemical composition and oil yield of the studied populations of *Z. clinopodioides* seemed to be a difference in environmental conditions and chemotypes as plant populations collected from close geographical areas were classified in a cluster. Generally essential oils of *Z. clinopodioides* aerial parts were characterized by high levels of oxygenated monoterpenes, especially pulegone.

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Conflicts of interest

All authors have none to declare.

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