# Diversity in the chemical composition of essential oils of *Ziziphora tenuior* as a potential source of pulegone

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<sup>2</sup> Medicinal Plants Program, College of Natural Sciences, University of Massachusetts, Amherst, MA, 01003, USA

<sup>3</sup> Department of Plant Science, Fars Sciences and Research Branch, Islamic Azad University, Shiraz, Iran Hydro-distillated essential oils of wild populations of *Ziziphora tenuior* L. aerial parts collected from various natural habitats in the alpine regions, Kerman province, Iran were investigated for their chemical components and oil yield diversity. The essential oil yields ranged from 0.61 to 0.75 ml/100 g dry plants. GC–MS analyses revealed 16 compounds, constituting 89–97% of the essential oils. Strong chemical variability, depending on the origin of the samples, was observed. The main constituents of the essential oils 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%). Two different chemotypes were characterized: Chemotype I (pulegone) and Chemotype II (pulegone/limonene). The main source of variability in chemical composition and oil yield of the studied populations of *Z. tenuior* seemed to be a difference in environmental conditions and chemotypes as plant populations collected from close geographical areas were classified in a cluster. In conclusion, *Z. tenuior* is a good raw source of pulegone which has been widely used in food and drug industries.

Key words: chemotype, essential oil, pulegone, variation, Ziziphora tenuior L.

# **INTRODUCTION**

The genius Ziziphora L. belongs to the Lamiaceae family (mint family) and consists of four species, including Z. tenuior L., Z. clinopodioides Lam., Z. capitata L., and Z. persica Bunge in Iran. These species plants are known as "Kakouti" in Persian. Ziziphora species are medicinal and aromatic plants that are traditionally used for the treatment of hypertension, fever, edema, heart disease, neurasthenia, insomnia, tracheitis, lung abscess, and hemorrhoids [1]. Other ethnobotanical uses of Ziziphora species have been recorded in Iranian and Turkish folk medicines as stomachic, aperitive, carminative, and wound healing material [2–4]. In Iran and Turkey, the dried aerial parts of the plants are used as flavouring agents, especially in yogurt and cheese [2–5]. In addition, in Iranian folk medicine, *Ziziphora* species have been used as an infusion for various purposes such as antiasthmatic, stomachic, carminative, etc. [4, 6]. Phytochemical investigations on the *Ziziphora* genus mainly focused on its essential oil composition [2, 3, 7]. The *Ziziphora* species are source of essential oils, flavonoids, caffeoyl derivatives, fatty acids and sterols [3, 8–10]. Many literature surveys indicated that the oils of *Ziziphora* species have been found to be rich in pulegone.

Results of several studies [11–14] have shown that some characteristics of the medicinal and aromatic plants can be affected by genetic and ecological factors, including precipitation, temperature, plant competition and nitrogen concentration in the soil. The composition of *Z. tenuior* essential oil may therefore vary with plant genetics, environmental conditions, extraction methods, and geographic origin, including climate,

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soil, elevation, and topography. Knowledge of population diversity is the main prerequisite and the first step in plant breeding and domestication [15]. A local population of plants is a suitable germplasm for improving plant breeding and domestication programs. To our knowledge, no documented reports on diversity of chemical composition and oil yields of the essential oils of various populations of *Z. tenuior* in the alpine regions in Iran are available. The aim of this study was to determine the variation of chemical constitutes and oil yield of different populations of *Z. tenuior* collected from the alpine regions of Iran and to assess the relationships between variations of chemical composition contents and the environmental factors involved in different geo-ecological regions.

### **EXPERIMENTAL**

# Plant material

Samples of *Ziziphora tenuior* L. wild population, collected in various regions of the alpine region, Kerman province, Iran, were used in this study (Fig. 1). A total of three replicates samples of 30 plants each were gathered at flowering for four natural habitats. The aerial parts of the plants (2–3 cm above ground level) were harvested from 10 April to 15 June 2011. Selected geographic characteristics of accessions differed (Table 1). Each sample was labeled and the location was recorded using a Global Positioning System (GPS) receiver. Soil physical and chemical characteristics, including



Fig. 1. Sampling locations (\*) of wild populations of Ziziphora tenuior L. in Kerman provinces, Iran

Table 1.	Environment conditions of nat	ural habitats of various	s populations of <i>Zi</i>	ziphora teniur L.
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Region	Altitude, m	Longitude	Latitude	Pa	рН	E. C. <sup>b</sup>	<b>0. C.</b> <sup>c</sup>	Clay	Silt	Sand
Kohpayeh1	2 210	57°12′ E	30°92′ N	276	7.61	1.320	0.6	12	26	62
Kohpayeh2	2 235	57°12′ E	30°92′ N	276	7.48	0.834	0.69	16	34	50
Kohpayeh3	2 244	57°12′ E	30°92′ N	276	7.53	1.590	0.75	10	21	69
Khebr1	2 377	56°22′ E	28°51′ N	237	7.87	0.548	0.72	18	36	46
Khebr2	2 389	56°22′ E	28°51′ N	237	7.93	1.070	0.45	10	17	73
Khebr3	2 228	56°36′ E	29°13′ N	237	7.95	0.663	0.76	14	37	49
Rabor1	2 398	56°92′ E	29°32´ N	308	7.56	0.690	0.75	14	30	56
Rabor2	2 372	56°93′E	29°31′ N	308	7.42	0.789	0.75	18	31	51
Rabor3	2 650	56°95′E	29°33´ N	308	7.54	2.320	0.55	20	34	46
Sirch1	2 609	57°24′ E	30°11′ N	215	7.78	1.830	0.4	14	6	80
Sirch2	2 200	57°28′ E	30°12′ N	215	7.63	2.630	0.45	12	40	48
Sirch3	1 804	57°33′ E	30°11′ N	215	7.80	0.969	0.81	18	31	51

<sup>a</sup> Annual precipitation (mm); <sup>b</sup> Electrical conductivity (dSm<sup>-1</sup>); <sup>c</sup>Organic carbon (%), clay, silt, and sand (%).

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 identities were confirmed by Mr. H. A. Shirmardi, and a representative voucher specimen (No. 231) has been placed in the Herbarium of Islamic Azad University, Shahrekord Branch, Iran.

### Essential oil extraction

The fresh aerial parts of *Z. tenuior* were dried inside for four days at room temperature (30 °C), and ground to fine powder using a food processor. The essential oil was extracted from 30 g of ground tissue in 0.5 liter of water contained in a 1 liter flask and heated by a heating jacket at 100 °C for 3 h 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

GC and GC-MS analysis methods were used to determine the composition of the essential oils. GC analysis was done on an Agilent Technologies 7890 gas chromatograph equipped with a Flame Ionization Detector (FID) and a HP-5MS 5% capillary column ( $30.00 \text{ m} \times 0.25 \text{ mm}$ , 0.25 µm film thicknesses). The carrier gas was helium at a flow of 0.8 ml/min. The initial column temperature was 60 °C programmed to increase at 4 °C/min

Table 2. Essential oil composition of various populations of Ziziphora teniur L.

to 280 °C. The split ratio was 40 : 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 Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV. Mass range was from m/z 50–550. Retention indices were calculated for all components using a homologous series of n-alkanes (C<sub>5</sub>–C<sub>24</sub>) injected under conditions used with the oil samples. Identification of oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) [16].

# Statistical analyses

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

### **RESULTS AND DISCUSSION**

The yields of clear, light yellow oils of studied *Z. tenuior* populations ranged between 0.61 ml/100 g dry plants in the Kohpayeh population to 0.75 ml/100 g dry plants in the Sirch population (Table 2). The yield of the essential oils from

			Kohpayeh	Khebr	Rabor	Sirch	ANOVA
	Monoterpenes	,					
	Hydrocarbons						
1.	a-Pinene	940	tr‡	$0.09\pm0.16^{\rm d}$	$0.12 \pm 0.21$	$0.19 \pm 0.16$	N. S
2.	Camphene	951	$0.51 \pm 0.87$	$0.87 \pm 0.19$	$2.52 \pm 4.38$	$0.09 \pm 0.14$	N. S
3.	Sabinene	973	tr	$0.07 \pm 0.13$	tr	tr	N. S
4.	β-Pinene	977	$0.54\pm0.20$	$0.38\pm0.19$	$0.29\pm0.27$	$0.47 \pm 0.11$	N. S
5.	Limonene	1 0 2 8	7.82 ± 11.61 a	$\textbf{4.31} \pm \textbf{0.27} \text{ b}$	$\textbf{0.51} \pm \textbf{0.46} \text{ b}$	$\textbf{0.58} \pm \textbf{0.12} \text{ b}$	$p \le 0.05$
6.	γ-Terpinene	1056	$0.56\pm0.98$	tr	tr	tr	N. S
	Oxides						
7.	1,8-Cineole	1032	$1.01 \pm 0.53$	$1.86 \pm 1.18$	$0.65 \pm 0.55$	$0.98\pm0.06$	N. S
8.	Piperitenone oxide	1 363	$0.14 \pm 0.23$	$0.46 \pm 0.40$	$0.49\pm0.44$	$0.10 \pm 0.15$	N. S
	Alcohols						
9.	Isopulegol	1146	$1.72 \pm 0.65$	$1.53 \pm 0.27$	$1.39 \pm 1.27$	$1.42 \pm 0.24$	N. S
10.	Mentol	1 1 8 0	$0.39 \pm 0.45$	$1.76 \pm 0.71$	$1.19 \pm 1.41$	$0.61 \pm 0.28$	N. S
	Ketones						
11.	Menthone	1157	$0.01 \pm 0.02 \text{ c}$	3.46 ± 0.92 bc	3.74 ± 1.76 a	2.96 ± 0.17 ab	$p \le 0.05$
12.	Pulegone	1 2 3 5	81.19 ± 1.78 a	71.16 ± 8.51 b	79.82 ± 3.14 a	85.27 ± 1.53 a	$p \le 0.05$
13.	Piperitone	1 2 5 0	tr	tr	$0.59\pm0.58$	$0.39\pm0.34$	N. S
	Phenol						
14.	Thymol	1 283	4.31 ± 3.35	$2.99 \pm 0.81$	$1.50 \pm 2.59$	1.25 ± 1.19	N. S
15.	Carvacrol	1 301	$0.63\pm0.20$	$0.44 \pm 0.26$	$0.31 \pm 0.52$	$0.54\pm0.49$	N. S
	Sesquiterpenes						
	Hydrocarbons						
16.	β-Caryophyllene	1 4 1 3	$0.56\pm0.52$	$0.09 \pm 0.14$	tr	tr	N. S
	0il yield, ml/100 g dry matter		$0.61 \pm 0.23$	$0.68 \pm 0.16$	$0.69 \pm 0.03$	$0.75 \pm 0.07$	N. S

Retention indices (RI) relative to C5–C24 *n*-alkanes on HP-5MS capillary column. <sup>‡</sup>Trace (<0.01%).

\* Means with different letter in a row are statistically significant at 5% level probability. Values of the compounds are given as means  $\pm$  SD.

% GC peak, area percent was obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

	yield, %	tial oil Chemical composition, %		Literature
Z. tenuior	0.6–0.8	Pulegone (71.2–85.3), limonene (0.51-7.8), thymol (1.0–4.3)	South Iran	Our study
Z. tenuior	N. R <sup>+</sup>	Pulegone (86.3–87.1)	Turkey	[17]
Z. tenuior	N. R	Pulegone (85.0), limonene (5.1)	North Iran	[18]
Z. brevicalyx	0.2–0.8	Pulegone (75.0–88.0), menthone (3.2–9.6), menthol (0.5–4.7)	Uzbekistan	[19]
Z. taurica	1.6	Pulegone (81.9), limonene (4.5)	Turkey	[2]
Z. taurica	1.2	Pulegone (46.7), isomenthone (19.2)	Turkey	[20]
Z. capitata ssp. capitata	N. R	Germacrene D (31.1), (Ζ)-β-ocimene (15.4), (Ε)-β-ocimene (9.7)	Iran	[7]
Z. clinopodioides ssp. rigida	1.0	Pulegone (45.8), piperitenone (17.4),		[21]
Z. clinopodioides	0.9	Pulegone (34.4), piperitenone (15.1), 1,8- cineole	Iran	[22]
. clinopodioides (four ecotypes)	Pulegone (22.3–60.1), 1.8-cineole (6.3–29.9).		Iran	[23]
Z. clinopodioides	0.4	Pulegone (31.9), 1,8-cineole (12.2), limonene (10.5)	Turkey	[3]
Z. clinopodioides ssp. bungeana	1.0	Pulegone (65.2), <i>iso</i> -menthone (11.9)	Iran	[24]
Z. clinopodioides ssp. Rigida	Pulegone (0.7–44.5), 1.8-cineole (2.1–26.0).		Iran	[25]
Z. pedicellata	N. R	Pulegone (62.0), isomenthone (11.5), menthol (9.2)	Kazakhstan	[26]
Z. persica	0.4 Pulegone (79.3), limonene (6.8), piperitenone (4.2)		Turkey	[27]
Z. persica	N. R	N. R Pulegone (57.5), isomenthone (5.1), α-terpinene (4.8)		[26]
Z. persica	N. R	Pulegone (61.3), isomenthone (5.6), α-terpinene (5.8)	Azerbaijan	[26]
Z. persica	Z. persica 0.8 Pulegone (77.3–78.1), 1,8-cineole (11.7–13.2), α-pinene (3.9–4.3)		Iran	[28]
Z. persica	_	Pulegone (27.8), neomenthol (22.5), <i>p</i> -menth-3-en-8-ol (18.1)	Iran	[29]
Z. vychodceviana	N. R	Pulegone (66.0), isomenthone (15.7), menthone (2.3)	Kazakhstan	[26]

Table 3. Previous studies on the essential oil	vields and com	positions of the Zizi	ohora species, sub	species and ecotypes

† Not reported.

other species, subspecies and ecotypes of *Ziziphora* reported by other researchers [2, 3, 7, 17–30] was used for comparison with the oil yields obtained from studied populations of *Z. tenuior* (Table 3).

GC and GC-MS analysis resulted in identification of 16 constituents of the oil composition (Table 2). The sum of the oil composition constituted the bulk of the oils and ranged from 89 to 97% of total oil. Results of the analysis of essential oil indicated that the major compounds were pulegone (71.2 to 85.3%), limonene (0.51–7.8%), thymol (1.0–4.3%), and menthone (tr – 3.7%). We selected several reports of the species and subspecies of *Ziziphora* for comparison with the chemical compounds in *Z. tenuior* oil (Table 3). Generally, pulegone is the major constituent in species of *Ziziphora* oil, with the exception of a report by Aghajani et al. [7] that indicated that germacrene-D was the main compound in *Z. capitata* oil. This chemical differentiation was in agreement with the substantial inter-populations chemical polymorphism observed by other researchers [2, 3, 7, 17–30].

(+)-Pulegone ( $C_{10}H_{16}O$ ) is a natural monoterpene found in essential oils from plants of the Lamiaceae family. This compound is the major constituent of *Ziziphora* species oil. De Sousa et al. [30] reported that (+)-pulegone is a psychoactive compound and has the profile of an analgesic drug. (+)–Pulegone is used in flavoring agents, perfumery, and aromatherapy [31]. Previous studies [32] show that (+)–pulegone is considered one of the most powerful allele-chemicals and has been demonstrated to be about twice as toxic as HCN. Pulegone and pulegone-derived lactones have anti-feedant, antibacterial, antifungal and insecticide activity [33–37].

A hierarchical cluster analysis of the content of the main compounds in the essential oils of Z. tenuior could be grouped into two distinctive clusters (Fig. 2). The first cluster is formed by the essential oils of the populations of Z. tenuior from three natural habitats, including Sirch, Khebr, and Rabor. This cluster had higher pulegone concentration as compared with other cluster (Chemotype I: pulegone). The second cluster formed by the essential oil of one population (Kohpayeh) contained pulegone (81.2%) and limonene (7.8%) (Chemotype II: pulegone/limonene). The chemical variation between the populations can be attributed to genetics (ecotype and chemotype), and environmental factors (climate, seasonal, and geographical) is frequently encountered in aromatic plants [38], these factors influence biosynthetic pathways and consequently the relative proportion of the main characteristic compounds. Genetic differences cannot be directly deduced from the varying amounts of a secondary plant product [39]. Probably, the environmental factors,





including temperature, radiation, air humidity, topography, etc. influenced the chemical constitution of *Z. tenuior* oils collected from the four alpine regions, Iran.

In conclusion, the current study indicates that the essential oil components of wild populations of *Z. tenuior* vary with chemotypes, environmental conditions, and geographic origin. Variation in oil composition of *Z. tenuior* can result from genetic diversity and differences in environmental conditions and their interactions. The main source of variability in chemical composition of the studied populations seemed to be due to differences in environmental conditions. Essential oils of *Z. tenuior* 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.

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# ZIZIPHORA TENUIOR ETERINIŲ ALIEJŲ, POTENCIALAUS PULEGONO ŠALTINIO, ĮVAIROVĖ IR CHEMINĖ SUDĖTIS

### Santrauka

Ziziphora tenuior L. laukinių populiacijų antžeminės dalys buvo rinktos įvairiose gamtinėse kalnų augimvietėse Irane, Kermano provincijoje. Hidrodistiliacijos būdu iš jų išskirti eteriniai aliejai buvo tiriami dujų chromatografijos-masių spektrometrijos būdu. Nustatyta 16 junginių, sudarančių 89–97 % eterinių aliejų masės. Identifikuoti du šių augalų chemotipai.