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Original Article

Chemical Variation of *Ziziphora clinopodioides* subsp. *rigida* (Boiss) Rech. f. from the Central of Iran

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Abstract

Because of the special situation of medicinal plants and their natural product on human health, researchers are interested to discover useful constituents. The present research aims to discover the level of variation in oil of *Ziziphora clinopodioides* subsp. *rigida* (Boiss) Rech. f. at full flowering stage in Yazd province. All samples were approved in herbarium of Yazd University and dried out in shade and laboratory situation. Then 50 grams of each sample were powdered. In order to extract the oil of each community, the dried materials were subjected by hydro-distillation method (clavenger types). Resulted oils were dried by anhydrous sodium sulfate and kept in tightly closed vials at 4 °C before chemical analyses. The GC-FID and GC–MS analyses identified 29 compounds among which pulegone (18.1-51.4%), piperitenone (10.3-23.3%), and 1,8-Cineole (8.6-15.6%) were the major component in all populations. It is indicated that the present result is similar to the previous study. In addition, Damgahan and Tezerjan had the highest amount of essential oil and pulegone in all studied populations, respectively.

Keywords: Composition, Variation, *Ziziphora*, Iran.

Introduction

Due to the importance of medicinal plants and their metabolites on human health, researchers are interested to discover new components. Therefore, the commercial development of medicinal plants as new source of bioactive products to enhance human health and food preservation is one of the key roles. One of the largest and most distinctive families of plants in the world is Lamiaceae. Some of species in Lamiaceae are one of the major sources of culinary, food flavoring, vegetable, and medicinal plants all over the world. Wide ranges of compounds, such as terpenoids, phenolic compounds, and flavonoids, have been isolated from the members of the family [1,2]. Labiateae family contains of 258 genera and 6970 species. The family, with 46 genera and ca. 420 species and subspecies, has also a great diversity and distribution in the flora of Iran [3-5]. This family is important for their essential oils, and many biological activities. Most species of Lamiaceae have being used in perfumery, confectionary and pharmaceutical preparations. They are also used in different traditional medicinal treatments as herbal remedies and in the food industry as food additives and taste enhancers because of their good odors [6]. On the other hand, there has been a worldwide trend towards the use of essential oils and natural phytochemical for their pharmacologic efficacies [7,8].

Ziziphora clinopodioides ubsp. *rigida* (Boiss) Rech. f. with the common Persian name “kakuti-e kuhi” belongs to Lamiaceae family. *Ziziphora* is represented widespread all over Iran with three annual species (*Z. tenuior, Z. persica* and *Z. capitate*) and a perennial species (*Z. clinopodioides*) [9]. The species is an edible medicinal plant that leaves, flowers and stems are commonly used as wild vegetable or additive in foods. In Iranian folklore, the dried aerial parts of this plant have been frequently used as culinary, cold, cough treatments, various ailments such as antiseptic and wound healing [10]. *Z.*

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clinopodioides is used for the treatment of stomachic, anti-fever, anti-inflammatory, sedative and flavoring in Traditional Medicine of Iran [11, 12]. These treatment efficacies are because of compound that is called natural product. Natural products as secondary metabolite like essential oil, which are aromatic substances that gained from aromatic and medicinal plant has attracted great interests due to their well-known biological properties. In other word, many researchers focus on chemical composition in different plants [13]. On the other hand, the variation of essential oil compositions have been studied in several medicinal plant species such as *Z. clinopodioides* subsp. *rigida* [14], *Satureja khuzistanica* jamzad [15], *Stachys lavandulifolia* Vahl [16], *Salvia tebesana* Bunge [17], and *Nepeta asterotricha* Rech. f. [18].

A literature review showed that most component in the oil of *Ziziphora* is pulegone. The main constituents in the oil of *Z. vychodceviana* and *Z. persica* from Kazakhestan were pulegone (57.5-66%) and isomenthone (5.1.-15.7%) [19]. Essential oil of Turkish Endemic *Z. taurica* subsp. *clenioides* was pulegone (81.9%), limonene (4.5%), and piperitenone (2.3%) [20]. The most important constituent in the oil of *Z. tenuior* L. was pulegone (87.1%), too [21]. The chemical composition of essential oil of *Z. persica* and *Z. canescens* was pulegone [22]. Regarding main component of the oils of different *Ziziphora* species, a research group founded *Z. capitata* containing germacrene D (21.3 %) as major constituent in contrast to pulegone for other species were chemically different. The research showed that the other main compositions of *Z. capitata* were limonene, β-caryophyllene, bicyclogermacrene and spathulenol 7.8, 7.5, 5.5, and 4.5%, respectively [23]. The essential oil of *Z. clinopodioides* was investigated in Razan, Lorestan, Iran. In this research were identified twenty-three constituents accounting to 96.6% of the total oil. The major components of the oil were pulegon (30.1%), thymol (21.3%), p-mentha-3-en-8-ol (12.9%), piperitenone (9.3%) and 1, 8-cineol (4.1%) [24]. In Kermanshah province *Z. clinopodioide* was examined. The results discovered the main components were dissimilar with other outcome. According to the study eighteen components were categorized also, carvacrol (64.2%), thymol (19.2%), p-cymene (4.8%) and γ-terpinene (4.6%) were the abundant components of the oil [25]. The oil of *Z. clinopodioide* in Dehbala, Yazd was studied and the main compound was carvacrol (52.7%) [26]. Related survey in Taftan, Baluchestan, Iran was done. In this research confirmed thirty-two ingredients that the major components were pulegone, cis-caran-trans-2-ol, 1, 8-cineole, and 2-β-pinene 61.67, 12.66, 10.23, and 2.16%, respectively [27].

As can be seen unlike regions are dissimilar in quality and quantity of essential oil. Growth and development of plants in different ecosystems and natural habitats are effected by disparate environmental factors such as topography, climate and soil. For this purpose, the present study aims to investigate *Z. clinopodioides* in dissimilar area to characterize the level of variation in Yazd, Iran.

Material and Method

The Selective Site

Five ecotypes of *Ziziphora clinopodioides* subsp. *rigida* (Boiss.) Rech.f. were studied in natural habitats of Yazd, Iran. Geographical properties such as altitude, latitude, and direction of each study area were considered, which could be presented in the table 1.

Table 1 Geographical property of the studied areas

Parameter / Habitat	Geographical properties		
	Latitude	Longitude	Altitude (m)
Damgahan	31° 30' 54" N	54° 18' 46" E	2414
Dehbala	31° 34' 53" N	54° 05' 22" E	2791
Taghi Abad	31° 34' 24" N	54° 07' 14" E	2736
Tezerjan	31° 34' 28" N	54° 09' 30" E	2532
Zardein	31° 30' 04" N	54° 14' 17" E	2610

Plant parameters and extraction the essential oil

All samples were compared with flora of Iran to be approved. The samples were dried in the shade at room temperature. Then 50 grams of each community was powdered after those essential oils were obtained by hydro distillation using a Clevenger for 3 hours. The obtained essential oils were dried by filtration over anhydrous sodium sulfate and kept in tightly closed vials at 4 °C. The percentage of the oils was calculated based on dry weight. Finally, the oils were analyzed by GC-FID and GC-MS to identify each compounds.

Gas Chromatography

The GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). This analysis was carried out on a fused silica capillary BPX-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The injector and detector temperatures were kept at 250 ºC and 300 ºC, respectively. Nitrogen has been used as the carrier gas at a flow rate of 1.1 ml/min; the oven temperature program was 60–250 ºC at the rate of 4 ºC /min and finally held isothermally for 10 min; split ratio was 1:50.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out by a Thermoquest-Finnigan gas chromatograph equipped with same form as mentioned above for the GC by a TRACE mass

(Manchester, UK). Helium was used as the carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 ºC and 250 ºC, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same as the GC.

Identification and Quantification of the Essential Oil Compounds

Chemical compositions of essential oil were achieved by comparison of retention indexes (determined relative to the retention times of a series of n-alkanes) on a BPX-5 column and recorded mass spectra with those available in the system. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST, Adams and Wiley 7.0). The yield of essential oil in each study area is presented in table 2 and it varied between 0.7 – 1.7%. The highest and lowest quantity of yield was obtained from Damgahan (1.7) and Zardein (0.7), respectively. The GC-FID and GC-MS analyses showed 29 compounds that the identified rang varied between 91.7% up to 97.9% of total essential oils. As can be seen in table 2 the major compositions were pulegone (Figure 1), piperitenone (Figure 2), and 1,8-cineole (Figure 3) in all populations. Taghi Abad and Tezerjan had the highest amount of pulegone. It should be said that the main composition in the oil of *Z. vychodceviana* and *Z. persica* from Kazakhestan were pulegone (57.5-66%), which is perfect [19].

Table 2 Chemical compounds of the essential oil of *Ziziphora clinopodioides* subsp. *rigida* (Boiss) Rech. f. in the study areas

No	Compound	RI	Damgahan	Deh-Bala	Taghi Abad	Tezerjan	Zardein
1	*α*-Thujene	925	0	0.1	0.0	Tr	Tr
2	*α*-Pinene	933	0.9	1.3	1.2	1.3	0.9
3	Camphene	948	1.1	0.6	0.4	0.2	0.4
4	Sabinene	973	1.1	2.3	1.4	1.2	1.1
5	*β*-Pinene	978	1.2	1.6	1.7	1.3	1.5
6	Myrcene	989	0.4	0.9	0.6	0.6	0.5
7	*α*-Terpinene	1017	0.3	0.8	Tr	0	0
8	*o*-Cymene	1026	0	0.6	Tr	Tr	0
9	1,8-Cineole	1034	11.4	15.6	11.8	8.6	10.9
10	*(E)*-*β*-ocimene	1046	1.1	0.2	0.1	0.2	0.2
11	*γ*-Terpinene	1058	1.1	1.8	0.1	0.3	0.2
12	*cis*-Sabinene hydrate	1069	5.3	8.2	0.2	0.3	9.1
13	Terpinolene	1089	0.4	0.5	0.1	0	0
14	*trans*-Sabinene hydrate	1101	Tr	0.4	0.0	0	0.1
15	*p*-mentha-3-en-8-ol	1151	5.2	6.1	0.0	Tr	10.6
16	Isomenthone	1160	2.9	2.9	0.0	Tr	0.8
17	Borneol	1168	3.9	tr	3.8	0	Tr
18	Isopulegone	1170	1.5	1.8	0.0	Tr	1.1
19	Terpinen-4-ol	1177	3.1	4.1	Tr	1.8	3.8
20	Iso-Menthol	1185	3.2	7.5	0.0	6.1	7.1
21	Pulegone	1234	40.9	18.1	50.2	51.4	22.5
22	Piperitone	1252	0	1.1	0.0	0.8	0.8
23	Geranyl formate	1304	0.3	0.4	Tr	0	0.5
24	Piperitenone	1351	10.3	11.8	21.7	23.3	18.3
25	*β*-Bourbonene	1388	0.2	0.1	0.0	0	0.2
26	*E*-Caryophyllene	1423	Tr	0.1	0.1	0	0.9
27	Germacrene D	1484	1.2	0.7	0.8	0	0.6
28	Bicyclogermacrene	1500	0.2	0.6	0.2	0	0.3
29	Spathulenol	1584	0.7	0.4	0.2	0.4	0.4
	Total		97.9	90.1	94.8	97.8	92.8
	Yield %		1.7	1.5	1.69	1.4	0.7

tr: Trace (< 0.05%)

Results and Discussion

On the other hand, the oil of *Z. taurica* subsp. *clenioides*, as an endemic plant in Turkey, was full of pulegone (81.9%). In other words, *Z. taurica* subsp. *clenioides* can be used as a source of pulegone [20]. The most important constituent in the oil of *Z. tenuior* L. was pulegone (87.1%), too [21]. The chemical composition of essential oil of *Z. persica* and *Z. canescens* was pulegone [22]. Briefly, Ziziphora is famous for this monoterpene compound. Pulegone could be found in *Mentha piperita* L., *Mentha pulegium* L., and *Ziziphora clinopodioides* essential oils (60–90%). Additionally, another source of human exposure to pulegone is the consumption of beverage flavoring and confections [28-30]. On the other hand, the lowest amount of pulegone was found in Dehbala (18.1%). This variation is directly affected by environmental factor and genotype. In other words, secondary metabolite are strongly influenced by several intrinsic and extrinsic factors, the variation of quantity and quality of essential oils in the present research can be attributed partly to genetic factor or environmental condition of the studied region. Tezerjan not only has the highest amount of pulegone but also it has the highest quantity of Piperitenone. Additionally, the main compound of *Z. clinopodioides* oil in Razan were pulegon (30.1%). The identified chemical compounds were twenty-three accounting to 96.6% of the total oil [24]. The oil of *Z. clinopodioide* in Dehbala, Yazd was studied and the main compound was carvacrol (52.7%) [26]. Related survey in Taftan, Baluchestan, Iran was done. In this research confirmed thirty-two ingredients that the major components were pulegone, cis-caran-trans-2-ol, 1, 8-cineole, and 2-β-pinene 61.67, 12.66, 10.23, and 2.16%, respectively [27]. A research group in Kermanshah province was examined the oil of *Z. clinopodioide*. They discovered the main components were dissimilar with other outcome. According to their study eighteen components were categorized, which carvacrol (64.2%), thymol (19.2%), p-cymene (4.8%) and ɣ-terpinene (4.6%) were the abundant components [25]. These variations are be because of collection time, drying conditions, geographic or climatic factors, and mode of distillation. On the other hands, different researchers state that the chemical variation between the populations can be attributed to environmental and ecological factors such as geography, climate, and topography [15,16,18]. It is indicated that the present result is similar to the previous study. In addition, Damgahan and Tezerjan had the highest amount of essential oil and pulegone in all studied populations, respectively.

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