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Research Article

Extraction and determination of content and composition of essential oils of vegetative and reproductive organs of *Zataria multiflora*

Mansoureh Jiyanpour[1], Alireza Yavari[2*]
Department of Horticulture Science and Engineering, College of Agriculture and Natural Resources, University of Hormozagan, Bandar Abbas, Iran
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Abstract

Zataria multiflora **is one of the valuable medicinal plants belonging to the Lamiaceae family. The aromatic aerial parts of this species are traditionally used by endemic folks to flavor some native foods, also for numerous therapeutic functions. In the present study, different plant organs (leaf, flower, stalk and whole aerial organ) were collected from Fanuj region of Sistan and Balouchestan province, in the southeast of Iran, and then the variability in the essential oil contents and compositions of them were studied. The essential oils of air-dried samples were extracted by hydro-distillation. The experiment was arranged in a completely randomized design with three replications for the essential oil contents. The essential oil yields were calculated based on dry weight and the oils were analyzed by a combination of GC-FID and GC-MS techniques, investigate for chemical variability. The essential oil yields of leaf, flower, stalk and aerial part were 4.8, 5.3, 0.6 and 2.7 % (w/w), respectively. The total number of compounds identified and quantified were 17 in leaf, 17 in flower, 19 in stalk, and 19 in whole aerial organ, representing 99.0, 99.2, 97.3, and 96.2% of the total essential oil, respectively. Results of essential oil compound analysis demonstrated that thymol (31.0 – 67.1 %), carvacrol (8.4 – 36.7 %) and *p*- cymene (6.5 – 23.2 %) were the main compounds in the evaluated plant organs of *Z. multiflora*. Chemical diversity of the essential oil of *Z. multiflora* plant parts can be considered by medicinal plants breeders and pharmaceutical, food and cosmetic industries for breeding and processing uses.**

Keywords: Chemical variation, Essential oil, Plant organ, Thymol, *Zataria multiflora*

Introduction

Zataria multiflora Boiss. is a medicinal and aromatic perennial shrub belonging to the Lamiaceae family that grows naturally in dry and rough inclines from central to southern parts of Iran, furthermore Afghanistan and Pakistan (Sajed *et al*., 2013). This aromatic plant is known by the Persian name of Avishan Shirazi that is additionally entitled Sattar or Zattar, which means thyme (Pourhosseini *et al*., 2020). *Z. multiflora* grows up to 80 cm as a shrub with dark green leaves and white flowers and a lifespan of several years (Jamzad, 2012). The biological activities of the plant are associated to the essences in epidermal glands of leaves, stalks and other organs, along with the phenolic compounds stored in the whole aerial organ (Golmakani and Rezaei, 2008; Pourhosseini *et al*., 2020). The *Z. multiflora* species has a wide range of biological properties; It has anti-inflammatory, anti-microbial and anti-spasm effects and is an analgesic (Dashipour *et al*., 2015; Azadi *et al*., 2020; Barghi *et al*., 2021). Meanwhile, due to its pleasant odor, this plant is also used as a seasoning for yogurt, numerous types of fast foods and many Iranian local cuisines (Nazaryanpour and Nejad Ebrahimi, 2020). Many studies have focused on *Z. multiflora* essential oil as the predominant secondary metabolite of this species, which have reported three compounds of thymol, linalool and carvacrol as the main compounds (Saharkhiz *et al*., 2010; Hadian *et al*., 2011; Sadeghi e*t al*., 2015; Mahmoudvand *et al*., 2017; Meamari *et al*., 2020). *Z. multiflora* essential oil has anti-oxidant properties due to reducing oxidative stress (Saleem *et al*., 2004; Saei-Dehkordi *et al.*, 2010; Mojaddar Langroodi *et al*., 2019). This species is used to treat respiratory disorders, gastrointestinal disorders, fever, premature labor pain, rupture, bone and joint pain, headache, diarrhea, vomiting and colds (Khazdair *et al*., 2018; Golkar *et al*., 2020).

Biosynthesis of the secondary metabolites starts from basic pathways, such as the glycolysis or shikimic acid pathways, and subsequently diversifies, largely depending on cell type, developmental stage and environmental cues, these compounds are widely distributed in different plants cells, tissues and organs. However, different cells, tissues and organs of medicinal plants may possess different medicinal properties at different developmental stages (Li *et al*., 2020). Given the considerable applications of volatile compounds and essential oils in different industries such as medicine, food and cosmetics, the extraction and study of their constituents in various medicinal plants

*Corresponding Author, Email: yavari@hormozgan.ac.ir – yavari313@gmail.com

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have become significantly important (Jalali *et al*., 2021). To date, numerous reports on the study of the diversity in chemical compounds of *Z. multiflora*'s aerial organ essential oil, collected from its different natural habitats, have been published in Iran. In one study, the performance efficiency and chemical compounds of aerial organ essential oil of *Z. multiflora* were assessed during the plant's flowering phase; samples were collected from six natural habitats in Hormozgan Province including Roudkhaneh, Lavar-e-sheikh, Faryab, Tang-e Zagh, Bashagerd and Bandar Khamir. Based on the results, the highest and lowest essential oil efficiencies were found in the ecotypes of Roudkhaneh (6.5%) and Bandar Khamir (3.9%), respectively; moreover, four compounds including thymol, linalool, carvacrol and para-cymene were reported as the main constituent compounds of the essential oil (Meamari *et al*., 2020). In another study, the compounds and contents in the essential oil of aerial organ of *Z. multiflora*, collected from four habitats of Estahban, Neyriz, Fasa and Larestan, were examined; the results showed five compounds including thymol (34.41-54.35%), para-cymene (9.49-19.85%), gamma-terpinene (7.34-16.70%), carvacrol (5.35-15.34%) and alpha-pinene (1.63-5.25%) as the major essential oil compounds in the collected samples (Niczad *et al*., 2019).

Considering the extensive application of this plant in the traditional Iranian medicine, conducting more phytochemical studies on this subject appears extremely essential. To date, there has been a variety of inquiries on *Z. multiflora* such as the identification of sesquiterpenes and phenolic compounds (Hadian *et al*., 2011; Sadeghi *et al*., 2015; Golkar *et al*., 2020; Pourhosseini *et al*., 2020). However, there have been no studies specifically conducted to compare the chemical compounds of essential oils in different *Z. multiflora* organs including leaf, flower and stalk. Therefore, the present study attempted to examine the chemical compounds of different organs of *Z. multiflora* including leaf, flower and stalk; in addition, these compounds were compared with those of the whole aerial organ for further applications in breeding and different parts of industries.

Materials and methods

Plant material and habitat specifications: Initially, the natural habitat of *Z. multiflora* was identified by directly observing its different single shrubs in Fanouj, located in Balouchestan region of Sistan and Balouchestan Province (719 m above mean sea level), with geographical coordinates of 26°34′ 33″ North and 59°38′ 42″ East (Rechinger, 1963; Jamzad, 2012). To determine the harvest time at the full flowering stage of the plant, phenological information of Fanuj ecotype was collected. Then, the aerial organs of 30 whole shrubs were collected during their complete flowering phase on late April of 2019. Voucher specimen was authenticated and deposited in the Herbarium of Agricultural and Natural Resources Research Center of Hormozgan Province and registered under the herbarium code of 2228. The shrubs were divided into four separate sample groups consisting of leaves, flowers, stalks and the whole aerial organs. Then, the samples were dried in darkness, under room temperature (24° C). Finally, the samples were conserved in closed containers placed within a moisture-free environment until the time of application.

Isolation of the essential oils: The distillation method with water was employed to extract the essential oils and to determine their percentages. Importantly, it was essential to provide a condition of maximum contact with the water in the apparatus balloon. Consequently, the dried samples of each organ (leaf, flower, stalk and the whole aerial organ) were grinded separately using a grinder. Then, 50 g of the resulting powder of leaf and flower organs along with 200 and 100 g of stalk and the whole aerial organ powders, respectively, were added to a specific volume of distilled water using the distillation method (Jalali *et al*., 2021). The essential oils of samples were extracted by hydro-distillation for 3 h with three replications for each sample, using a Clevenger-type apparatus (5 L volume; Shot, Germany) according to the method recommended in British Pharmacopoeia (Pharmacopoeia, 2007). Finally, the essential oil yield was calculated based on the dry weight of the plant material according to 3 repetitions, using Equation (1) (Medjahed *et al*., 2016).

$$\text{Essential oil yield (\%)} = \frac{(\text{Essential oil weight} \times 100)}{\text{Plant material weight}} \quad (1)$$

Anhydrous sodium sulfate was used to remove the existing moisture in the resulting essential oils and then they were stored at 4 °C in sealed glass containers for analysis in the refrigerator (Binava *et al*., 2020).

Separation and identification of essential oil constituent compounds: Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) devices were used to identify and separate the constituent compounds of the essential oils, in the Medicinal Plant Research Department of Iran Research Institute of Forests and Rangelands, Tehran, Iran. After separation, the percentages of the constituent compounds of each essential oil were calculated along with the inhibition index. To conduct a qualitative examination (identification), the mass spectrums related to the compounds of essential oils were obtained. The spectrums were identified by calculating Kovats retention indices, carried out by injecting normal hydrocarbon (C_7-C_{25}) under the same conditions as the injection of essential oils; then, they were compared with values published in various references. Mass spectrums were also examined to identify the compounds and the identifications were confirmed based on standard compound mass spectrums and different library sources. The relative percentages of each essential oil constituent compound were obtained for the area under the curve in the gas chromatography spectrum and then compared with values in different reference by considering the Kovats index (Shibamoto, 1987).

Gas chromatography device (GC): The employed GC device (Model: Thermo-UMF) was equipped with a Chrom-card 2006 processor and capillary column with a length of 10 m, internal diameter of 0.1 mm, and stationary phase layer thickness of 0.25 µ, under the commercial name, 'Ph-5'. The temperature planning of the column entailed a starting temperature of 60 °C and adding 3 °C to the temperature every 3 minutes until reaching 210 °C. Then, the temperature was ramped up by 20 °C every minute and stopped at 240 °C for 8.5 minutes. The injection chamber and detector temperatures were adjusted as 280 and 300 °C, respectively. The type of detector used in the GC device was FID (Flame Ionization Detector); helium gas was used as the carrier gas and its input pressure to the column was set as 3 kg/cm (Davies, 1990).

Gas chromatography-mass spectrometry device (GC/MS): A GC device (variant 3400) installed to MS (Saturn II, GC/MS) was employed. The used column was a Ph-5 type with a length of 10 m, an internal diameter of 0.1 mm, and a stationary phase layer thickness of 0.25 µ. The temperature planning involved 50-240°C with temperature raising pace of 3°C per minute, and the temperature of the injection chamber and transfer line were 250°C and 260°C, respectively, with helium used as the carrier gas. The helium gas speed was 31.5 cm/s, the ion trap detector ionization energy was 70 eV, the scanning time was 1s and the mass range was 40-300 (Adams, 2012).

Statistical analysis: The data obtained from the essential oil yield of different organs and the entire aerial organ to variance analysis were analyzed in a completely random design, with three replications, involving a comparison of mean average performance using Duncan's multiple range test at 1% probability level via SAS ver. 9.4 software.

Results

The results of variance analysis showed a significant difference in terms of the essential oil yield at 1% level among different organs as well as the whole aerial organ of *Z. multiflora* under examination (Table 1).

The average essential oil yield of different organs including leaf, flower, stalk and the whole aerial organ of *Z. multiflora* were 4.8, 5.3, 0.6 and 2.7 % (w/w), respectively (Table 2). As can be seen, the essential oil yield of flowers were higher than those of others, while the lowest efficient yield belonged to stalks.

Comparison of different studied organs of *Z. multiflora* with whole aerial organ showed significant variations in terms of type and percentage of constituents of essential oil (Table 3). A total of 23 compounds were observed in different organs of *Z. multiflora* in which 14 compounds were common. The highest number of identified compounds was observed in the essential oil of the whole aerial organ and stalks with 19 compounds and the lowest in leaf and flower organs with 17 compounds.

The identified compounds from the leaf accounted for 99.0 % of the total essential oil. The major constituent compounds of leaf in *Z. multiflora* included thymol (55.8 %), paracymene (23.2 %), carvacrol (9.6 %) and gamma-terpinene (3.1 %). Other compounds constituted less than 3 % of the essential oil which are listed in Table 3. The identified compounds in the flower constituted 99.2 % of the essence. The major compounds of this sample were thymol (56.7 %), para-cymene (18.8 %), carvacrol (8.4 %) and gamma-terpinene (4.3 %). 97.3 % of the whole essential oil was identified in the stalk's essential oil and the major compounds were found to be carvacrol (67.1 %), thymol (13.1 %), para-cymene (6.5 %). Finally, 96.2 % of the whole essential oil was identified in the essential oil of the whole aerial organ of *Z. multiflora* and the major compounds entailed carvacrol (36.7 %), thymol (31 %), para-cymene (14.9 %) and gamma-terpinene (3.5 %).

The constituent compounds of the essential oil in different organs of *Z. multiflora* as well as in the whole aerial organs were classified in terms of chemical formula which are listed in Table 3. Considering the different compounds identified in the essential oil of these four samples, it was shown that oxygenated monoterpenes were the main group that constituted the stalks (84.2 %), the whole aerial organ (70.2 %), the flower (69.2 %) and the leaf (66.6 %), followed by monoterpenes hydrocarbons and sesquiterpene hydrocarbons, respectively. Out of the fourteen mutual constituent compounds of essential oils in the examined organs of *Z. multiflora*, the major compounds included thymol, carvacrol and para-cymene.

Discussion

An examination of references shows that there are limited studies on the quantitative and qualitative features of essential oils in different organs of *Z. multiflora* in Iran. According to the results obtained from assessing the essential oil performance of different organs, the flower and the stem had the highest and lowest essential oil yield with 5.3 % and 0.6 %, respectively. The average yield of essential oil production in the aerial organ was found to be 2.7 %. The comparison of the results in this study which were conducted for the first time on different organs of *Z. multiflora* as well as the whole aerial organ showed similarities as well as differences with the findings of previous studies. In one study on the essential oils acquired from the flowering aerial parts of *Z. multiflora*, collected from different habitats in Hormozgan province, the essential oil yield in habitats including Roudkhaneh, Lowersheik, Fariab, Tang-e Zagh, Bashagerd and Bandar Khamir were 6.5, 6.2, 4.7, 4.3, 4 and 3.9 %, respectively (Meamari e*t al*., 2020). Another study reported the essential oil yield of aerial organs of 12 different *Z. multiflora* populations collected from their natural habitats in Fars province as 2.91-4.0 % in which the highest and lowest essential oil yields

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Table 1. Analysis of variance for essential oil percent of *Z. multiflora* plant parts

Source of variation (S.O.V)	df	Mean of Squares
Essential oil yield	3	5.79 **
Error	8	1.17

** **Significantly different at the 1 % probability level**

Table 2. Average of the essential oil content (w/w) in different plant parts of *Z. multiflora*

Plant organ	Leaf	Flower	Stalk	Whole aerial organ
Essential oil yield (%)	4.8 ± 0.18 [a]	5.3 ± 0.21 [a]	0.6 ± 0.11 [c]	2.7 ± 0.19 [b]

***Means with different letters are significant according to the Duncan's multiple range test at P ≤ 0.01. The values are mean of three replicates ± standard deviation (SD).**

Table 3. Essential oil compositions of different organs of *Z. multiflora*

No.	Compound name	Class of Compounds	RI**	Oil content (%)				Identification*
				Leaf	Flower	Stalk	Whole aerial organ	
1	*α*- thujene	MH	978	0.3	0.4	0.2	0.1	MS, RI
2	*α*- pinene	MH	995	1.1	1.2	1.2	2.8	MS, RI
3	camphene	MH	997	0.1	-	-	0.1	MS, RI
4	*β*- pinene	MH	1015	-	-	0.1	0.1	MS, RI
5	3- octanone	MH	1030	-	-	0.1	1.3	MS, RI
6	myrcene	MH	1033	1.2	1.3	0.3	0.2	MS, RI
7	*α*- phellandrene	MH	1038	0.2	-	-	-	MS, RI
8	*α*- terpinene	MH	1076	1.5	2.0	0.7	1.2	MS, RI
9	*p*- cymene	MH	1085	23.2	18.8	6.5	14.9	MS, RI
10	limonene	MH	1100	0.4	0.5	0.2	0.3	MS, RI
11	1,8- cineole	OM	1194	0.1	0.2	0.2	-	MS, RI
12	γ- terpinene	MH	1260	3.1	4.3	1.8	3.5	MS, RI
13	linalool	OM	1345	-	0.8	0.7	-	MS, RI
14	terpinene- 4-ol	OM	1386	0.7	0.9	1.3	0.5	MS, RI
15	α- terpineol	OM	1392	0.2	0.4	0.9	0.2	MS, RI
16	methyl ether thymol	OM	1413	-	-	0.7	-	MS, RI
17	methyl ether carvacrol	OM	1435	-	-	-	0.1	MS, RI
18	thymol	OM	1440	55.8	56.7	67.1	31.0	MS, RI
19	carvacrol	OM	1503	9.6	8.4	13.1	36.7	MS, RI
20	thymyol acetate	OM	1504	0.2	1.7	0.2	0.9	MS, RI
21	carvacrol acetate	OM	1517	-	0.1	-	0.8	MS, RI
22	*e*- caryophyllene	SH	1530	0.8	1.1	1.0	1.1	MS, RI
23	aromadendrene	SH	1538	0.5	0.4	0.7	0.4	MS, RI
	Monoterpene hydrocarbons (MH)			31.1	28.5	11.4	24.5	
	Oxygenated monoterpens (OM)			66.6	69.2	84.2	70.2	
	Sesquiterpene hydrocarbons (SH)			1.3	1.5	1.7	1.5	
	Total identified			99.0	99.2	97.3	96.2	

***) Mode of identification: retention index (RI), mass spectrometery (MS), and co-injection (CoI) with some available authentic compounds.**
****) RI: retention indices determined in the present work relative to C_7-C_{25} *n*-alkanes on the Ph-5 column.**

belonged to Zarghan and Sivand ecotypes, respectively. Such diversity in the percentage of essential oil in the aerial organs of various *Z. multiflora* ecotypes could be due to the plant's genetics, the climate conditions of its habitat, altitude from the sea level, harvesting time, drying method, essential oil extraction method, and the interaction between these factors (Bigdeloo *et al*., 2017).

Studies have shown that in addition to the roles of genetic and environmental factors in the production and diversity of essential oil compounds in quantitative and qualitative terms, there is another factor called the different plant organs and the significantly effective development stage. Different organs of aromatic plants have different potentials for essential oil production; consequently, gaining information on specific plant organs with high percentages of essential oil is necessary for achieving maximum essential oil performance (Barra, 2009). While medicinal plants breeders can focus on this matter for breeding purposes

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regarding the performance of organs' dry materials, it can also be beneficial in medicine, food and cosmetic industries (Binava *et al*., 2020). In terms of appearance, different organs of *Z. multiflora* are covered by secretory trichomes which are shown to be one of the main places of essential oil accumulation across various types of the Lamiaceae family (Werker, 1993; Anackov *et al*., 2009). Therefore, the higher amounts of essential oil found in the flower (5.3 %) compared to the other organs can be associated with a large number of flowers and inflorescences followed by the numerous secretory trichomes in the reproductive organs of *Z. multiflora*. In addition, the high amount of essential oil in the flower organ could also represent the active role of these compounds in attracting insects for pollination purposes (Cseke *et al*., 2007). The closest essential oil yield to that of the flower belonged to the leaf (4.8 %) in which secretory trichomes are abundant as well (Jamzad, 2012). In addition, leaves are vitally important for the existence of plants due to their roles in photosynthesis and maintaining their resulting compounds. Known as the products of photosynthesis, carbohydrates are used to produce secondary compounds in medicinal plants. Consequently, the higher number of leaves in this species, the higher the possibility to receive sunlight; in such a case, given the larger surfaces of the existing leaves and the higher number of mesophilic cells containing chloroplast, higher amounts of carbohydrates are produced through photosynthesis. In turn, this provides the possibility of generating secondary compounds (such as essential oils) to a higher extent (Verma and Shukla, 2015). Therefore, one breeding goal in line with a maximum increase in the essential oil efficiency of *Z. multiflora* could involve focusing on features including the number of flowers, inflorescences and leaves along with leaf surface area; indeed, medicinal plant breeders can focus on increasing these factors. Given *Z. multiflora*'s multiyear lifespan, lignin is formed on its stalks during the maturity period. Such a development in the plant's organ could alter the structure of external secretory trichomes, involving tears and ruptures and the loss of accumulated essential oils (Figueiredo *et al*., 2008). Moreover, a large portion of this plant appears wooden which suggests the high ratio of stalk compared to the other parts in the aerial organ of this species (Jamzad, 2012). Accordingly, this can justify this study's findings regarding the low percentage of essential oil in the stalks (0.6 %) compared to other organs of *Z. multiflora*.

According to the comparison between the constituent compounds of essential oils in the examined samples, the main compounds in leaf, flower, stalk and the whole aerial organ of *Z. multiflora* respectively included thymol (55.8, 56.7 and 67.1 %) and carvacrol (36.7 %). The second and third prevalent compounds in leaf and flower essential oils were found to be para-cymene (23.2 and 18.8 %) and carvacrol (9.6 and 8.4 %), respectively; meanwhile, carvacrol (13.1 %) and thymol (31 %) were observed as the second and the third main compounds in essential oils of the stalk and the whole aerial organ, followed by para-cymene (6.5 and 14.9 %), respectively. The third main compound in stalk and the whole aerial organ essential oils were found to be para-cymene (6.5 %) and 1,8-cineole (11.4 %). In one study on the identification of essential oil compounds of the aerial organs of 14 *Z. multiflora* populations collected from five provinces of Isfahan, Yazd, Fars, Kerman and Hormozgan, four main compounds were reported including carvacrol (10.56-73.31 %), thymol (3.51-48.12 %), linalool (0.90-55.38 %) and para-cymene (1.66-13.96 %). The findings of the present study are consistent with these results in terms of three main compounds (thymol, carvacrol and para-cymene) (Karimi *et al*., 2020). Another study assessed the essential oil compounds obtained from aerial organs of 18 accessions of *Z. multiflora*, collected from seven provinces including Isfahan, Yazd, Fars, Kerman, Sistan and Balouchestan, Hormozgan and Boushehr; out of the 56 identified compounds using GC and GC-MS devices, four compounds were found as the main constituents which included thymol, carvacrol, linalool and para-cymene. The prevalent essential oil compounds of Nikshahr accession, as the only accession from Sistan and Balouchestan in this study, were found to be carvacrol (36.8 %) and linalool (25.5 %) (Hadian *et al*., 2011). The findings of the present study regarding the whole aerial organ are consistent with the results of the previous study, in terms of high amounts of carvacrol in the essential oil of the whole aerial organ of *Z. multiflora* collected from Sistan and Balouchestan province. Identification of compounds in the essential oils of different organs of *Z. multiflora* along with whole plant showed that in all of the organs the percentage of monoterpenes was higher than sesquiterpenes. Among the grouping of monoterpenes, the essential oils of all organs had more oxygenated monoterpenes than hydrocarbon monoterpenes. Sesquiterpene compounds are divided into hydrocarbon and oxygenated groups (Abu-Izneid *et al*., 2020). Considering the sesquiterpene compounds group, only two sesquiterpene compounds were identified in the entire examined organs which included (E)-caryophyllene and aromadendrene; unlike sesquiterpene hydrocarbons, no oxygenated sesquiterpenes were identified in the examined organs of *Z. multiflora* in this study. The findings of the present study are consistent with results of previous research regarding the high percentages of monoterpenes in the essential oil of *Z. multiflora* aerial organ at the full flowering phase (Najafpour Navaei and Mirza, 2014; Sadeghi *et al*., 2015; Niczad *et al*., 2019; Golkar *et al*., 2020; Karimi *et al*., 2020; Meamari *et al*., 2020). Based on the comparison of previous assessments conducted on the essential oil of *Z. multiflora* with the results of the present study, the qualitative combination of the chemical constituents of essential oil appeared to be similar, with minor differences in certain cases. The chemical compounds of essential oil may change according to genetic diversity, epigenetic factors, different plant organs and a large number of environmental factors such as climatic characteristics, temperature, humidity, type of soil and altitude from sea level (Barra, 2009; Dastan *et al*., 2021).

Conclusion

In general, the results of the present study provided distinctions between different aerial organs of *Z. multiflora* as well as the whole aerial plant in terms of the main constituent compounds of its essential oil. These findings suggested that the high potential of this plant in essential oil production; the flower and leaf of *Z. multiflora* had higher essential oil production potentials compared to the stalk (5.3 and 4.8 %, respectively), which can be considered for applications. On the other hand, given the high ratio of stalk to flower and leaf in this species that results in low essential oil yield of the entire aerial organ, paying attention to this feature in compiling breeding plans to find genotypes or ecotypes with a lower stalk to leaf and flower ratios appear essential. The observed diversity in the qualitative features of the plant was clear as well. Consequently, detailed identification of quantitative and qualitative indices of essential oil followed by introducing the most suitable chemical type would be an effective step towards correct applications, provision of desirable domestication conditions and conservation of natural resources; additionally, this would provide the required settings for the suitable introduction of these valuable resources to industries such as medicine, food and cosmetics.

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