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Original Article

Comparison of Chemical Composition and Physical Properties of *Satureja khuzistanica* Essential Oil in Different Growing Conditions (Wild and Cultivated) in Lorestan Province

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ABSTRACT

Satureja khuzistanica Jamzad is a species native to Iran and is highly important in south western regions. It belongs to the Lamiaceae family which is widely used in various pharmaceutical, food, cosmetic and health industries. Quantitative comparison of the essential oil extracted from the flowering branches of *S. khuzistanica* shows that the amount of essential oil varies between 2.5 % and 3.7 %. Chemical analysis of essential oils revealed that the main and common constituents of essential oils are carvacrol (92.21 to 95.75%), γ-terpinene (0.37 to 1.93%), *p*-cymene (0.48 to 1.64%), β-bisabolene (0.64 to 0.83%), and myrcene (0.26 to 0.67%). The relative density of *S. khuzistanica* essential oil at 20 °C is from 0.953 to 0.987, and the refractive index of essential oil at 20 °C is from 1.5064 to 1.5147, the optical rotation of essential oil at 20 °C is from -1.00 to -1.40 degrees. If one volume of *S. khuzistanica* essential oil is mixed with 1.4 volume of 80% ethanol at a temperature of 20 °C, a clear liquid is obtained.

INTRODUCTION

Plant essential oils are one of the most widely used medicinal and aromatic products, which, in addition to being used in the pharmaceutical industry, are also used in cosmetic-hygiene and food products, for this reason, in terms of production and consumption, they are at the top of medicinal plant products list. The global essential oils market size was valued at USD 8.8 billion in 2022 growing at a CAGR (compound annual growth rate) of 11.8% to reach USD 15.3 billion in 2027 [1].

Satureja khuzistanica belongs to the family Lamiaceae is an endemic plant to Iran that is widely distributed in the southern part of Iran. It is a subshrub with a branched stem of about 30 cm high and dense leaves [2]. In traditional medicine, *S. khuzistanica* has been used as analgesic and antiseptic among the inhabitants of southern parts of Iran [3]. Until today, several biological activities such anti-inflammatory and anti-nociceptive [4], anti-thyroid [5], anti-microbial [6], antioxidant [7], anti-diabetic and anti-hyperlipidemic [8] have been reported for this plant. Most of these features are attributed to carvacrol, which is the active ingredient of *S. khuzistanica* essential oil. A literature survey has shown that there are several reports on the volatile of *S. khuzistanica*. For example, Doosti evaluated *S. khuzistanica* essential oils in full flowering stage from 3 different habitats in west and south west of Iran. The aerial parts were subjected to hydro distillation using a Clevenger apparatus. Analysis of the essential oils were carried out with GC/MS system. The average yield of essential oil in 3 habitats was 1.4 to 2.8 % (Lives, Mongere and Takhtejan). In the oil obtained from Lives, carvacrol (90.74%), β-bisabolene (2.73 %), γ-terpinene (1.19 %) and *p*-cymene (1.10 %) were detected, while in Mongere essential oil, carvacrol (93.60 %) and β-bisabolene (1.47 %) were characterized as the main components. In Takhtejan essential oil carvacrol (89.60 %), caryophellene oxid (3.01%), β-bisabolene (2.56 %), γ-terpinene (1.78 %) and *p*-cymene (1.78 %) were detected as the major components [9].

In another study, a stable carvacrol-rich *S. khuzistanica* essential oil nano-emulsion was prepared and its antibacterial activity against food borne pathogens were evaluated. Twenty-one components were reported in *S. khuzistanica* essential oil used in this research, and carvacrol with a frequency of 87.16 % and *p*-cymene with a frequency of 6.39% were the main components of this essential oil [10]. In the study of Hasheminya *et al.*, Kefiran-carboxymethyl cellulose bio-composite films were developed with *S. khuzistanica* essential oil. Results indicated that increase in the concentration of the essential oil increased ultimate contact angle and tensile strength but decreased moisture content, elongation at break and water vapor permeability. It also significantly altered color parameters and the percentage of light transmission in the visible and ultraviolet range. From the total of 24 identified compounds in used *S. khuzistanica* essential oil, the highest amounts belonged to carvacrol (80.55%), *p*-cymene (6.43%), beta-bisabolene (3.25%), citronellal (1.8%) and linalool (1.35%) [11]. Many of the previous studies demonstrated that the ecological and climatic conditions effect on the quantity and quality of plant essential oil but none of these articles have compared the essential oil of wild plants with cultivated ones. The purpose of this article is quantitative and qualitative comparison of wild and cultivated *S. khuzistanica* essential oil to determine whether the plant growing conditions have any effect on the results or not.

Experimental

Plant Material

According to Flora of Iran, number 76 (Lamiaceae), the natural habitats of the plant were identified in Lorestan province, and the flowering branches of the plant were collected during the flowering stage. Also, from information about cultivated area of this plant in research projects of the Research Institute of Forests and Rangeland, the area of cultivating of this plant in Lorestan province were determined. Based on this, the collection of plants was done in fall of 2020 from Poldokhtar (takhtejan, 1058 m altitude) and Khoramabad farm (1202 m altitude) and identified by the herbarium of the Research Institute of Forests and Rangelands.

Isolation of Essential Oil

The flowering branches of *S. khuzistanica* were air-dried at ambient temperature in the shade. Essential oils were collected using a Clevenger-type apparatus for 3 hours by hydro distilation method. The yields of oils were 2.53-3.70% (w/w). The yellow essential oils were dried over anhydrous sodium sulphate and stored in refrigerator until analysis.

Relative Density at 20 °C

The relative density measurement test at 20 °C was performed according to the Iranian national standard INSO 2274-9, Essential oils - Determination of relative density at 20 °C –Reference method (ISO 279:1998) [12, 13].

Refractive Index at 20 °C

The refractive index measurement test at 20 °C was performed according to the Iranian national standard ISIRI 2274-6, Essential oils – Determination of refractive index- Test method (ISO 280:1998) [14, 15].

Optical Rotation at 20 °C

The optical rotation measurement test at 20 °C was performed according to the Iranian National Standard ISIRI 2274-5, Essential oils - Optical rotation measurement - Test method (ISO 592: 1998) [16, 17].

Evaluation of Miscibility in Ethanol (80%) at 20 °C

The test to measure the mixing of the product with 80% ethanol at 20 °C was performed according to the Iranian National Standard ISIRI 2274-1, Essential oils - Evaluation of miscibility in ethanol - Test method (ISO 875: 1999) [18, 19].

Gas Chromatography (GC)

Analytical GC was carried out on an Agilent 7890A equipped with a Flame Ionization Detector (FID) and a DB-5 column (methyl phenyl siloxane, 30 m x 0.25 mm, 0.25 μm film thickness). Nitrogen was used as the carrier gas with 0.7 ml/min flow rate. Temperature programming was performed from 60 °C and increased to 220 °C by 4 °C/min rate, then increased to 260 °C by 20 °C/min rate, and hold at this temperature for 10 min. The injector and detector temperatures were 260 °C. The split ratio was 1:40.

Gas Chromatography/Mass Spectrometry (GC/MS)

Gas Chromatography/Mass spectrometry was carried out on an Agilent 7890A equipped with a 5975C Mass Detector and a DB-5 ms column (methyl phenyl siloxane, 30 m x 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas with 0.7 ml/min flow rate. The temperature program was exactly the same as the GC program. Quadrupole mass spectrometer was operated at 70 eV ionization energy. EIMS spectra were obtained in scan mode in 40-340 m/z range.

Quantification and Identification of Components

The components of the essential oil were identified based on their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra and those of reference compounds from Adams Library [20]. Also, their identification was confirmed by co-injection of available compounds. The amount of identified compounds was computed from the GC peak area without any correction factor.

RESULTS

Physical Properties of Essential Oils

The percentage yield of essential oil obtained from the water distillation process of the air-dried aerial part of *S. khuzistanica* ranged from 2.53-3.70% (w/w). The results showed cultivated samples had the higher oil yield, and lower optical rotation, refractive index and relative density (Table 1).

Chemical Composition

The total number of compounds identified in the essential oils of the air-dried aerial part of *S. khuzistanica* were 14 compounds (Fig. 1 and Table 2). The main and common constituents of essential oils were carvacrol (92.21 to 95.75%), γ-terpinene (0.37 to 1.93%), *p*-cymene (0.48 to 1.64%), β-bisabolene (0.64 to 0.83%), and myrcene (0.26 to 0.67%).

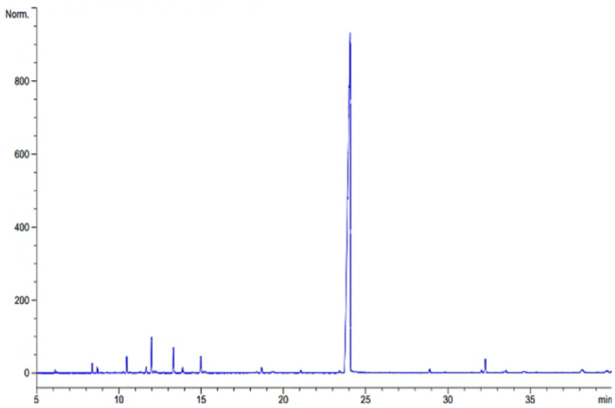

Fig. 1 Gas chromatogram of essential oil of *S. Khuzistanica*

DISCUSSION

A quantitative comparison of essential oil shows that the amount of essential oil varies between 2.5% and 3.7%, that higher amount of essential oil belongs to cultivated samples which may be related to the difference in the amount of irrigation of samples.

Table 1 Physical properties of *S. khuzistanica* Essential oil

Entry	Growing condition	Number of samples	Essential oil percent (%)	Optical rotation (°)	Refractive index	Relative density	Min volume of ethanol 80% for miscibility in 1 ml essential oil
1	wild	2	2.53-2.71	-1.30 to -1.40	1.5064-1.5138	0.964-0.987	1.1-1.2
2	cultivated	2	3.13-3.70	-1.00 to -1.20	1.5145-1.5147	0.952-0.961	1.2

Table 2 Percentage composition of wild and cultivated *S. khuzistanica* Essential oil

Entry	Compounds	Retention indices	Wild (%)	Cultivated (%)
	α-thujene	928	0.31-0.41	0.16-0.17
	α-pinene	936	0.19-0.27	0.10-0.11
	myrcene	989	0.55-0.67	0.26-0.29
	α-phellandrene	978	0.00-0.11	0.10-0.11
	α-terpinene	1017	0.34-0.43	0.16-0.18
	p-cymene	1024	1.38-1.64	0.48-0.56
	γ-terpinene	1060	1.58-1.93	0.37-0.40
	cis-sabinene hydrate	1067	0.22-0.28	0.26-0.31
	linalool	1099	0.45-0.62	0.58-0.67
	terpinen-4-ol	1177	0.19-0.31	0.22-0.32
	carvacrol	1300	92.21-93.51	95.75-96.16
	E-caryophyllene	1420	0.22-0.27	0.19-0.20
	β-bisabolene	1508	0.71-0.83	0.64-0.65
	E-γ-bisabolene	1533	0.12-0.14	0.10-0.11

Chemical analysis of essential oils shows that the main and common constituents of essential oils were carvacrol (92.21 to 95.75%), γ-terpinene (0.37 to 1.93%), *p*-cymene (0.48 to 1.64%), β-bisabolene (0.64 to 0.83%), and myrcene (0.26 to 0.67%). Higher amounts of carvacrol in the cultivated samples affected other physical properties such as optical rotation and relative density. Considering that carvacrol is an optically inactive compound, it is clear that by increasing the amount of carvacrol, the optical rotation of the essential oil will be closer to zero. Also, considering that the density of pure carvacrol is 0.977 kg/m^3, with the increase in the amount of carvacrol, the density also approaches the mentioned number.

The results of this research show that there is not a significant difference in yields, chemical compositions and concentrations of essential oils obtained from nature and farm in similar geographical regions. It means *S. khuzistanica* is a plant that the same geographical regions, even with a change in cultivation conditions (wild or cultivated), does not have much effect on the amount and composition of the essential oil, while reports indicate that the change in geographical regions has a great impact on the results. For example, *S. khuzistanica* essential oil from Ilam province was collected in full blooming and before flowering stages and their antioxidant activity were evaluated. The major compound *S. khuzistanica* essential oils at both stages was the phenolic monoterpene carvacrol (93.7 and 94.3 % respectively) [7]. In another report, the effects of 28-homobrassinolide (HBR) on growth, photosynthesis, chlorophyll content, carbohydrate fractions and essential oil content of *S. khuzistanica* were investigated. 28-Homobrassinolide at 10^{-6} and 10^{-8} M concentrations, incremented the total content of essential oils. The quantitative analysis of essential oil revealed an increase in carvacrol content from 81.17 to 85.98% and *p*-cymene content from 3.18 to 4.11% and a decrease in the γ-terpinene content from 3.47 to 1.88% [21].In another study, the application of various concentrations of essential oil of *S. khuzistanica* collected from Mazhin in Lorestan province was reported on the oxidative stability of sunflower oil. Gas chromatography and Gas chromatography-mass spectrometry analyses of essential oil defined that carvacrol (87.7%) was the major component of essential oil [22] which confirms our results.

CONCLUSION

The results of this research show that there is not a significant difference in yields, chemical compositions and concentrations of essential oils obtained from nature and farm in similar geographical regions. A quantitative comparison of essential oil shows that the amount of essential oil varies between 2.5% and 3.7%. GC and GC/MS analysis of essential oils shows that the main and common constituents of essential oils were carvacrol (92.21 to 95.75%), γ-terpinene (0.37 to 1.93%), *p*-cymene (0.48 to 1.64%), β-bisabolene (0.64 to 0.83%), and myrcene (0.26 to 0.67%). The amount of carvacrol in the cultivated samples were higher than wild ones which affected physical properties such as optical rotation and relative density.

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