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Chemical compounds of essential oil in *Satureja mutica* and *Satureja spicigera* under dry farming: extraction, identification and comparison

Borzoo Yousefi[*1], Fatemah Sefidkon[2], Hassan Khamisabadi[3], Nastaran Jalilian[3]

[1]*Kermanshah Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Kermanshah, Iran;*
Email:** ***borzooyoosefi@yahoo.com

[2]*Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Iran;*
[3]*Kermanshah Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Iran;*

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ABSTRACT

Background & Aim: The genus Satureja has 38 species distributed throughout the Mediterranean Area, Caucasus and West Asia. *Satureja Mutica* and *Satureja spicigera* is two Iranian native species that are distributed in the North of Iran. Essential oil (EO) of Satureja specious was used to some medicinal, food and industrial purposes. The aim of this study was to determine the chemical compounds of EO in *Satureja mutica* and *Satureja spicigera* under dry farming**.**

Experimental: In early April, before the effective rain fall, the seedlings were transferred to the main land in mid-March. In the %50 flowering stage, plants were harvested and 100 g of plant dried powder used for EO extraction. The EO was extracted by water distillation method and chemical components of essential oils were identified and subsequently characterized using GC and GC/MS techniques.

Results: The EO percent in *S. spicigera* was 2.52% in first year and 3.08% in second years. The EO percent of *S. mutica* was 2.04% in first year and 2% in second year. In creeping savory EO, thirteen compounds were identified that were formed the major constituents of EO (about 98.74% in first year and 97.53% in second year). The main compounds of essential oil (more than 5%) were thymol (28.60- 28.96%), carvacrol (23.18- 24.47%), ρ-cymene (21.00- 24.25%) and γ-terpinene (18.57-13.05%). In white savory EO, nine different chemical compounds were identified which made up more than about 95.32% of EO content in the first year and 97.48% in the second year. The major compounds of EO were Thymol (48.25-48.60%), γ-terpinene (20.84- 21.89%), ρ-cymene (12.34- 12.61%) and Carvacrol (6.71- 6.95%) respectively.

Recommended applications/industries: Thymol and carvacrol contents in savory essential oil are the two important factors in pharmaceutical properties of savory EO. White and Creeping Savory can be used to pharmaceutical and food industries. Also we recommended the increase of EO content, thymol and carvacrol compounds in white and creeping savory using different cropping and breeding methods for further studies.

1. Introduction

The genus Satureja have 38 species distributed throughout the Mediterranean Area, Caucasus and west Asia (Martín-Mosquero *et al.*, 2006). *S. Mutica* and *Satureja spicigera* is two Iranian native species that are distributed in the North of Iran.

The some factors including photosynthesis, light period and quality, climatic conditions, nutritional materials, growth regulators, humidity and temperature are effective for the production of EO (Khorshidy *et al*., 2009). Not much research has been done on the chemical composition of EO in the dryland cultivation of medicinal plants, especially savory specious (Bahreininejad *et al*., 2022).

Tabaei - Aghdaei *et al.* (2017) concluded that *S. mutica* have a vegetative superiority than *S. rechingeri* under rainfed conditions and is suitable for rainfed cultivation The yield traits at *S. khuzestanica* and *S. rechingeri* under rainfed conditions were significantly lower than irrigated conditions (Nooshkam *et al*., 2014). In *Cuminium cyminium* under dry farming, thirteen components including non-oxygenated and oxygenated monoterpens identified and Cuminyl aldehyde and P-Mentha-1, 4-dien-7-al were the main compounds in the oil (Ahmadi *et al*., 2001).

In previous study the main compounds of EO in *S. spicigera* has reported as: thymol, ρ-cymene, γ-terpinene and carvacrol (Gokturk, 2021; Sefidkon and Jamzad, 2004), thymol, p-cymene, carvacrol, methyl carvacrol and γ-terpinene (Tümen and Baser, 1996), Thymol and carvacrol methyl ether (Farzaneh *et al*., 2015), carvacrol, γ-terpinene, carvacrol methyl ether, ρ-cymene and β-caryophyllene (Bahtiyarca Bagda, 2010). The EOs derived from plants of Satureja species possess strong antibacterial activities (Miladi *et al*., 2013). The satureja EOs may find industrial applications as natural preservatives (Haroutounian, 2004) and as active ingredients in medical uses (Chorianopoulos *et al*., 2004).

2. Materials and methods

2.1. Experimental material

The seeds of *s. mutica* and *S. spicigera* were prepared from seed bank of Forests and Rangelands Research Institute of Iran. The seeds were disinfected with 5% sodium hypochlorite for 2 minutes and dried. Then it was planted in a tray and in peat moss bed, in a greenhouse at a temperature of 18-24 C° and a humidity of 35%. The main land was plowed and leveled in the fall of last year, and then heaps were piled at 50 cm intervals. In early April, before the effective rain fall, the seedlings were transferred to the main land in mid-March before effective rainfall.

2.2. Experimental design

This experiment was performed as a randomized complete block design (RCB) with three replications during the 2016-2018 crop years. The area of each plot is $4 \times 3 = 12$ m^2.

2.3. Experimental conditions

Experiment was conducted at Mehregan research station in 20 km of Kermanshah to Sanandaj road, at 34°.9′ latitude and 47°.9′ longitude, 1270m altitude, average annual rainfall 470.7, absolute minimum temperature -13°C, absolute maximum temperature + 40.5°C, average annual temperature 13.8°C and has a semi-arid steppe climate class. No chemical fertilizers, herbicides or pesticides were used during the project and mechanical methods used to control weeds. Rainfall in the first crop year (2018-2019) was 394.5 mm and in the second year (2019-2020) was 434 mm.

2.4. Soil analysis

The soil analyze was done in soil laboratory of Kermanshah agriculture and natural resources research and education center. The Table 1 showed Physicochemical properties of farm soil.

Table 1. Physicochemical properties of farm soil (results of soil test).

Soil texture	Soluble salts EC×10^3	Total saturated acidity	Absorbable P (ppm)	Organic C (%)	Absorbable K (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Clay %	Silt %	Sand %
Silty/ clay	0.70	7.03	1.13	12.2	520	11.00	12.52	0.72	2.70	42.3	47.4	10.3

2.5. Essential oil extraction

After 50% flowering, in late August, savory plants were collected, dried and weighed for EO extraction. The 100 g of crushed plant powder was used for EO extraction. EO extracted by water distillation using Clevenger system according to British Pharmacopoeia (1993) and for 2 hours. The essential oil samples were dehydrated with dry sodium sulfate (Na_2So_4) and kept in a refrigerator (4°C) until injection into a chromatographic apparatus.

The EO content was calculated by W/W method from the following formula: %EO= EO weight (g)/plant dry weight (g) ×100.

2.6. GC and GC/MS analysis

The EOs of three replications mixed and after diluting 1 µL of essential oil in 2 µL of dichloromethane, samples analysed by a gas chromatograph with specifications (Ultra Fast Model) Thermo-UFM and with Chrom-Card A/D data processor, Ph-I column made by thermo company with a length of 5 m and an inner diameter of 0.1 mm, 0.1 µm film thickness, (non-polar) when the inner surface of the device was coated with a stationary phase of 5% dimethyl siloxane phenyl made by thermo company, Italy. The oven temperature was programmed as follows: the initial temperature of 60 °C was immediately increased to 220 °C at a rate of 10 °C/ min, subsequently the temperature was increased to 285 °C at a rate of 40°C/min and held at this temperature for 5 min. Detector (FID) temperature and injector temperature was 280 °C; helium was used as carrier gas with a linear velocity of 0.5 mL/min. Quantification data was obtained from GC-FID area percentages without the use of correction factors.

GC/MS device was Varian 3400 connected to mass spectrometer (Saturn II), with ion telephoto system and ionization energy of 70 electron volts. It has a DB-5 column which is a semi-polar column (length 30 m, inner diameter 0.25 mm and thickness of static phase layer equal to 0.25 microns). Column head gas pressure was set at 35 pounds per square inch, temperature 40 °C to 250 °C with increasing speed of 4 °C per minute, injection chamber temperature 260 °C and line transfer temperature 270 °C. The retention indices were calculated by injection of normal hydrocarbons (C_7-C_{25}) under the same conditions as essential oil injection, by computer program and in BASIC language.

The chemical compounds identified by comparison of the mass spectra data with library spectra (Wiley 275 software, D.03.00). Further identification confirmations were made referring to RI data generated from a series of known standards of *n*-alkanes mixture C8 to C26 (Adams, 2017; Davies, 1990).

3. Results and discussion

In the present study, in *Satureja spicigera* EO, twelve compounds in sample of 2018 (Fig. 1) and thirteen compounds in sample of 2019 (Fig. 2) were identified including p-cymene, γ-terpinene, thymol, carvacrol, trans-caryophyllene, ρ-cymene, α-pinene, myrcene, α-thujone, β-bisabolene, terpinolene, terpinene-4-ol, methyl ether thymol and methyl ether carvacrol (only in sample of 2019) were the constituents comprising about 98.74% in first year and 97.53% in second year.

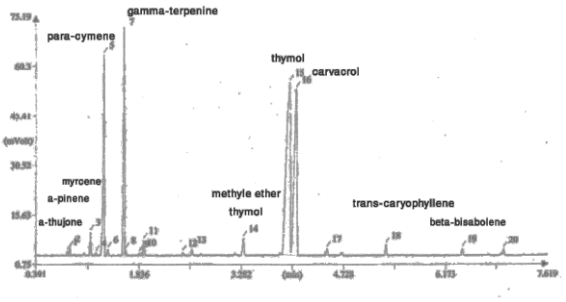

Fig. 1. The chromatogram of EO chemical compounds of *S. spicigera* under dry land conditions in 2018 crop year, 1= α--thujone, 2= α-pinene, 3= myrcene, 5- ρ-cymene, 7= γ-terpenine, 8= terpinolene, 13= terpenine-4-ol, 14= methyl ether thymol, 15= thymol, 16= carvacrol, 18= trans- caryophyllene, 19= β-bisabolene.

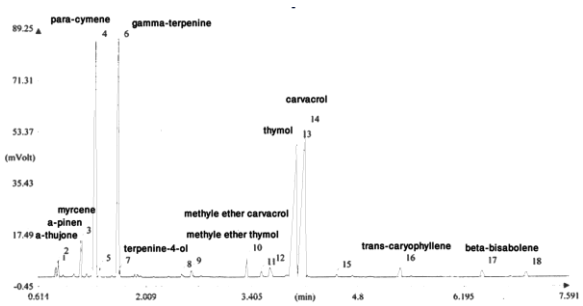

Fig. 2. The chromatogram of EO chemical compounds of *S. spicigera* under dry land conditions in 2019 crop year, 1= α--thujone, 2= α-pinene, 3= myrcene, 4- ρ-cymene, 6= γ-terpenine, 7= terpinolene, 9= terpenine-4-ol, 10= methyl ether thymol, 12=methyl ether carvacrol, 13= thymol, 14= carvacrol, 16= trans-caryophyllene, 17= β-bisabolene.

Amounts and specification of chemical components were presented in Table 2.

Table 2. Chemical compounds profile of EO in *S. spicigera* under dryland cultivation conditions.

Classification	Chemical compounds	% of chemical compounds		RT	RI	Formula
		2018	2019			
Monoterpene	Terpinolene	0.39	0.44	1.65	1054.11	$C_{10}H_{16}$
	Myrcene	1.58	1.66	1.16	986.46	$C_{10}H_{16}$
	Terpinene-4-ol	0.58	0.59	2.58	1146.53	$C_{10}H_{18}O$
Isometric monoterpene	γ-terpinene	18.57	13.05	1.63	1051.59	$C_{10}H_{16}$
Phenol monoterpenoids	Carvacrol	23.18	24.47	4.07	1289.96	$C_{10}H_{14}O$
	Thymol	28.96	28.60	3.96	1281.22	$C_{10}H_{14}O$
	Methyl ether thymol	1.26	1.46	3.32	1224.97	$C_{11}H_{16}O$
	Methyl ether carvacrol	0.00	0.32	3.6	1250.81	$C_{11}H_{16}O$
Bicyclic monoterpene	α-pinene	1.36	0.76	0.87	931.13	$C_{10}H_{16}$
ketonic monoterpene	-thujoneα	0.68	0.78	0.84	924.38	$C_{10}H_{16}O$
Sesquiterpenes.	β-bisabolene	0.00	0.43	6.42	1493.57	$C_{15}H_{24}$
Bicyclic sesquiterpenes.	Trans-caryophyllene	1.18	0.72	5.33	1376.02	$C_{15}H_{24}$
Benzene alkyl	ρ-cymene	21.00	24.25	1.36	1014.15	$C_{10}H_{14}$
-	Thymol+carvacrol	52.14	53.07	-	-	-
-	Total percent of EO	98.74	97.53	-	-	-

Methyl ether carvacrol was present only in the second year and in a small amount. The main compounds of essential oil (up to 5%) were thymol (28.60- 28.96%), carvacrol (23.18- 24.47%), ρ-cymene (21.00- 24.25%) and γ-terpinene (18.57-13.05%). Total values of two important components in creeping savory EO, carvacrol and thymol, which are mainly responsible for the medicinal properties of the EO, in the first and second years were 52.14% and 53.14%, respectively.

In the white savory EO, in each 2 years, nine compounds were identified (about 95.32% in the first year and 97.48% in the second year), include carvacrol, thymol, γ-terpinene, α-terpinene, ρ-cymene, α-pinene, β-pinene, Camphene and trans- caryophyllene (Fig. 3 and Fig. 4). The average of major compounds were thymol (48.25- 48.60%), γ-terpinene (20.84- 21.89%), ρ-cymene (12.34-12.61%) and Carvacrol (6.71-6.95%), respectively. Total carvacrol and thymol in the first year was (55.20%) and in the second year 55.31% (Table 3).

The Comparison of chemical components of EO between *S. mutica* and *S. spicigera* showed that the main compounds of each two specious were thymol, carvacrol, ρ-cymene and γ-terpenine. The content of thymol in white savory was very higher (nearly 2 times) than creeping savory. On the other hand, the carvacrol content in *S. spicigera* was higher than *S. mutica* (nearly 3/3 times), although it seems that the total of this two compounds in *S. spicigra* and *S. mutica* was nearly the same (52.64% in *S. spicigera* and 55.26% in *S. mutica*).

The γ-terpenine content in *S. mutica* was a little more than *S. spicigera* and ρ-cymene contents in *S. spicigera* was very higher than *S. mutica* (nearly 2 times).

The chemical compounds of terpinolene, myrcen, terpinen-4-ol, α-thujone, β-bisabolene, α-pinene, Methyl ether carvacrol and Methyl ether thymol were found only in *S. spicigera* EOs. The three chemical components that include Camphene, β-pentene and α-trepanned were find in White savory EOs but no in creeping savory EOs.

Table 3. Chemical compounds of EO in *S. mutica* plants cultivated under dryland conditions**.**

Classification	Chemical compounds	Average of chemical compounds (%)		RT	RI	Molecular formula
		2018	2019			
Bicyclic sesquiterpenes.	Trans caryophyllene	1.22	1.35	3.92	1471.49	$C_{15}H_{24}$
Bicyclic monoterpene	β-pinene	1.84	2.09	1.31	980.66	$C_{10}H_{16}$
	α-pinene	0.91	1.30	1.88	935.57	$C_{10}H_{16}$
	Camphene	0.71	0.69	1.94	947.36	$C_{10}H_{16}$
Phenolic monoterpenoids	Carvacrol	6.95	6.71	3.45	1329.22	$C_{10}H_{14}O$
	Thymol	48.25	48.60	3.41	1319.59	$C_{10}H_{14}O$
Isometric monoterpene	γ-terpinene	20.84	21.89	2.47	1081.82	$C_{10}H_{16}$
	α-terpinene	2.24	2.25	1.72	1041.1	$C_{10}H_{14}$
Alkyl benzene monoterpene	ρ-cymene	12.34	12.61	2.34	1050.97	$C_{10}H_{14}$
Thymol+ Carvacrol		55.20	55.31	-	-	-
Total percent of EO		95.32	97.48	-	-	-

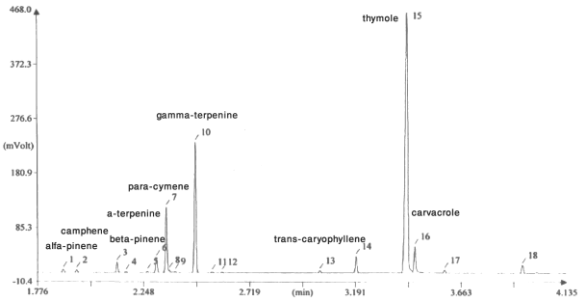

Fig. 3. The chromatogram of EO chemical compounds in *S.mutica* under dry condition in 2018 crop year, 1= α-pinene, 2= camphene, 3= β-pinene, 6= α-terpenine, 7= ρ-cymene, 10= γ-terpenine, 13= trans-caryophyllene, 15= thymol, 16= carvacrol.

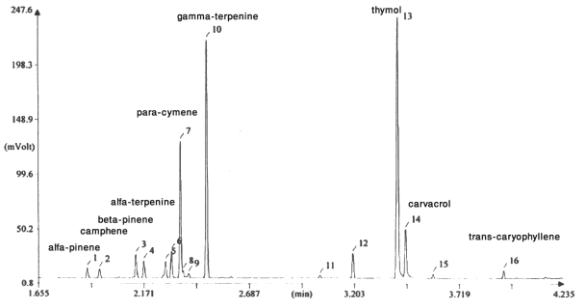

Fig. 4. The chromatogram of EO chemical compounds in *S.mutica* under dry condition in 2019 crop year, 1= α-pinene, 2= camphene, 3= β-pinene, 6= α-terpenine, 7= ρ-cymene, 10= γ-terpenine, 13= thymol, 14= carvacrol, 16= trans- caryophyllene.

The chemical compounds and specifications of EO in different Satureja species were reported by some authors (Hadian *et al*., 2010; Chorianopoulos *et al*., 1994; Hejja *et al*., 2002; Pank *et al*., 2004) and many different chemotypes can be detected. In *S. mutica* population, Thymol (6.5-74.6%), carvacrol (0.9-70.4%), borneol (0.1-10.9%), p-cymene (0.30-14.2%), and γterpinene (0.1-9.9%) were identified as the major predominant constituents of essential oils (Karimi *et al*., 2016). p-cymene, γ-terpinene, thymol, and carvacrol reported as main components of EO in different specious of Satureja (Ghorbanpour *et al*., 2016), S. bachtiarica (Salehi-Arjmand *et al*., 2014), *S. hortensis* (Sefidkon *et al*., 2006), *S. khuzistanica* (Hadian *et al*., 2011), *S. montana* (Ibraliu *et al*., 2011), *S. pilosa* (Dardioti *et al*., 2012), and *S. mutica*, *S. macrantha* and *S. intermedia* (Sefidkon and Jamzad, 2005). In one study, twenty nine components identified in EO of *S. hortensis* and carvacrol, γ-terpinene and p-cymene were the main chemical compounds. In *S. khuzistanica* EO, carvacrol (89.59-95.41%) have been had the high percentage as main component (Hadian *et al*., 2011).

Gohari *et al.* (2011) reported the main components for *S. atropatana* as: thymol (62.1%), p-cymene (6.1%) and spathulenol (5.2%), and, for *S. mutica*, thymol (62.6%), p-cymene (9.4%), carvacrol (6.6%) and methyl thymol (5.4%). Thirty-seven compounds were identified in *S. atropanata* EO and carvone (21.5%), menthol (18.1%), 1, 8-cineol (13.1%), methyl chavicol (11.1%) and menthone (10.5%) being the major compounds and in the oil of *S. mutica* 39 components were identified that characterized by higher amount of menthol (37.4%), menthone (17.2%) and 1, 8-cineol 9.3% (Rustaiyan *et al*., 2004).

It should be noted that variability in the EO constituents related to some different factors such as: genetic, geographical conditions, feeding, developmental stage, climatic conditions, as well as extraction methods. (Hadian *et al*., 2011; Runyoro *et al*., 2010; Sefidkon *et al*., 2007).

The carvacrol, thymol, p-cymene and γ-terpinene are the major compounds of EO in Satureja specious. The antimicrobial activities of EO in Satureja species mainly related to carvacrol and thymol (Juven *et al*., 1994; Kim *et al*., 1995) and to some extent related to other compounds and as well as interaction between the compounents (Palmer *et al*., 2000). Also Skočibušić *et al.* (2006) reported the relationship between high antimicrobial activity and the presence of phenolic components

4. Conclusion

The main compounds of EO in each two specious, *S. spicigera* and *S. mutica* under rainfed conditions were p-cymene, γ-terpinene, thymol and carvacrol. Thymol and carvacrol contents in savory essential oil are the two important factors in pharmaceutical properties of savory EO. Finding specious, variety, landraces and populations with high content of Thymol and carvacrol or increasing the amounts of these two compounds by breeding program or treatments is a key goal in research about savory EO.

5. Acknowledgements

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