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Quantitative and qualitative evaluation of *Satureja mutica* essential oil at different growth stages

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Abstract

Background: *Satureja mutica* is an annual plant that belongs to the family Labiatae and has been widely used in various industries. In this research, variations in the content, quality and antioxidant activity of the essential oil of *S. mutica* at different growth stages i.e. pre-flowering (E_1), flowering (E_2) and post-flowering (E_3), are reported.

Materials and Methods: *S. mutica* was collected at three different stages of growth from North Khorasan Province of Iran. Essential oils were obtained from the aerial parts of the plant using steam distillation. Then, the chemical composition of the oils were analyzed by GC-MS. The antioxidant activity and total phenolic content of the essential oils were examined by DPPH and Folin-Ciocalteu assays, respectively.

Results: The results showed that plants collected at the flowering stage, had the highest yield whereas the plants collected at the post-flowering stage had the lowest yield. Analysis of the obtained oils revealed the presence of 29 compounds, mainly monoterpenes specially carvacrol, thymol, p-cymene and γ-terpinene. The essential oil of the plants collected at pre-flowering stage which had the highest amount of phenolic compounds such as thymol and carvacrol, showed the strongest antioxidant activity with the lowest amount of EC_{50}.

Conclusion: this study showed that growth stage had significant effects on chemical composition and antioxidant properties of *S. mutica* essential oil.

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Introduction

The genus *Satureja* (family Lamiaceae) has 13 species in Iran and the essential oil obtained from this plant has industrial applications (e.g. flavors, medicines and perfumes) (Cantina et al, 1992; Rechinger, 1982). *Satureja* species grow in the mountains of Iran, mainly in the Western and Northern parts. *S. mutica* is found on calcareous rocks in the Northeast of Iran and is generally called "white or forest savory" (Rechinger, 1982; Mozaffarian, 1996). Several members of this genus are well known for their aromatic compounds and medicinal properties and have been used in traditional medicine to treat many diseases such as nausea, diarrhea, cramps, and muscle pains (Gulluce et al, 2003). *Satureja* species contain cytotoxic and antitumor agents (Gohari et al, 2006). The essential oils isolated from various species of *Satureja* have biological properties such as antibacterial (Kotan et al, 2013), antioxidant (Grosso et al, 2009) and anti-HIV-1 activities (Yamasaki et al, 1998). The essential oil of *S. horvatii* is a potent inhibitor of food spoiling microorganisms, and can be considered as a useful source of natural antioxidants such as thymol and p-cymene (Bukvičkia et al, 2014). Carvacrol, thymol, γ-terpinene and p-cymene are the main compounds of *Stureja genus* (Khadivi et al, 2014; Economoua et al, 2011). *Satureja* genus has strong antibacterial activity because of the presence of γ -terpinene and carvacrol (Adiguzel et al, 2007). The main constituents of *S. mutica* are carvacrol, p-cymene and thymol (Sefidkon, Jamzad, 2005). In the essential oil of *S. montana*, thymol, p-cymene, linalool and carvacrol are the main compounds (Oliveira et al, 2012).

Carvacrol and thymol have strong antibacterial activities against *Pseudomonas aeruginosa*, *in vitro* (Kotan et al, 2013). Moreover, carvacrol exhibits antioxidant, antibacterial and antifungal activities (Ramak, 2013). Other components present in the essential oil of this genus are γ-terpinene, p-cymene, limonene, 1, 8-cineol, and borneol which are cyclic monoterpenes (Mahboubi et al, 2011).

The chemical composition of plants is known to be influenced by several external factors such as climate and seasonal variations and plant material collected at different times of the year may contain different compounds with different bioactivities (Hussain et al, 2008). The effects of seasonal variations on the essential oils of the family Lamiaceae have been reported in the literature (Hussain et al, 2008). So far, several articles on the essential oil of *S. mutica* have been published but there is no report on the effect of growth stages on the antioxidant activity and total phenolic content of the essential oil of *S. mutica*. Therefore, in the present study,

chemical composition of the essential oil of *S. mutica* collected at different growth stages (pre-flowering (E_1), flowering (E_2) and post-flowering (E_3)) and the antioxidant activities of them, were investigated.

Materials and Methods

Materials

Foline-Ciocalteu (FC) reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate anhydrous and gallic acid were purchased from Sigma-Aldrich (Germany).

Plant materials

The aerial parts of *S. mutica* were collected in May 2013 (pre-flowering stage), July 2013 (flowering stage) and November 2013 (post-flowering stage), from the Havar Mountain, near Darkesh village (at 1300 meters above sea level), Iran. A voucher specimen of the plant (voucher No. MP- 248) was deposited at Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran. The aerial parts were air-dried at ambient temperature in the shade (Sahena et al, 2009).

Preparation of the essential oil

For this purpose, 80 g of dried aerial parts of the plant was submitted to Clevenger apparatus. The volatile distillate was collected by anhydrous sodium sulfate and refrigerated prior to further analysis (Yamini et al, 2008).

GC-MS analysis

GC-MS analysis was done by Shimadzu-QP2010SE, using operating at 70 eV ionization energy, Rtx-5MS Poly (methyl phenyl siloxane) 30 m × 0.25 mm, and 0.25 µm film thickness) with He as the carrier gas and a flow rate of 0.9 ml/min. Acquisition mass range was 35-300 with a 0.5 sec scan time. Retention indices were determined by using retention times of *n*-alkanes that were injected after the oil, under the same chromatographic conditions. The retention indices for all components were calculated based on the previous reports (Kamali et al, 2013). The compounds were identified by comparing of their retention indices (RI) with those reported in the literature and searching Nist and Wiley libraries (Adams, 2005).

Free radical scavenging assay (DPPH)

The antioxidant activity of the essential oils was assayed according to the method of Wang et al (2003). The essential oils at different concentration (20–100 µg) were mixed with 0.5 mM DPPH in methanol. After 30 min, the absorbance was measured at 517 nm. The antioxidant activity was calculated by the following equation:

$$\%\text{DPPH scavenging activity} = \frac{(\text{Absorbance}_{517} \text{ of blank} - \text{Absorbance}_{517} \text{ of sample})}{\text{Absorbance}_{517} \text{ of blank}} \times 100$$

Concentration of sample necessary to scavenge 50% of the DPPH radicals was calculated using Bio-DataFit online software. BHT and ascorbic acid were used as the reference antioxidants. The EC50 of BHT and ascorbic acid were 20.2 and 18.0 µg/ml, respectively.

Determination of total phenolic content

The total phenolic content was measured by using Folin-Ciocalteu method (Hayouni et al, 2007). Here, 100µl of the essential oil (1000 mg/l) was added to Folin-Ciocalteu reagent (1/10, 500µl) and then, 1.5 ml sodium carbonate (Na_2CO_3) (20%) was added to the mixture. Then, the tubes were incubated at room temperature for 120 min. The absorbance was read at 760 nm using UV-VIS spectrophotometer (Cecil CE 1011; UK). The analysis was done in triplicates. Also the standard curve of gallic acid in methanol (50-500 mg/l) was prepared. Total phenolic content was expressed as gallic acid (GA) equivalents (mg GA/g of dried material).

Results

GC-MS analysis

The results showed that the highest yield of oil was obtained for the plants collected at the flowering stage [(flowering stage essential oil (E_2)] (3.78%) and the lowest was for the plants collected at the post-flowering stage [(post flowering stage essential oil (E_3)] (Table 1.).

Table 1. Yield, DPPH EC_{50} and total phenolic content of the essential oil of *S. mutica* collected at different growth stages

Stages	Observed yield (W/W %)	Total phenolic content (mg GAE/ gram dried material)	EC_{50} (µg/ml)
Pre- flowering stage (E1)	3.26%±0.767	0.57±0.178	52.41±0.411
Flowering stage (E2)	3.78%±0.255	0.341±0.62	79.05±0.134
Post- flowering stage (E3)	2.59%±0.978	0.433±0.356	74.18±0.231

Data obtained from qualitative and quantitative evaluation of the oil samples are shown in Table 2.

Table 2. Seasonal variation of the chemical composition of *S. mutica* essential oils.

No.	Compound	RI (Retention indices)	Chemical constituents (%) of the essential oil of the plants collected at pre-flowering stage (E1)	Chemical constituents (%) of the essential oil of the plants collected at flowering stage (E2)	Chemical constituents (%) of the essential oil of the plants collected at post-flowering stage (E3)
1	β-myrcene	990	2.88	1.94	2
2	α-phellandrene	1001	0.45	0.23	0.2
3	α -terpinene	1018	3.75	0.68	0.88
4	p-cymene	1026	6.88	13.12	19.24
5	Trans- β -ocimene	1051	0.24	0.06	-------
6	γ-terpinene	1062	19.77	16.71	7.58
7	Cis-sabinene hydrate	1073	0.16	0.13	0.3
8	Terpinolene	1089	0.25	0.29	0.76
9	Trans-sabinene hydrate	1098	0.55	0.16	0.2
10	Linalool	1105	-------	0.18	------
11	Borneol	1165	0.42	0.26	0.48
12	Terpinene-4-ol	1177	0.87	1.46	1.54
13	α -terpineol	1196	---------	0.23	0.27
14	Dihydrocarvone	1203	--------	--------	0.13
15	Carvacrol methyl ether	1248	3.06	0.81	1.03
16	Thymol	1292	20.09	18.58	14.67
17	Carvacrol	1298	33.96	29.55	34.51
18	Thymol acetate	1357	--------	0.32	0.08
19	Carvacrol acetate	1359	--------	0.55	0.17
20	β -caryophyllene	1397	2.84	1.02	0.73
21	α -humulene	1458	0.16	0.14	0.22
22	Germacrene-D	1480	0.16	0.05	0.08
23	tert-butylcatechol	1493	-------	-------	0.25
24	α -bisabolene	1503	0.25	0.14	------
25	β -bisabolene	1509	1.73	1.16	0.97
26	γ -cadinene	1510	-------	-------	0.11
27	Delta-cadinene	1529	0.25	0.06	0.19

28	Caryophyllene oxide	1587	0.26	0.2	1.35
29	Viridiflorol	1603	-----	-----	0.15
	Monoterpene hydrocarbons	-------	%34.22	%33.05	%30.66
	Oxygenated monoterpenes	-------	%59.11	%52.23	%53.71
	Sesquiterpene hydrocarbons	-------	%5.39	%2.57	%2.22
	Oxygenated sesquiterpenes	-------	%0.26	%0.2	%1.5
	Total	-------	%98.98	%88.05	%88.09

Total phenolic content

In this study, total phenolic content was determined in terms of gallic acid equivalents (GAE) (Table 1). The essential oil that displayed the highest concentration of total phenols was E1 (pre-flowering stage) (0.57 mg GAE/ g dry weight of the extract) and the essential oil that showed the lowest concentration of total phenols was E2 (0.341 mg GAE/ g dry weight of extract).

Radical scavenging effects

The radical scavenging effects of the essential oils were concentration-dependent and the highest concentration of them had the strongest radical scavenging effect (Figure 1). Results were reported as EC_{50} (the amount of antioxidant required to inhibit 50% of DPPH free radicals) (Table 1). E1 samples exhibited the highest radical scavenging potential as they had the lowest value of EC_{50} (EC_{50} = 52.41 µg/ml) followed by the E3 (EC_{50} = 74.18 µg/ml) and E2 (EC_{50} = 79.05 µg/ml).

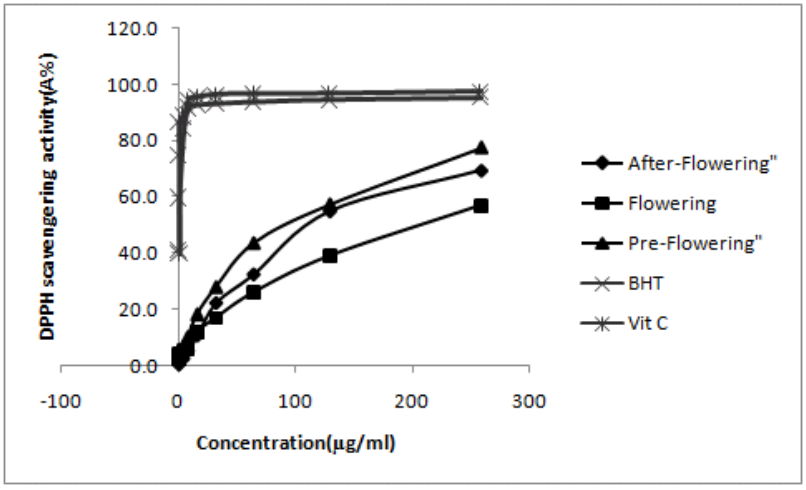

Figure 1. Radical scavenging activity of *S. mutica* essential oils.

Discussion

There are many reports on the essential oil of *Satureja*, and in this study a high yield was obtained while other species had lower yields as reported by other researches e.g. 1.8% (v/w) for *S. thymbra* at flowering stage (Cetin et al, 2010). Our results are in agreement with other studies, for example *Mentha spicata* and *Artemisia annua* L. exhibited maximum essential oils yield during flowering stage (Kofidis et al, 2004; Verdian-rizi, 2008). In this study, 21, 25 and 26 compounds were identified in the essential oils of the E1, E2 and E3 samples, representing 98.98%, 88.03% and 88.09 % of total oils, respectively. The main compounds in the oils were thymol, carvacrol, p-cymene and γ -terpinene. As shown in Table 2, oxygen-containing monoterpenes formed 59.11% of the oil of E1 samples, 52.23% of E2 samples and 53.71% of E3 samples. As shown in Table 2, the main compounds identified from plants collected at different growth stages, were almost the same but the amounts of them were different. In all samples, the most abundant compounds were oxygenated monoterpenes while the least abundant compounds were oxygenated sesquiterpenes and 19 compounds were similar at all three stages. main compounds in essential oils from the 3 stages were carvacrol (33.96%, 29.55%, and 34.51%) thymol (20.09%, 18.58%, and 14.67%), p-cymene (6.88%, 13.12%, and 19.24%), γ-terpinene (19.77%, 16.71%, and 7.58%) (Figure 2).

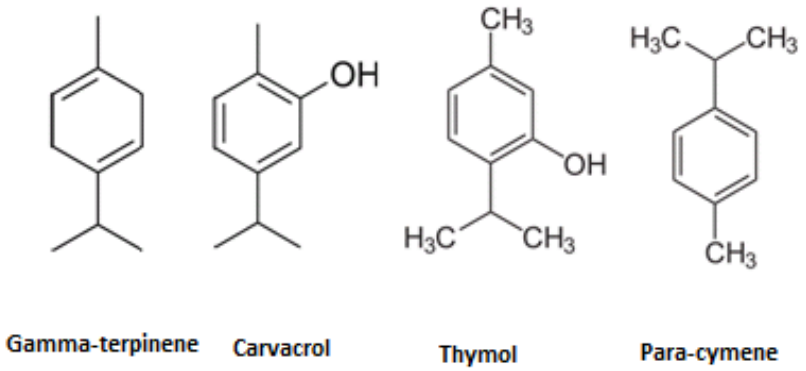

Figure 2. Chemical structures of the main compounds of the essential oil of *S. mutica*.

In a study on the essential oil of *S. biflora*, the major compounds were linalool (50.60%) and germacrene-D (10.63 %) (Matasyoh et al., 2007) while in *S. cuneifolia,* carvacrol and p-

cymene were major compounds (Oke et al, 2009). The oil of *S. monata* was evaluated and it was shown that this oil contains 38 components with carvacrol being the main compound (2.21-55.95%) (Ibraliu et al, 2010). also, the major components of *S. cuneifolia* were carvacrol (44.99%) and p-cymene (21.61%) (Oke et al., 2009). Moreover, it was reported that in the essential oil obtained from *S. montana*, thymol, p-cymene, linalool and carvacrol were the major chemical constituents (Oliveira et al, 2012). The major constituents of *S. thymbra* were carvacrol (40%) and γ-terpinene (27%) (Goren et al, 2004). Besides, it was demonstrated that the essential oil of *S. mutica* had high amounts of thymol, carvacrol, and p-cymene (Sefidkon et al., 2005). In the essential oil of *S. subspicata*, carvacrol (16.76%), α-pinene (13.58%), p-cymene (10.76%), γ -terpinene (9.54%) and thymol methyl ether (8.83%) were the main components (Skocˇibusˇic et al, 2006).

Folin-Ciocalteu method is a widely used assay for quantitative determination of phenolic compounds (Tawaha et al, 2007; Pourmorad et al, 2006). This method involves the oxidation of phenols in an alkaline solution and colorimetric measurement of the blue product (Cicco et al, 2009). Based on the data, standard curve was plotted and the regression was calculated (Y= 0.003 X+ 0.068 (R^2=0.98)). The difference in total phenolic content might be due to growth time, environmental conditions and seasonal changes. The phenolic content of some of *Satureja* plants has already been determined (e.g. the amounts of total phenol components in the methanolic extract and the oil of *S. cuneifolia* were 222.5 µg/mg and 185.5 µg/mg, respectively) (Oke et al, 2009). DPPH is a synthetic free radical that shows maximum absorption at 517 nm. Antioxidants can scavenge DPPH free radicals by providing a hydrogen atom and converting them to a colorless product resulting in a reduction in absorbance (Antolovich et al, 2002). E1 had the highest amount of phenolic content (especially thymol and carvacrol), and this essential oil had the highest radical scavenging potential. Our results were in agreement with the results of previous studies; For example, based on DPPH test system data, the antioxidant activity of *S. spicigera* was more pronounced than that of *S. cuneifolia* because major constituents of the oils of *S. spicigera* and *S. cuneifolia* were carvacrol (42.5% and 67.1%), γ-terpinene (21.5% and 15.2%) and p-cymene (20.9% and 6.7%), respectively (Eminagaoglu et al, 2007).

Some of the compounds present in the essential oil have been reported to exhibit antioxidant activity and carvacrol, thymol, γ-terpinene and p-cymene that are strong antioxidants, were the major compounds in the essential oil of *S. mutica* that constituted

80.7% of E1, 77.96% of E2, and 76% of E3. Among the identified compounds, carvacrol and thymol were the most abundant chemicals. These chemicals are phenolic monoterpenes which exhibit antioxidant activity due to their phenolic structures but p-cymene lacks a hydroxyl group and did not show antioxidant and antimicrobial activity (Ultee et al, 2002). Studies on the antimicrobial activity of carvacrol have shown that carvacrol has a broad spectrum of antimicrobial activity against almost every Gram-positive and Gram-negative bacteria tested (Friedman et al, 2002). Similar to carvacrol, thymol contains both a hydroxyl group and a system of delocalized electrons and was found to possess strong antimicrobial and antioxidant activity because the presence of a hydroxyl group (bound to a benzene ring) is important for antimicrobial and antioxidant activities and these activities are enhanced by the presence of double bonds (Pruthi, 1980**)**. The antioxidant properties of carvacrol and thymol are important as they play the role of active food additives, and also they can protect the polymer against oxidative degradation during processing and use (Ramos et al, 2013). γ-terpinene, is a monoterpene hydrocarbone that shows antioxidant activity and possesses good lipid peroxidation inhibitory properties (Öztürk, 2012). Therefore, the antioxidant activity of this essential oil could be due to the presence of γ-terpinene , carvacrol and thymol.

Conclusion

The results of our investigation of antioxidant activities and chemical composition of the oils were consistent with that expected due to the presence of phenolic compounds in essential oil and there is a close correlation between antioxidant activities and total phenolic content obtained from various essential oils. Moreover, this study showed that some compounds such as carvacrol, thymol and γ-terpinene were responsible for antioxidant activity of the essential oil of the plant collected at the pre-flowering stage.

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Conflict of interest

The authors have no competing interests to declare.

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