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Full Length Research Paper

Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran

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The effects of different amounts of compelet fertilizer on the yield, fresh and dry weight, essential oil composition, total phenolic content and antioxidant activity of *Satureja hortensis* L. was investigated. Different amounts of compelet fertilizer (0, 500, 1000 and 1500 mg/plant) were applied. The results showed that the use of fertilizer increases fresh and dry weigh in *S. hortensis.* One thousand and five hundred mg/plant compelet fertilizer enhanced the essential oil yield and essential oil efficiency. Nineteen components were identified in the essential oil of *S. hortensis* underwent at different treatments, that represented 97.58 - 99.24% of the oils. The major components were carvacrol (43.9 - 59.2%), γ-terpinene (30.7 - 40.2%), α-terpinene (2.8 - 4%) and *P*-cymene (1.8 - 2.2%). The effect of different amounts of fertilizer on the essential oil composition was very slight and was not significant. But the amount of some component such as carvacrol, γ-terpinene and α-terpinene was changed with using of fertilizer. Total phenolics and antioxidant activity of plant extracts were measured by the Folin-Ciocalteu and DPPH free radical scavenging assays, respectively. The total phenolic content varied from 23.58 to 24.52 (mg gallic acid equivalent/g dw) and the highest value was found in 1000 mg/plant compelet fertilizer treatment. IC_{50} values in the DPPH assay ranged from 8.45 to 8.6 µg/ml and the highest activity was observed in 1000 mg/plant compelet fertilizer treatment. The use of fertilizer increased total phenolic content and antioxidant activity in *S. hortensis.* Also, We have found a positive correlation between the total phenolic content and antioxidant activity in *S. hortensis* (R^2 = 0.55).

Key words: *Satureja hortensis,* fertilizer, essential oil composition, total phenolic content, antioxidant activity.

INTRODUCTION

Satureja hortensis L. is an annual, herbaceous aromatic and medicinal plant belonging to the family Lamiaceae. It is known as summer savory, native to southern Europe and naturalized in parts of North America (Sefidkon et al., 2006). This plant is traditionally used as carminative, digestive, antispasmodic and antitussive in Iran (Zargari, 1990). The aerial parts of some *Satureja* plants have been widely used in foods for herbal tea and flavor component and in folk and traditional medicine, to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Gulluce et al., 2003; Madsen et al., 1996; Zargari, 1990). Literature review, on essential oil composition in *Satureja* species show to be rich in phenolic components such as carvacrol, γ-terpinene, thymol, *p*-cymene, β-aryophyllene, linalool and other terpenoids. But chemical composition and the amount of components have variation between of different *Satureja* species oils (Baher et al., 2002; Baser et al., 2004; Chalchat et al., 1999; Kurcuoglu et al., 2001;

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Table 1. The results of soil analysis used in this study.

EC (ms)	pH	Sand (%)		Silt (%)	Clay (%)	Soil Texture	
1.85	7.77	71.4		9.96	18.46	Sandy	
Mn (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)	OC (%)	K (ppm)	P (ppm)	N (%)
7.4	0.6	0.22	17.2	3.64	168.7	32.2	0.32

Novak et al., 2006; Rojas and Usubillaga, 2000; Sefidkon et al., 2006; Svoboda et al., 2006; Tumen et al., 1998; Viturro et al., 2000).

Some researches show that the essential oil and extract of *Satureja* species have demonstrated a variety of activities including antibacterial and antifungal properties and show a strong inhibition on a wide range of bacteria and fungi in human, food and plant pathogens (Baydar et al., 2004; Boyraz and Özcan, 2006; Deans and Svoboda, 1989; Gulluce et al., 2003; Hajhashemi et al., 2000; Helander et al., 1998; Sahin et al., 2003). Recent studies show that some plants from the lamiaceae families are very rich in phenolic compounds, such as flavonoids, phenolic acids and phenolic diterpenes and possess high antioxidant activities (Aaby et al., 2004; Lu and Foo, 2001; Rice-Evans et al., 1997; Wong et al., 2006; Zheng and Wang, 2001). Flavonoids and phenolic compounds exert multiple biological effects such as antioxidant, free radical scavenging and anti-inflammatory properties (Jose del bano et al., 2003; Miliauskas et al., 2004; Shahidi, 2000). Oxidative damage in the human body plays an important causative role in disease initiation and progression (Gülcin et al., 2005; Jacob and Burri, 1996). Phenolic compounds are secondary plant metabolites and naturally present in almost all plant materials (Gülcin, 2005; Psomiadou and Tsimidou, 2002). These compounds can delay or inhibit the oxidative damage caused by free radicals such as superoxide anion radicals, hydroxyl radicals and non free-radical species such as H_2O_2 and singled oxygen, by inhibiting the initiation or propagation of oxidative chain reactions (Gülcin et al., 2002; Velioglu, 1998) and can protect us against major diseases such as coronary heart disease and cancer in human (Ames, 1983; Kris-Etherton et al., 2002). Also, a number of studies have suggested that the *Satureja* species have antioxidant properties (Dorman and Hiltunen, 2004; Madsen et al., 1996; Radonic and Milos, 2003).

The biosynthesis of secondary metabolites in medicinal and aromatic plants are strongly influenced by environmental factors (Stutte, 2006). These conditions cause variations in the fresh and dry weight, as well as active components (Cabo et al., 1982; Christensen and Grevsen, 2006; Ozguven and Stahl-Biskup, 1989). In this context, the use of organic and chemical fertilizers can increase the yield of the essential oil and main components of medicinal plants (Anwar et al., 2005; Arabaci and Bayram, 2004; Economakis, 2005; Khalid et al., 2006; Naghdibadi et al., 2004; Shalby and Razin, 1992). Due to the fast growth of the demand for medicinal plants and herbal remedies in the world, the cultivation of these plants has significantly increased in recent years. Meanwhile, utilization of chemical fertilizers and nutrients to increase the yield has become very popular (Yazdani et al., 2004). But the effect of different amounts of chemical fertilizers on bioactive components and secondary metabolites in aromatic and medicinal plants have has attracted less attention.

In this study, we have cultured *Satureja hortensis* L. as one of the most popular medicinal and aromatic plants in Iran and examined the effects of fertilizer on essential oil composition, total phenolic content and antioxidant activity in this plant. This study can provide useful information for improvement of culture conditions of medicinal plants and investigation on the effects of fertilizer on yield and bioactive component in medicinal plants.

MATERIALS AND METHODS

Chemical reagents

The chemical reagent DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid monohydrate (3,4,5-Trihydroxybenzoic acid) and sodium carbonate were purchased from Sigma chemical Co. (St. Louis, USA). Folin Ciocalteu reagent, methanol and acetone were purchased from Merck Co. (Darmstadt, Germany). Trolox and Quercetin hydrate were purchased from Acros organics Co. (New Jersey, USA).

Plant material

Seeds of *S. hortensis* L. were obtianed from institute of medicinal plants, Esfahan, Iran and were grown in green house conditions in streel soil containing clay, sand and peat moss (1:1:1, v:v:v). Before plant culture, the soil was analysed for determination of texture and macro and micro element components (Table 1). One seedling of plants was transplanted in to pots (20 × 20 cm, about 4 kg soil) and arranged in randomized complete block design in four replicates. Each replicate contained 8 pots. After 10 days of plant transition, different concentrations of chemical fertilizer (0, 500, 1000 and 1500 mg/plant) were used for plant nutriton. The chemical composition of the fertilizer used in this study is shown in Table 2. Plants were irrigated to pot capacity and maintained at day and night temperatures of 26 - 30 and 18 - 22ºC, respectively. Green house plants were harvested in the preflowering stage. Every plant in each treatment was weighed, bulked and placed in paper bag and dried at room temperature for 15 days to determine fresh and dry weight. Dry plants were stored in a dry place until analysis.

Table 2. Chemical composition of the fertilizer used in this study.

Amount	Elements
20%	N
20%	P (P_2O_5)
20%	K (K_2O)
1000 *	Fe
320 *	Mn
230 *	Zn
100 *	B
75 *	Cu

* Expressed as ppm.

Essential oil extraction

Essential oil was obtained from dried aerial parts from *S. hortensis* L. by steam distillation using Clevenger system during 3 h. The extracted essential oil was dried with anhydrous sodium sulfate. Then, the oil was weighed and stored in sealed amber flasks at 4°C until analysis.

Essential oil analysis

Gas chromatography (GC)

Gas Chromatography analysis was performed on an Agilent technologist model (6990 USA) series II gas chromatograph equipped with flame ionization detector and capillary column HP-5 (30 m × 0.25 mm, 0.25 µm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 240ºC at a rate of 3ºC/min. The injector and detector temperatures were 240 and 250ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:20. Quantitative data was obtained from electronic integration of peck areas without the use of correction factors.

Gas Chromatography- Mass Spectrometry (GC/MS)

Essential oil was also analysed by Hewlett- Packard GC-MS (model 6890 series II) operating at 70_e V ionization energy. Equipped with a HP-5 capillary column (phenyl methyl siloxane (30 m × 0.25 mm, 0.25 µm film thickness) with He as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra.

Extracts preparation

Grounded air dried plant material (7.5 g) was weighed in a glass and then defatted with petroleum benzine for 3 h. Each Sample was twice extracted with 200 ml of 90% aqueous methanol, each time for 24 h at room temperature. Each extraction was filterated through whatman filter paper (Whatman Ltd., England). Supernatants were combined and evaporated to dryness using a rotary evaporator to a volume of about 1 ml. These concenterated extracts were freeze-dried and weighed to determine the yield. These samples were stored for further experiments.

Total phenolic content analysis

The total phenolic content was determined with the Folin-Ciocalteu reagent as described previously (Singleton and Rossi, 1965). Briefly, 200 µl of plant extract dissolved in methanol (1 mg/ml) were mixed with 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times in distilled water) in glass tubes in triplicate. The samples were incubated at room temperature for 5 min and vortex mixed at least 2 times. Then, 2 ml of Na_2CO_3 7.5% was added and the glass tubes were incubated in the dark for 90 min with continuous shaking. The absorbances of samples were measured at 765 nm using a Spectrophotometer (Perkin-Elmer UV/Vis double beam lambda 1, USA) against a blanck of distilled water. Different concentrations of garlic acid in methanol were tested in parallel to obtain an standard curve. Total phenolic contents were expressed as milligram's of garlic acid equivalent per gram of dry weight (mg GAE/g dw).

Free radical scavenging capacity

The antioxidant activity was determined by DPPH free radical scavenging assay as described previously with some modifications (Brand-Williams et al., 1995). Briefly, 4 different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH 100 µM in a total volume of 4 ml. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Methanol was used as blank and all measurements were carried out in triplicate. Trolox, a water-soluble equivalent of vitamin E and quercetin were used as reference compounds. All solutions were made daily. The percent inhibition of DPPH free radical was calculated by the formula:

$$\text{Percentage inhibition (\%I)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where, A_{blank} is the absorbance of the control reaction (DPPH alone) and A_{sample} is the absorbance of DPPH solution in the presence of the test compound.

IC_{50} values denote the concentration of the sample, required to scavenge 50% of DPPH free radicals (Elmastas et al., 2006; Gülçin et al., 2006).

Statistical analysis

All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA by the software SAS (version 9.2 for windows). Significant differences between means were determined by Duncans new multiple-range test. A significant difference was considered at the level of $P < 0.05$. Correlation analyses of antioxidant activity versus the total phenolic content were carried out using the Microsoft Office Excel program. IC_{50} values of the antioxidant activity were calculated by the software Sigma Plot (version 8.0 for Windows).

RESULTS AND DISCUSSION

Effects of fertilizer on fresh and dry weight

Effects of different amounts of chemical fertilizer on fresh and dry weights (g/plant) are presented in Table 5. Generally increasing fertilizer level progressively increased the fresh and dry weight. The fertilizer application had positive effect on fresh and dry weight.

The values of fresh and dry weight were significantly

Table 3. Simple ANOVA between traits in *Satureja hortensis L*.

Traits		Fresh weight	Dry weight	Essential oil yield	Essential oil efficiency	Total phenolic content	Antioxidant activity
Sov	df			MS			
Rep.	3	0.27 ^{ns}	0.016 ^{ns}	0.003 ^{ns}	22.44 ^{ns}	0.07^{ns}	0.006 ^{ns}
Treatment	3	24.29 **	1.21**	0.78 **	4142.14 **	0.58**	0.01 ^{ns}
Error	9	0.16	0.018	0.006	26.53	0.01	0.006
CV%		2.01	3.14	3.37	5.07	0.41	0.97

Ns: Not significant.
** : Significant at 5% probability.

Table 4. Pearson correlation between traits in *Satureja hortensis* L.

Traits	Fresh weight	Dry weight	Essential oil yield	Essential oil efficiency	Total phenolic content	Antioxidant activity
Fresh weight	---					
Dry weight	0.98 **	---				
Essential oil Yield	0.96 **	0.96**	---			
Essential oil efficiency	0.98 **	0.98 **	0.99**	---		
Total phenolic content	0.57*	0.57 *	0.63 **	0.58*	---	
Antioxidant activity	0.41 ^{ns}	0.39 ^{ns}	0.57 *	0.49 ^{ns}	0.55*	---

Ns: No significant.
* : Significant at 5% probability.
** : Significant at 1% probability.

increased by increasing of fertilizer. Fertilization of *S. hortensis* with 1500 mg fertilizer /plant increased fresh and dry weight by 23.22 and 5 g. compared with control treatment 17.8 and 3.75 g. respectively. Thus in the present investigation an application of recommended dose of fertilizer nutrient (1500 mg/plant) recorded significantly higher fresh and dry weight as compared to control and other treatment.

Effects of fertilizer on essential oil yield and efficiency

The effects of fertilizer amounts on essential oil yield and efficiency in *S. hortensis* was significant (Tables 3 and 5). Essential oil percentage was significantly increased by increasing levels up to 1500 mg/plant. Using complete fertilizer up to 1500 mg/plant induced significant increase in the essential oil percentage. The highest value of essential oil percentage (2.81%) was observed from the treatment of 1500 mg/plant compared with control (1.82%) treatment. Essential oil efficiency was calculated by essential oil yield (percentage) x plant dry weight / 100 and expressed as mg/plant. According to essential oil yield, essential oil efficiency was increased by increasing fertilizer levels up to 1500 mg/plant. The highest essential oil efficiency (0.14 mg/plant) was obtained in 1500 mg/plant treatment and control treatment with 0.07 mg/plant the lowest essential oil efficiency in all treatments. This results suggested that using fertilizer up to 1500 mg/plant caused about 2 time increased in essential oil efficiency and 1.5 time increasing in essential oil percentage than control treatment. Omidbaigi and Arjmandi (2002) suggested that the use of nitrogen and phosphorous fertilizers increased the yield and influenced the essential oil components in thyme (*Thymus vulgaris*). Our results showed findings to this research.

Effects of fertilizer on essential oil composition

The effect of fertilizer on chemical composition of the essential oil of *S. hortensis* and retention indices are given in Table 6. The essential oil isolated by hydro-distillation of the aerial part of *S. hortensis*, with different levels of fertilizer, was found to be a yellow liquids, obtained in yield of 1.82 - 2.81 (v/w), based on dry weight respectively.

Nineteen components were identified in the essential oil of *S. hortensis* underwent at different treatments, that represented 97.58 - 99.24% of the oils. The major components were carvacrol (43.9 - 59.2%), γ-terpinene (30.7 - 40.2%), α-terpinene (2.8 - 4%) and *P*-cymene (1.8 - 2.2%). Other components were present in amounts less than 2%. The results depicted in Table 6 show that using

Table 5. Effects of different amounts of chemical fertilizer on the fresh and dry weight, essential oil yield and efficiency of *Satureja hortensis* L.

Chemical fertilizer Treatment	Fresh Weight[a] (g)	Dry Weight[a] (g)	Essential oil yield[b] (%)	Essential oil efficiency[c] (mg/ plant)
0 mg/plant	17.8 ± 0.4 d	3.75 ± 0.14 d	1.82± 0.02 d	68.37± 0.002 d
500 mg/plant	18.83 ± 0.38 c	4.02 ± 0.13 c	2.07± 0.06 c	83.32 ± 0.003 c
1000 mg/plant	21.43 ± 0.6 b	4.54 ± 0.13 b	2.5 ± 0.06 b	113.89 ± 0.005 b
1500 mg/plant	23.22 ± 0.32 a	5 ± 0.12 a	2.81 ± 0.1 a	140.55 ± 0.007 a

Each value in the table was obtained by calculating the average of four experiments ± standard deviation.
[a] Fresh and Dry weight. Data expressed as grams.
[b] Essential oil yield. Data expressed as mg per 100 g dry weight (DW).
[c] Essential oil efficiency. Data expressed as mg per g dry weight (DW).
Means with different letters were significantly different at the level of $p < 0.05$.

Table 6. Effects of different levels of chemical fertilizer on the yield and composition of the essential oil in *Satureja hortensis* L.

NO	Compound	RI[a]	0 mg/plant	500 mg/plant	1000 mg/plant	1500 mg/plant
1	α-Thujene	928	0.5 ± 0.3	1.13 ± 0.02	1.1 ± 0.09	1.1 ± 0.02
2	α -Pinene	934	0.4 ± 0.2	1.08 ± 0.01	1.1 ± 0.3	0.9 ± 0.01
3	Camphene	950	0.05 ± 0.003	0.07 ± 0.005	0.06 ± 0.01	0.07 ± 0.001
4	β-Pinene	978	0.4 ± 0.1	0.7 ± 0.06	0.5 ± 0.3	0.7 ± 0.01
5	Myrcene	990	1.3 ± 0.5	2.0 ± 0.06	2.0 ± 0.1	1.9 ± 0.06
6	α-Phellandrene	1002	0.2 ± 0.07	0.4 ± 0.03	0.3 ± 0.03	0.3 ± 0.04
7	α-Terpinene	1015	2.8 ± 0.7	4.0 ± 0.02	3.7 ± 0.3	3.8 ± 0.2
8	*P*-Cymene	1024	1.8 ± 0.2	2.2 ± 0.09	2.0 ± 0.3	2.2 ± 0.2
9	δ-Terpinene	1057	30.7 ± 4.5	40.2 ± 2.4	37.2 ± 3.6	38.8 ± 1.9
10	(E)-Sabinene hydrate	1061	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02
11	Terpinolene	1087	0.08 ± 0.009	0.1 ± 0.003	0.06 ± 0.01	0.07 ± 0.006
12	Terpinene-4-OL	1177	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.04
13	Thymyl methyl ether	1237	0.06 ± 0.003	0.06 ± 0.001	0	0.01 ± 0.002
14	Carvacrol	1303	59.2 ± 6.4	43.9 ± 1.2	49.3 ± 3.9	46.9 ± 2.5
15	Carvacryl acetate	1374	0.05 ± 0.006	0.2 ± 0.04	0.2 ± 0.02	0.1 ± 0.01
16	β-caryophyllene	1417	0.3 ± 0.02	0.4 ± 0.02	0.3 ± 0.05	0.4 ± 0.02
17	bicyclogermacrene	1496	0.3 ± 0.2	0.08 ± 0.02	0.06 ± 0.05	0.1 ± 0.04
18	β -bisabolene	1503	0.3 ± 0.2	0.7 ± 0.09	0.6 ± 0.1	0.8 ± 0.3
19	(E)-α -bisabolene	1533	0.5 ± 0.2	0.06 ± 0.008	0.02 ± 0.002	0.08 ± 0.01
	Oil Yield (%w/w)		1.82%	2.07%	2.5%	2.81%
	Total		99.24	97.58	98.8	98.53

[a] RI, retention indices in elution order from HP-5 column.
Each value in the table was obtained by calculating the average of four experiments ± standard deviation. Data expressed as percentage of total.

complete fertilizer in *S. hortensis* caused a very slight and non significant change in essential oil composition. But the amount of some component such as carvacrol, δ-terpinene and α-terpinene was changed with using of fertilizer. Using complete fertilizer up to 1500 mg/plant induced significant decrease in carvacrol, but caused increased in δ-terpinene and α-terpinene values. The chemical fertilizer that was used in this study, contained micro and macroelement nutrients (Table 2). It is already known that nitrogen, phosphorous and potassium affect the growth and essential oil synthesis in medicinal plants.

These components influence the levels of enzymes that are very important in the biosynthesis of important terpenoides such as thymol and carvacrol (Sell, 2003). The use of chemical fertilizer in our study seemed to cause change in some of the main components of

Table 7. Effects of different amounts of chemical fertilizer on total phenolic content and antioxidant activity of *Satureja hortensis* L.

Chemical fertilizer Treatment	Total phenolic content [1] (mg GAE/g DW.)	IC_{50} [2] (µg/ml)
0 mg/plant	23.58 ± 0.08 c	8.6 ± 0.06 a
500 mg/plant	23.99 ± 0.115 b	8.51± 0.06 a
1000 mg/plant	24.52 ± 0.2 a	8.45 ± 0.11 a
1500 mg/plant	24.08 ± 0.18 b	8.47 ± 0.08 a

[1]Data expressed as mg of gallic acid equivalents per g dry weight (DW).
[2]IC_{50}: Data expressed as µg per millilitre. Lower IC_{50} values indicated the highest radical scavenging activity. Means with different letters were significantly different at the level of $p < 0.05$.
Each value in the table was obtained by calculating the average of four experiments ± standard deviation. Values followed by the same letter under the same row, are not significantly different ($p > 0.05$).

Satureja essential oil in our study. This finding is similar to the results of another study on *T. vulgaris* (baranauskiene et al., 2003) and a report on *Ocimum basilicum* (Arabaci et al., 2004).

Effects of fertilizer on total phenolic content and antioxidant activity

Phenolic compounds are a class of antioxidant agents which act as free radical scavengers and are responsible for antioxidant activity in medicinal plants (Shahidi and Wanasundara, 1992). Free radical may cause many disease conditions such as cancer and coronary heart disease in human (Javanmardi et al., 2003; Löliger, 1991). Many plants extracts containing bioactive compounds including phenolics and flavonoid exhibit efficient antioxidant properties and prevent from free radical damage (Larson, 1998; Koleva et al., 2002). The total phenolic content was measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 0.0287x + 0.2384$, $R^2 = 0.9949$). The effects of fertilizers on total phenolic content and antioxidant activity are shown in Table 7. The total phenolic content was increased with using chemical fertilizer. The highest total phenolic content was observed with using 1000 mg fertilizer per plants (24.52 mg GAE/g dw) compared with control treatment without using chemical fertilizers (23.58 ō.09 mg GAE/g dw). The results show that the using of fertilizer caused incresed in total phenolic content in *S. hortensis*.

The antioxidant activity of plant extracts were assessed by the DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging method. This assay determines the scavenging of stable radical species of DPPH by antioxidant (Othman et al., 2007). The capacity of reducing DPPH radical by antioxidants was determined by monitoring the decrease in its absorbance at 517 nm.

The effect of different amounts of chemical fertilizer on radical scavenging activity of *S. hortensis* is shown in Table 7. In all treatments, the radical scavenging activity of extracts increased with increasing concentrations of the plant extracts. The highest radical scavenging activity was observed in using 1000 mg fertilizer/plant with an IC_{50} of 8.45 µg/ml and the lowest value was detected in the control treatment without using chemical fertilizer group. These results showed that *S. hortensis* possesses strong antioxidant activity and can be used as a good source of natural antioxidants for medicinal purposes.

According to our results, the use of chemical fertilizer in *S. hortensis* increased the antioxidant activity in all treatments but not significant (Tables 3 and 7). Also we have founded the coefficient correlation between total phenolic content and antioxidant activity in *S. hortensis* ($R^2 = 0.55$ in Table 4). This result suggest that 55% of antioxidant activity in *S. hortensis* results from the phenolic compounds and it can be concluded that antioxidant activity on plant extracts is not limited to phenolics and activity may also come from the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids and vitamins that in *S. hortensis* contributed to 45% of the antioxidant capacity. This results are in agreement with those reported by Javanmardi et al. (2003) in *Ocimum basilicum* and Zheng (2001) in selected herbs.

Conclusion

S. hortensis L. is an aromatic and medicinal plant belonging to the family Lamiaceae used in the Iranian folk medicine for various purposes.

Our results showed that chemical fertilizers increased fresh and dry plant weight. The use of 1500 mg/plant of compelet fertilizer increased the essential oil yield and efficiency. Nineteen component were identified in *S. hortensis* essential oils. The effect of chemical fertilizer on essential oil composition was very slight and was not significant, but the amount of some component such as carvacrol, δ-terpinene and α-terpinene was changed with

using of fertilizer. Total phenolic content increased by use of chemical fertilizer. But no significant effect on antioxidant activity. The results of this study can be useful to investigation of using of chemical fertilizer on yield and bioactive component in medicinal plants.

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REFERENCES

Ames BN (1983). Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases Sci. 221: 1256–1264.

Arabaci O, Bayram E (2004). The effect of nitrogen fertilization and different plant densites on some agronomic and technologic characteristic of *Ocimum basilicum* L. (Basil). J. Agro. 3: 255-262.

Baher ZF, Mirza M, Ghorbanli M, Rezaii M (2002). The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. Flav. Frag. J. 17: 275–277.

Baser KHC, Ozek T, Kirimer N, Tumen G (2004). Comperative study of the essential oils of wild and cultivated *Satureja hortensis* L. J. Essen.Oil Reas. 6: 422-424.

Baydar H, Sagdic O, Ozkan G, Karadogan T (2004). Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Cont. 15: 169–172.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol. 28: 25-30.

Boyraz N, Özcan M (2006). Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey, Inter. J. Food Microbiol. 107: 238-242.

Chalchat JC, Gorunovic MS, Maksimovic ZA (1999). Essential oil of *Satureja kitaibelii* Wierzb. F. aristata (Vand) Hayek, lamiaceae from eastern Serbia. J. Essen. Oil Reas. 11: 691-692.

Deans SG, Svoboda KP (1989). Antibacterial activity of summer savory (*Satureja hortensis* L.) essential oil. J. Hort. Sci. 64: 205–210.

Dorman HJD, Hiltunen R (2004). Fe (III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis* L.) extract and subfractions. Food Chem. 88: 193-199.

Economakis C (2005). Effects of solution conductivity on the volatile constituents of *Origanum dictamnus* L. in Nutrient Film Culture. J. Agric. Food Chem. 53: 1656-1660.

Elmastas M, Gülçin I, Işildak Ö, Küfrevioğlu Öİ, İbaoğlu K, Aboul-Enein HY (2006). Radical scavenging activity and antioxidant capacity of bay leaf extracts, J. Iranian Chem. Soc. 3: 258-266.

Helander IM, Alakomi HL, Latva-Kala K, Mattila- Sandholm T, Pol I, Smid EJ, Gorris LGM, Von wright A (1998). Characterization of the action of selected essential oil component on gram- negative bacteria. J. Agric. Food Chem. 46: 3590-3595.

Gülçin I, Oktay M, Kufrevioglu OI, Aslan A (2002). Determination of antioxidant activity of Lichen *Cetraria islandica* (L.). Ach. J. Ethnopharmacol. 79: 325-329.

Gülçin I (2005). The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds, Inter. J. Food Sci. Nut. 56: 491-499.

Gülçin I (2006). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), Toxicol. 217: 213–220.

Gülçin I, Berashvili D, Gepdiremen A (2005). Antiradical and antioxidant activity of total anthocyanins from *Perilla pankinensis* decne. J. Ethnopharmacol. 101: 287–293.

Gülçin I, Elias R, Gepdiremen A, Boyer L, Köksal E (2007). A comparative study on the antioxidant activity of fringe tree (*Chionanthus virginicus* L.) extracts, Afr. J. Biotechnol. 6: 410-418.

Gülçin I, Mshvildadze V, Gepdiremen A, Elias R (2006). Screening of antiradical and antioxidant activity of monodesmosides and crude extract from *Leontice smirnowii* tuber. Phytomed.13: 343–351.

Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N (2003). In vitro antibacterial, antifungal and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J. Agric. Food Chem. 51: 3958–3965.

Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M (2000). Antispasmodic and antidiarrheal effect of *Satureja hortensis* L. essential oil. J. Ethnopharmacol. 71: 187–192.

Jacob RA, Burri BJ (1996). Oxidative damage and defense. Am. J.. Clin. Nutrit. 63: 985–990.

Javanmardi J, Stushnoff C, Locke E, Vivanco JM (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem. 83: 547-550.

Jose del bano M, Lorente J, Castillo J, Benavente-Garcia O, Antonio del Rio J, Ortuno A, Quirin KW, Gerard D (2003). Phenolic diterpenes, flavones and rosmarinic acid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis*. Antioxidant activity. J. Agric. Food Chem. 51: 4247-4253.

Khalid KhA, Hendawy SF, El-Gezawy E (2006). *Ocimum basilicum* L. production under organic farming. Res. J. Agri. Biol. Sci. 2: 25-32.

Koleva LL, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Analys. 13: 8-17.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer, Am. J. Med. 113 (Suppl. 9B): 71S–88S.

Kurcuoglu M, Tumen G, Baser KHC (2001). Essential oil constituents of *Satureja Biossieri* from Turkey. *Khimiya Prirodnykh Soedinenii* 37: 280–281.

Larson RA (1998). The antioxidant of higher plants. Phytochem. 27: 969-978.

Löliger J (1991). The use of antioxidants in food. Hn O.I. Aruoma & B. Halliwell (Eds.), Free radicals and food additives. London, Taylor and Francis pp. 129-150.

Lu Y, Foo YL (2001). Antioxidant activities of polyphenols from sage (*Salvia officinalis*), Food Chem. 75: 197–202.

Madsen HL, Andersen L, Christiansen L, Brockhoff P, Bertelsen G (1996). Antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat. Food Res. Technol. 203: 333–338.

Miliauskas G, Venskutonis PR, Van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chem. 85: 231–237.

Naghdibadi H, Yazdani D, Mohammad Ali S, Nazari F (2004). Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, (*Thymus vulgaris* L.) Indust. Crops Prod. 19: 231-236.

Novak J, Bahoo L, Mitteregger U, Franz Ch (2006). Composition of individual essential oil glands of savory (*Satureja hortensis* L., Lamiaceae) from Syria. Flav. Frag. J. 21: 731-734.

Omid baigi R (2000). Production and processing of medicinal plants; Astan Ghods Razavi Press. Tehran, Iran p. 397.

Othman A, Ismail A, Abdul Ghani N, Adenan I (2007). Antioxidant capacity and phenolic content of cocoa beans. Food Chem. 100: 1523-1530.

Psomiadou E, Tsimidou M (2002). Stability of virgin olive oil. 1. Autoxidation studies. J. Agric. Food Chem. 50: 716–721.

Radonic A, Milos M (2003). Chemical composition and *in vitro* evaluation of antioxidant effect of free volatile compounds from *Satureja montana* L., Free Radical Res. 37: 673–679.

Rojas LB, Usubillaga A (2000). Composition of the essential oil of *Satureja brownei* (SW.) Briq. From Venezuela. Flav. Frag. J. 15: 21–22.

Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A,

Ozturk S, Kotan R (2003). Evaluation of antimicrobial activites of *Satureja hortensis* L. J. Ethnopharmacol. 87: 61-65.
Sefidkon F, Abbasi K, Bakhshi Khaniki G (2006). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis.* Food Chem. 99: 19-23.
Sell CS (2003). A fragrant introduction to terpenoid chemistry. The Royal Society of Chemistry, Thomas Graham House, Scientific Park, Milton Road, Cambridge, UK p. 410.
Shahidi F (2000). Antioxidant in food and food antioxidants. Nahrung 44: 158–163.
Shahidi F, Wanasundara PKJPD (1992). Phenolic antioxidants. Crit. Rev. Food Sci. Nut. 32: 67-103.
Shalby AS, Razin AM (1992). Dense cultivation and fertilization for higher yield of thyme (*Thymus vulgaris* L.). J. Agro. Crop Sci. 168: 243–248.
Singleton VL, Rossi JA (1965). Colorimetry of total phenolic with phosphomolybdic- phosphotungestic acid reagents. Am. J. Enol. Vitic. 16: 144-158.
Stutte GW (2006). Process and product recirculation hydroponics and bioactive compounds in controlled environment. Hort. Sci. 41: 526-530.
Svoboda KP, Hay RKM, Waterman PG (2006). Growing summer savory (*Satureja hortensis*) in Scotland: Quantitative and qualitative analysis of the volatile oil and factors influencing oil production. J. Sci. Food Agri. 53: 193-202.
Tumen G, Baser KHC, Demirci B, Ermin N (1998). The essential oils of *Satureja coerulea Janka* and *Thymus aznavourii* Velen. Flav. Frag. J. 13: 65–67.
Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agri. Food Chem. 46: 4113- 4117.
Viturro CI, Molina A, Guy I, Charles B, Guinaudeau H, Fournet A (2000). Essential oils of *Satureja boliviana* and *Satureja pavifolia* growing in the region of Jujuy, Argentina. Flav. Frag. J. 15: 377–382.
Yazdani D, Shahnazi S, Seifi H (2004). Cultivation of medicinal plants medicinal plant Research Center press, Tehran, Iran p. 169.
Zargari A (1990). Medicinal plants Vol. IV. Tehran University Press, Tehran pp. 325-328.
Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49: 5165-5170.