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Research Article
Chemical Composition and Antioxidant Activity of Iranian *Satureja montana*

[1]Zahra Nemati, [2]Ebrahim Talebi, [1]Maryam Khosravinezhad and [2]Hojjat Golkari

[1]Shiraz branch, Islamic Azad University, Shiraz, Fars, Iran
[2]Darab branch, Islamic Azad University, Darab, Fars, Iran

Abstract

Background and Objective: The essential oils are vegetal metabolites with a wide range of biological properties and uses. *Satureja montana* (*S. montana*) is an aromatic, perennial shrub native in Iran. The objective of present study was to evaluate chemical composition and antioxidant activity of *Satureja montana* essential oil which is grown in Iran. **Methodology:** Chemical composition of *Satureja montana* essential oil was analyzed using gas chromatography-mass spectrometry (GC/MS). The antioxidant activity of *Satureja montana* was estimated by three methods and compared with TBHQ (TBHQ is a synthetic antioxidant). All calculations were performed using Minitab software and the paired student's t-test was used to compare the results of samples. **Results:** The essential oil of *Satureja montana* contains twenty compounds. The main compound was carvacrol (83.4%) and the results of TBHQ was shown that this plant have a good potential against oxidants. **Conclusion:** The concentration of carvacrol in *Satureja montana* essential oil which grown in Iran was higher than other area in the world. So, *Satureja montana* that grown in Iran could be used for a wide range of food and pharmaceutical industries.

Key words: *Satureja montana*, antioxidant activity, TBHQ, carvacrol, oxidants

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Corresponding Authors: Zahra Nemati, Shiraz branch, Islamic Azad University, Shiraz, Fars, Iran
Ebrahim Talebi, Darab branch, Islamic Azad University, Darab, Fars, Iran Tel: +989177312823

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The essential oils are a source of bioactive molecules and have been used widely traditionally. They are widely used to prevent and treat human diseases and also they have a good potential as preservative in food safety[1,2]. *Satureja montana* L. (perennial savory) is an essential oil containing species, belonging to the family Lamiaceae[3]. It is used in Mediterranean cooking, mainly as a seasoning for meats and fish and a flavoring agent for soups, sausages, canned meats and spicy sauces[2]. In the folk medicine uses for diuretic, stomachic, antiseptic and[3] the therapy of bronchitis[3], treating greasy skin or the associated aesthetic defects[4] and cold sensitivity[5]. Furthermore as antitussive, antidiarrheal[3,6], carminative and anthelmintic agent[3]. According to the report, *Satureja montana* have antioxidant[2,7-12], antimicrobial and cytotoxicity activities[4,13,14]. Chemical composition and antioxidant activity of *Satureja montana* essential oil from some area in the world such as Serbia and Montenegro[13,15], Croatia, Bosnia and Herzegovina[9,7,16,17], Bulgaria[18], Mt. Biokovo[8], South France mountain[2], Spain[19], Mediterranean area[20], Albania[21-23], Romanian[10,11] was evaluated. The researches have not been investigated on *Satureja montana* essential oil in Iran, yet. So the interest to research about medicinal plants of Iran, investigated chemical composition and antioxidant activity of *Satureja montana* essential oil that grown in Iran. In present research, chemical composition of *Satureja montana* essential oil that grown in Iran was analyzed using gas chromatography-mass spectrometry (GC/MS) device and also antioxidant activity of *Satureja montana* essential oil was evaluated by DPPH, potassium ferricyanide reaction and CUPRAC (cupric reducing antioxidant capacity) methods. Finally, these results were compared with TBHQ that is a synthetic antioxidant.

MATERIALS AND METHODS

Satureja montana (Fig. 1) was prepared and dried in spring season from Khoramabad, Iran in 2014. GC/MS instrument in model of 7890A, Agilent Technologies and Micro plate reader instrument in model of Beijing Beifen-Ruili, VIS-7220G/UV-9200 were used for analysis. TBHQ, DPPH, methanol, copper (II) chloride were purchased from Sigma-Aldrich company and potassium ferricyanide, trichloroacetic acid, phosphate buffer from Merck company.

Extraction of *Satureja montana*: For extraction of essential oil, 50 g of dried *Satureja montana* was added to 500 mL distilled water after that essential oil was extracted by Clevenger device. Finally, the essential oil was kept in a glass bottle at 18°C until research.

Fig. 1: *Satureja montana* plant

GC/MS system condition: The column was HP-5MS (30×0.25 mm, film thickness 0.25 μm). The column temperature was programmed at 60-210°C with rate of 3°C for 1 min and then temperature changed at 210-240°C with rate of 20°C for 1 min after that for 8 min stay in this temperature. The carrier gas was helium. The injection port and the detector temperature was 280°C (split ratio: 1/100).

Antioxidant activity by DPPH method: First of all, it added 20 μL of samples (essential oil of *Satureja montana* and TBHQ) with 6.25-800 concentrations into cells 1-8 of micro plate rows, then 200 μL of DPPH solution was added to the cells of row. Then 20 μL of samples with 6.25-800 concentrations were added into cells 1-8 of other row of micro plate with 200 μL of methanol as blank. As well as, 20 μL of methanol with 200 μL of DPPH solution was added into last cell of each row as control. This experiment was performed in 3 replicates. After that the micro plate was kept in dark area for 30 min. Finally, micro plate was placed into micro plate reader instrument.

The antioxidant activity of samples was calculated using the Eq[24]:

$$IC_{50} = 100\text{-}\left[\frac{AB_{sample} - AB_{blank}}{AB_{control}}\right]\times 100$$

IC_{50} values denoted the concentration of sample which was required to scavenge 50% of DPPH free radicals[25].

Determination of reducing power using potassium ferricyanide method: For determination of reducing power of *Satureja montana* essential oils by potassium ferricyanide

method, at first, 1 mL of *Satureja montana* essential oils was mixed with 2.5 mL of phosphate buffer (0.2 M, pH = 6.6) and 2.5 mL of 1% potassium ferricyanide in 10 mL test tubes. The mixtures were incubated for 20 min at 50°C. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixtures, followed by centrifuging at 5000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm[26].

Antioxidant activity by CUPRAC method: This method was based on reduction of Cu (II) to Cu (I) by reduction (antioxidants) present in a sample[25]. This method should be advantageous over the ferric reducing antioxidant power method because the redox chemistry of copper (II) as opposed to that of ferric ions involves faster kinetics. The method comprises mixing of the antioxidant solution with a copper (II) chloride solution, a neocuproine alcoholic solution and an ammonium acetate aqueous buffer at pH 7 and subsequent measurement of developed absorbance at 450 nm after 30 min[27]. Finally the cupric ions (Cu^{2+}) reducing capacity of samples was expressed as trolox equivalent (μg mL^{-1}).

Statistical analysis: All calculations were performed using Minitab software (Ver. 14). The paired student's t test was used to compare the results of samples. The values are reported as means ±SEM.

RESULTS

GC/MS analysis: The results of GC/MS *Satureja montana* essential oil was shown that this plant contains twenty compounds. The important compounds were carvacrol (83.4%), thymol methyl ether (1.12%), γ-terpinene (9.62%) and α-terpinen (1.70%) (Table 1). The results showed a high content of the phenolic compound of carvacrol in the essential oil of *Satureja montana* that grown in Iranian samples.

Antioxidant activity analysis: The results of antioxidant activity of *Satureja montana* essential oil and TBHQ by DPPH method exhibited that the amount of IC_{50} for *Satureja montana* essential oil and TBHQ are 32.42±0.459 and 23.83±2.44 μg mL^{-1}, respectively. The amount of IC_{50} for *Satureja montana* essential oil in comparison to TBHQ that is a synthetic antioxidant were very close. The results of antioxidant activates of samples by potassium ferricyanide method revealed that the amounts of *Satureja montana* essential oil and TBHQ are 563.84±23.54 and 610.24±12.67 μg mL^{-1}, respectively. Using of total CUPRAC antioxidant capacity showed that the amount of *Satureja montana* essential oil and TBHQ are 735.14±9.47 and 907.54±16.74 μg mL^{-1}, respectively.

Table 1: Chemical composition of *Satureja montana* essential oil using gas GC/MS

Component	Retention time	Area (%)
α-thujene	5.70	0.35
α-pinene	5.90	0.58
Camphene	6.30	0.43
β-pinene	7.10	0.27
Myrcene	7.40	0.61
Octanol	7.60	0.17
α-phellandrene	7.90	0.38
δ-3-carene	8.20	0.13
α-terpinen	8.30	1.70
Cymene(p)	8.60	0.75
Limonene	8.70	0.23
(Z)-β-ocimene	9.40	0.39
(E)-β-ocimene	9.50	0.08
γ-terpinene	9.80	9.62
Linalool	11.30	0.44
Thymol methyl ether	18.70	1.12
Carvacrol	19.90	83.40
Carvacrol acetate	22.60	0.28
Bisabolene	23.50	0.14
Hexadecane	25.20	0.11

DISCUSSION

Satureja montana contains several compounds in which the main one was carvacrol by 83.4(%). The TBHQ's consequences were revealed that this plant has a good potential against oxidants. Based on the report, carvacrol is the main constituent in the essential oil of *Satureja montana*. In the presence of high amounts of thymol and carvacrol in *Satureja* species, good aromas and simple cultivation, they were used as a flavoring compound in food, pharmaceutical and cosmetic industries[13]. The concentration of carvacrol of *Satureja montana* essential oil that grown in Iran, in contrast with *Satureja montana* that grown in Montenegro (15.19 and 24.46%)[25], Spain (59.72%)[14], Bulgaria[12], Croatia (45.7%)[11] Montenegro (15.47 and 4.40%)[10], South France Mountain (53.35%)[2], Mt. Biokovo (63.4%)[13], Mediterranean area (59.1%)[16], Albania (2.21-55.95 and 21.1-56.8%)[17-19] and Romanian (63.40 and 60.9%)[20,21] was significantly higher. But the carvacrol concentration of Iranian *Satureja montana* was similar to concentration of this essential oil of Croatia, Bosnia and Herzegovina (84.19%)[17]. Experiments revealed that the essential oil content in *Satureja montana* plants varies and depending on the geographic region and environmental conditions[4,10]. Generally, in a phytochemical study of the plants growing in natural habitat, it was difficult to isolate the

effect of individual factors such as temperature, light, water and nutrient supply from the rest of the environment where effect on the essential oil compositions[20].

According to the results, potassium ferricyanide and total CUPRAC methods exhibited that *Satureja montana* essential oil have a good potential for reducing of Fe^{+3} to Fe^{+2} and also Cu (II) to Cu (I) such as TBHQ. The essential oil of *Satureja montana* contains antioxidative compounds, namely carvacrol, thymol, β-caryophyllene, γ-terpinene, p-cymene, together with linalool, which was reported to possess a strong antioxidant activity[16]. Trifan *et al.*[10] suggested phenolic chemo type (carvacrol) that existing in *Satureja montana* essential oils was showed a remarkable antioxidant activity with potential applicability in the protection of susceptible matrices from free radical-mediated oxidative stress, including ionizing radiation-induced oxidative damage. The results of antioxidant activity of *Satureja montana* using three methods and also comparison with TBHQ was revealed this plant have a good potential against oxidants like TBHQ. Meanwhile, carvacrol was the main constituents of *Satureja montana* essential oil. It can be used for a wide range of food and pharmaceutical industries.

CONCLUSION

Twenty compounds found in the essential oil of Iranian *Satureja montana* and the important compound was carvacrol (83.4%). The concentration of carvacrol in *Satureja montana* that grown in Iran was higher than other area in the world. The *Satureja montana* which was collected in Iran could be used for different purposes.

SIGNIFICANCE STATEMENT

This study discovers the chemical composition and antioxidant activity of *Satureja montana* essential oil that grown in Iran which it can be beneficial for a wide range of food and pharmaceutical industries. This study will help the researchers to uncover the critical areas of some illness that be created by free radical. Thus, a new theory on antioxidant activity of *Satureja montana* essential oil that grown in Iran for control of oxidant that may be arrived at.

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