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# ESSENTIAL OIL CONSTITUENTS, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF TWO ENDEMIC *SATUREJA* SPECIES FROM IRAN

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**Abstract**

The volatile constituents of the aerial parts of *Satureja khuzistanica* and *S. bachtiarica* growing wild in Iran, were investigated by GC/MS. Carvacrol (77.21%), γ-terpinene (6.43%), β-bisabolene (2.30%) and *p*-cymene (2.24%) were found to be the major constituents of the oil of *S. khuzistanica* and the major components of *S. bachtiarica* essential oil were carvacrol (65.48%), thymol (15.70%), E-caryophyllene (4.82%), γ-terpinene (4.55%) and linalool (2.74%). The methanolic extract of two *Satureja* species were also subjected to screening for phenolic content and antioxidant activity by using folin-ciocalteau and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. The two *Satureja* species extracts having high phenolic content and antioxidant activity.

## Introduction

Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents, such as phenolic compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity (Velioglu *et al*. 1998, Cai *et al*. 2004). The genus *Satureja* belongs to the Lamiaceae consist of about 235 species of herbs, annual and perennials. This genus is represented in flora of Iran by 17 species, of which 10 are endemic. *Satureja khuzistanica* (Marzeh-e-Khuzestani) and *Satureja bachtiarica* (Marzeh-e-kohi or Marzeh-e- Bakhtiariin Persian) are two endemic *Satureja* species that grow in south-west of Iran (Jamzad 2010).The aerial parts of *S. khuzistanica* and *S. bachtiarica* are commonly used as medicinal and aromatic herb in traditional and folklore medicine as herbal tea, flavoring agents and medicinal purpose such as for treatment of cramps, indigestion, diarrhea and infectious diseases (Zargari 1990). Previous study on essential oil composition on two *Satureja* species showed that these are rich in terpenoids, such as carvacrol, γ-terpinene, thymol, p-cymene, β-caryophyllene, linalool, and otherterpenoids; However, the chemical composition and the amount of components vary among and within plants growth conditions (Moein *et al*. 2012, Ghasemi Pirbalouti *et al*. 2013, Saei-Dehkordi *et al*. 2012, Ghasemi Pirbalouti and Moalem 2013). Native medicinal and aromatic plant populations are frequently suitable as germplasm for domestication programs as genetic reserve and improving plant breeding (Alizadeh 2015, Allard 1999, Ghasemi Pirbalouti and Mohammadi 2013). The aim of this study is comparison of essential oil composition, phenolic content and antioxidant activities of *S. khuzistanica* and *S. bachtiarica* as two endemic medicinal plants growing wild in Iran for domestication programs as genetic reserve and possibility for medicinal use.

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## Materials and Methods

The aerial parts of plant tissues collected from wild population of *Satureja khuzistanica* and *S. bachtiarica* growing in Khuzestan and Fars regions were used in this study. Voucher specimen was deposited at the herbarium of medicinal and aromatic plants of Islamic Azad University, Estahban branch (Voucher no. 113 and 114).The harvested plants were dried at room temperature (25°C) for 2 weeks, then, air-dried plants were grind and powdered by grinder for essential oil extraction and other experiments.

The essential oil was extracted from 30 g of ground tissue in 500 ml of water contained in a 1 liter flask and heated using a heating jacket at 100°C for 3 hrs in a Clevenger-type apparatus.The collected essential oil was dried over anhydrous sodium sulfate and stored at 4°C until analyzed.

The essential oils were analyzed using an Agilent 7890 a gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The chromatographic conditions were as follows; the oven temperature increased from 60 to 240°C at a rate of 3°C/min. Injector and detector temperatures were set at 240 and 290°C, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s, and the samples were injected using split sampling technique by a ratio of 1 : 20. The percentage compositions were obtained from electronic integration of peak areas without the use of correction factors. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200°C. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard (Van Den DoolandKratz 1963). The compounds were identified by comparison of retention indices (RRI- HP-5) with those reported in the literature and by comparison of their mass spectra with the Willey (WILLEY /ChemStation data system) and mass finder 3 libraries or with the published mass spectra (Adams 2001).

Total phenolic content inplant extracts was determined by the Folin-Ciocalteau colorimetric method, as described by the method of Singleton and Rossi (1965). Different concentrations of gallic acid in methanol were tested in parallel to obtain a standard curve. Total phenolic contents were determined as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

Radical scavenging activity of plant extracts against the stable free radical DPPH was measured according to the method employed by Brand-Williams *et al*. (1995). Different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH (100 μM) in 96-well microplates. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Quercetin was used as reference compound. BHT, gallic acid and quercetin were used as positive control. Radical scavenging activity of the plant extracts was calculated according to the equation, 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 plant extract. The $IC_{50}$ values were calculated as the concentration of extracts causing a 50% inhibition of DPPH radical, a lower $IC_{50}$ value corresponds to a higher antioxidant activity of plant extract sample.

## Results and Discussion

The yield and composition of essential oils, isolated by hydro-distillation from the aerial parts of *S. bachtiarica* and *S. khuzistanica* were given in Table 1.The yellow oil of *S. bachtiarica* and *S. khuzistanica* obtained in the yields of 2.34 and 3.25% based on plant dry matter, respectively.

The GC/MS analysis of *Satureja bachtiarica* essential oil showed 48 components representing 98.60% of the total oil; the major constituents were carvacrol (65.48%), thymol (15.70%), E-caryophyllene (4.82%), γ-terpinene (4.55%) and linalool (2.74%). The major constituents of the oil were the phenolic monoterpenes. The essential oil yield and its chemical composition were expected as they are affected by several factors, including genetic (species and subspecies), geographical origins, environmental and climatic conditions, harvest time, plant growth period (ontogenesis) and essential oil extraction and quantification methods (Alizadeh 2015, Alizadeh *et al*. 2013, Loziene and Venskutonis 2005, Sefidkon *et al*. 2006). The essential oil yield and major constituents obtained from other *S. bachtiarica* populations that have been reported by other researchers were 2.7% EO yield and thymol (65.1%), γ-terpinene (15.0%), β-caryophyllene (4.85%), p-cymene (4.4%), linalool (3.5%) and borneol (3.05%) as major components by Moein *et al*. (2012) and 0.90 - 1.8% EO yield and carvacrol (57.4 - 71.4%) and *p*-cymene (8.6 - 12.5%) as major components by Salehi-Arjmand *et al*. (2012) in wild and cultivated *S. bachtiarica*. Ghasemi Pirbalouti *et al*. (2013) reported carvacrol (31.25 - 42.21%), thymol (11.74 - 19.43%), γ-terpinene (10.97 - 18.32%) and p-cymene (8.23 - 14.09%) were the major constituents in *S. bachtiarica* essential oil (1.9 - 2.28% EO yield) obtained with different drying methods. A comparison of our results with the previous reports suggests differences in the essential oil of the plant material could be attributed to the geographic origin of the plants, climate and soil composition and plant harvesting times. Forty two components in the EO of *S. khuzistanica* were identified. The major components were carvacrol (77.21%), γ-terpinene (6.43%), β-bisabolene (2.30%) and *p*-cymene (2.24%). Sefidkon and Ahmadi (2000) reported *p*-cymene (39.6%) and carvacrol (29.6%) were the major components of *S. khuzistanica* essential oil. Our results confirm earlier reports (Farsam *et al*. 2004, Kheirandish *et al*. 2011, Hashemi *et al*. 2012, Saei-Dehkordi *et al*. 2012) that major volatile constituents obtained from the aerial parts of *S. khuzistanica* were carvacrol, thymol, p-cymene and *γ*-terpinene. Carvacrolis a phenolic monoterpeneas major constituents in *S. khuzistanica* essential oil in all researches. In present study, the yellow oil yield of *S. khuzistanica* was 3.25%. The essential oil yield of from other ecotypes of *S. khuzistanica* that have been reported by other researchers were 1.12% (v/w) from Indimeshk (Khuzestan) (Saei-Dehkordi *et al*. 2012), 0.9% (v/w) from Khoarramabad (Lorestan) (Kheirandish *et al.* 2011), 1.1 - 1.4% of different ecotypes from Khuzestan (Ghasemi and Moalem, 2013) and 0.6 and 1.2% (v/w) for wild and cultivated plants, respectively (Farsam *et al*. 2004). In our research, the essential oil yield of *S. khuzistanica* was higher than previous report of this plant. Various factors, including ecotype, harvesting stage, drying, and extraction methods caused on this variation.

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 by inhibiting the initiation or propagation of oxidative chain reactions (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).

The total phenolic content of the methanolic extracts of *S. bachtiarica* and *S. khuzistanica* were measured by the Folin-Ciocalteau reagent and expressed as gallic acid equivalent/g dry weight. According to Table 2, the total phenolic content were 44.45 and 48.44 mg GAE/g DW for *S. bachtiarica* and *S. khuzistanica,* respectively. Previous study in some *Satureja* species indicated the different species have high phenolic content, total phenolic content of methanolic extract of Iranian *S. rechingeri* were 35.5 - 37.5 mg GAE/g DW (Alizadeh 2015). Phenolic content of wild and cultivated *S. bachtiarica* were 24.5 - 16.5 mg caffeic acid/g sample respectively (Salehi-Arjmand *et al*. 2014**)**. Sadeghi *et al*. (2012) reported the phenolic content of *S. sahendica* were 24.78 - 25.56 mg GAE/g DW in different phenological stages. Alizadeh*et al*. (2010) reported total

**Table 1. Chemical composition of the essential oils of two Iranian *Satureja* species.**

| No | Compound | RI[a] | *S. bachtiarica* | *S. khuzistanica* |
|---|---|---|---|---|
| 1 | α-thujene | 925 | 0.11 ± 0.03 | 0.50 ± 0.11 |
| 2 | α-pinene | 932 | 0.06 ± 0.02 | 0.29 ± 0.08 |
| 3 | Camphene | 947 | 0.08 ± 0.03 | 0.04 ± 0.02 |
| 4 | Sabinene | 972 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| 5 | β-pinene | 976 | 0.03 ± 0.01 | 0.13 ± 0.03 |
| 6 | 3-octanone | 984 | 0.05 ± 0.02 | 0 |
| 7 | Myrcene | 990 | 0.16 ± 0.03 | 1.04 ± 0.18 |
| 8 | 3-octanol | 993 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| 9 | n-decane | 999 | 0.16 ± 0.01 | 0.38 ± 0.11 |
| 10 | α-phellandrene | 1005 | 0.04 ± 0.02 | 0.22 ± 0.05 |
| 11 | δ-3-carene | 1010 | 0.008 ± 0.002 | 0.06 ± 0.02 |
| 12 | α-terpinene | 1016 | 0.52 ± 0.08 | 1.47± 0.19 |
| 13 | ρ-cymene | 1026 | 1.39 ± 0.24 | 2.24 ± 0.25 |
| 14 | Limonene | 1028 | 0.04 ± 0.02 | 0.16 ± 0.04 |
| 15 | β-phellandrene | 1029 | 0.04 ± 0.02 | 0.13 ± 0.03 |
| 16 | 1,8-cineole | 1030 | 0.02 ± 0.01 | 0.08 ± 0.02 |
| 17 | (z)-β-ocimene | 1035 | 0.02 ± 0.02 | 0.006 ± 0.002 |
| 18 | (e)-β-ocimene | 1045 | 0.03 ± 0.01 | 0.07 ± 0.01 |
| 19 | γ-terpinene | 1061 | 4.55 ± 0.32 | 6.43 ± 0.78 |
| 20 | *cis*-sabinene hydrate | 1067 | 0.45 ± 0.07 | 0.53 ± 0.07 |
| 21 | Terpinolene | 1087 | 0.06 ± 0.02 | 0.08 ± 0.02 |
| 22 | Linalool | 1103 | 2.74 ± 0.23 | 0.60 ± 0.16 |
| 23 | Borneol | 1166 | 1.25 ± 0.19 | 0.35 ± 0.11 |
| 24 | Terpinene-4-ol | 1176 | 0.44 ± 0.08 | 0.69 ± 0.18 |
| 25 | α-terpineol | 1189 | 0.08 ± 0.02 | 0.07 ± 0.02 |
| 26 | n-dodecane | 1198 | 0.07 ± 0.03 | 0.14 ± 0.05 |
| 27 | Trans-dihidrocarvone | 1205 | 0.03 ± 0.01 | 0.07 ± 0.02 |
| 28 | Nerol | 1230 | 0.02 ± 0.01 | 0 |
| 29 | Carvacrol methyl ether | 1242 | 0.11 ± 0.01 | 1.86±0.33 |
| 30 | Geraniol | 1250 | 0.03 ± 0.01 | 0 |
| 31 | Geranial | 1270 | 0.09 ± 0.04 | 0.05 ± 0.02 |
| 32 | Thymol | 1290 | 15.70 ± 1.12 | 0.42 ± 0.12 |
| 33 | Carvacrol | 1302 | 65.48 ± 2.23 | 77.21 ± 1.65 |
| 34 | δ-elemene | 1334 | 0.03 ± 0.01 | 0 |
| 35 | Eugenol | 1354 | 0.01 ± 0.01 | 0.03 ± 0.01 |
| 36 | Carvacrol acetate | 1370 | 0.04 ± 0.02 | 1.22 ± 0.25 |
| 37 | n-tetradecane | 1396 | 0 | 0.02 ± 0.01 |
| 38 | α- gurjunene | 1406 | 0.05 ± 0.01 | 0 |
| 39 | (E)-caryophyllene | 1421 | 4.82 ± 0.33 | 0.64 ± 0.19 |
| 40 | *Trans*-α- Bergamotene | 1434 | 0 | 0.02 ± 0.01 |
| 41 | Aromadendrene | 1438 | 0.07 ± 0.02 | 0 |
| 42 | α-humulene | 1452 | 0.22 ± 0.05 | 0.06 ± 0.02 |
| 43 | (E)-β-farnesene | 1455 | 0 | 0.01 ± 0.01 |
| 44 | Allo-aromadendrene | 1456 | 0.03 ± 0.01 | 0 |
| 45 | Germacrene D | 1480 | 0.05 ± 0.02 | 0 |
| 46 | Virdiflorene | 1491 | 0.05 ± 0.02 | 0.05 ± 0.02 |
| 47 | (Z,E)-α-farnesene | 1494 | 0 | 0.03 ± 0.01 |
| 48 | Bicyclogermacrene | 1495 | 0.08 ± 0.03 | 0 |
| 49 | β-bisabolene | 1507 | 0.05 ± 0.001 | 2.30 ± 0.25 |
| 50 | δ-cadinene | 1519 | 0.02 ± 0.001 | 0 |
| 51 | (E)-γ-bisabolene | 1539 | 0 | 0.17 ± 0.03 |
| 52 | Spathulenol | 1577 | 0.06 ± 0.01 | 0 |
| 53 | Caryophyllene oxide | 1582 | 0.11 ± 0.03 | 0.02 ± 0.01 |
| | Total | | 99.60 | 99.94 |
| | Essential oil yield (%) | | 2.34±0.03 | 3.25 ± 0.08 |

phenolic content of *S. hortensis* was 23.58 - 24.52 mg GAE/g DW by use different level of fertilizer. In present study, phenolic content of *S. bachtiarica* and *S. khuzistanica*was higher than previous reports of *Satureja* species.

The antioxidant activities of the methanolic extracts of two endemic *Satureja* species were assessed by the DPPH free radical scavenging methods. The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu *et al.* 2004). The $IC_{50}$ values were 27.56 and 30.24 µg/ml in *S. khuzistanica* and *S. bachtiarica,* respectively. BHT, Gallic acid and Quercetin as positive controls were exhibited $IC_{50}$ values equal to 23.38, 25.32 and 35.84 µg/ml, respectively (Table 2). The lower $IC_{50}$ value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher $IC_{50}$ value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. Previous study in some *Satureja* species indicated the different species have high antioxidant activity, the antioxidant activity of methanolic extract of Iranian *S. rechingeri* were 46.2 - 50.21 mg/ml in different phenological stages (Alizadeh 2015). The antioxidant activity of wild and cultivated *S. bachtiarica* was 29.04 - 16.15 mg Trolox/g, respectively (Salehi-Arjmand *et al*. 2014**)**. Ozkan *et al*. (2007) reported the antioxidant activity of *Satureja cilicica* growing in Turkey was 32.02 µg/ml.

**Table 2. Phenolic content and radical scavenging activity of two Iranian *Satureja* species.**

| | Total phenolic content [a] | $IC_{50}$ [b](µg/ml) |
|---|---|---|
| **Species** | | |
| *Satureja bachtiarica* | 44.45 ± 1.25 b | 30.24 ± 1.43 b |
| *S. khuzistanica* | 48.44 ± 1.23a | 27.56 ± 1.37c |
| **Synthetic antioxidant** | | |
| BHT | ND | 23.38 ± 0.53 d |
| Quercetin | ND | 35.84 ± 1.23a |
| Gallic acid | ND | 25.32 ± 1.24cd |

Each value in the table was obtained by calculating the average of three experiments ± standard deviation. Means with different letters were significantly different at the level of $p < 0.05$. [a]: Data expressed as mg of gallic acid equivalents per g dry weight (DW). [b]: $IC_{50:}$ Data expressed as µg per milliliter. Lower $IC_{50}$ values indicated the highest radical scavenging activity. ND = Not determined

As seen in Table 2, the extract of *S. khuzistanica* has higher phenolic content and was most effective in scavenging the DPPH radical, compare to *S. bachtiarica*. Thus, our results show that, a correlation between the antioxidant activities and the total phenolic contents was revealed. These results suggest that the major part of the antioxidant activity in two endemic *Satureja* species results from the phenolic compounds. This result is in line with those reported by Javanmardi *et al*. (2003) in Iranian *Ocimum* accessions, Nencini *et al*. (2007) in *Allium* species. Dorman and Hiltunen (2004) and Alizadeh *et al*. (2010) in *Satureja hortensis,* Alizadeh *et al*. (2011) in *Origanum majorana*, Sadeghi *et al*. (2012) in *Satureja sahendica*, Messaoud *et al*. (2012) in *Lavandula* species, Alizadeh (2013) in *Salvia virgate* and Méndez-Tovar *et al*. (2015) in *Thymus mastichina*, who found similar correlations between total phenolic content and antioxidant activity of various medicinal and aromatic plants. Furthermore, it should be taken into consideration that antioxidant activity might be pertained to the chemical structure of phenolic compounds, as well as synergistic or antagonistic effect of compounds present in the crude extract (Gharibi *et al*. 2013, Messaoud *et al*. 2012).

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