

## Essential Oils Composition, Antioxidant Activities and Phenolics Content of Wild and Cultivated *Satureja bachtiarica* Bunge Plants of Yazd Origin

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### Abstract

**Background:** *Satureja bachtiarica* Bunge, an endemic species with relatively wide distribution, is traditionally used as a medicinal and spice plant in Iran.

**Objective:** Essential oils composition, antioxidant activities and phenolics content of wild and cultivated *S. bachtiarica* of Yazd origin were determined in this study.

**Methods:** Hydrodistilled essential oils were analyzed by GC-FID and GC-MS. Folin-Ciocalteu and AlCl<sub>3</sub> methods were served to determine total phenolics and flavonoids of methanol extracts, respectively. Antioxidant activities of the extracts were examined by DPPH and FRAP methods and their rosmarinic acid content was measured by using HPLC.

**Results:** The oil content of cultivated and wild plants was 1.8% and 0.9% v/w, respectively. Oxygenated monoterpenes (78.3 - 79.5%) constituted the major fraction of essential oils with carvacrol (71.4% and 57.4% in cultivated and wild plants, respectively). *p* - Cymene (8.6 - 12.5%) was another major components of essential oils. Methanol extract from wild growing plants exhibited the higher levels antioxidant activities in both DPPH and FRAP methods (29.04 and 58.25 mg Trolox/g, respectively). This extract also contained the higher amounts of total phenolics (24.5 mg caffeic acid /g sample), total flavonoids (87.99 mg catechin /g sample) and rosmarinic acid (3.73 mg/g dry weight) in comparison to cultivated ones (16.2 mg caffeic acid /g sample, 40.55 mg catechin /g sample and 1.73 mg/g dry weight, respectively).

**Conclusion:** Obtained results may be helpful for domestication of this plant and development of natural antioxidants for use in different industries.

**Keywords:** *Satureja bachtiarica* Bunge, Antioxidant activity, Essential oil, Rosmarinic acid, Total flavonoids, Total phenolics



## Introduction

The genus *Satureja* belongs to the family Lamiaceae and comprises about 200 species of herbs and shrubs which are widely distributed in Mediterranean area, Asia and boreal America [1]. Members of this genus are among the well-known aromatic and medicinal plants. Some species of the genus *Satureja* have economic importance and are used as culinary herbs and flavouring agents in perfumery and cosmetics [2]. Additionally, *Satureja* species have been medicinally important and their essential oil, which contains variable levels of biologically active components such as carvacrol and thymol, are mainly responsible for therapeutic activities these plants [3].

The genus *Satureja* represented in the flora of Iran by at least 14 species, out of which eight are endemic [4, 5]. One endemic species is *S. bachtiarica* Bunge, a plant with relatively wide distribution in the country [6]. In earlier studies, the plant has been the subject of a set of analyses and some of its biological activities including antibacterial, antifungal and antioxidant are documented [3, 6-13]. Although chemical analysis revealed an inter-specific variability in the essential oil composition of this species, thymol and carvacrol have been mostly identified as the major components. In a study by Ahmadi et al. [14], essential oil composition of *S. bachtiarica* before and at the full flowering stages in field and provenance was investigated. These authors identified carvacrol as the principal components of analyzed samples except that extracted from wild growing plants before flowering stage in which *p*-cymene was dominant.

Essential oils are a mixture of different compounds especially terpenoids as the most abundant ones. A wide range of biological

activities of essential oils and their main components have been documented in an extensive body of research which may have the great importance in several fields from food chemistry to pharmacology and pharmaceuticals [15]. About 3000 essential oils are known at present, of them 300 are commercially important and used especially in pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries [16]. In the food industry, for example, they are used as a functional ingredient of foodstuffs to confer desirable aroma and protect them against spoiling, production of toxins and loss of quality which may result from the presence of microorganisms and unfavorable oxidative reactions.

From the chemical point of view, phenols are considered as the compounds with at least one aromatic ring directly attached to one or more hydroxyl groups. These components, which have been reported to have multiple biological effects as antioxidant activity, are commonly occur in both edible and nonedible plants [17]. In recent years, phenolic compounds have gained a growing interest because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foodstuffs and also because they show promise of being powerful antioxidants that can protect the human body against free radicals [17-18]. Rosmarinic acid is considered as a polyphenol compound and an ester of caffeic acid. It occurs mainly in the members of Boraginaceae and Lamiaceae families, although it is identified as a constituent of other 13 plant families. Rosmarinic acid is considered as a defense compound against pathogens and herbivores and has been revealed several biological activities, the most important one a high antioxidant capacity [19].



The biological activities, flavor and other features of crude extracts and essential oils depend on concentration of components present and overall composition. Moreover, production of such components is influenced by various parameters such as genetic factors, geographical origin and climatic conditions. Hence, we decided to investigate phytochemical features and antioxidant activities of essential oils and also methanol extracts from wild growing and cultivated plants of *S. bachtiarica*.

## Materials and Methods

### Antioxidant activity assay

To determine ferric reducing antioxidant power, which work on the base of ability antioxidant components present in the sample to reduce  $\text{Fe}^{3+}$  ions, 180  $\mu\text{L}$  of working reagent (containing 25 mL of 0.3 M acetate buffer pH 3.6 which mixed with 2.5 mL of 10 mM TPTZ in 40 mM HCl and 2.5 mL of freshly prepared 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was mixed with 9  $\mu\text{L}$  of sample or calibration standard and 15  $\mu\text{L}$  of distilled water. After 4 min, the absorbance of tested samples was measured at 595 nm by using mentioned microplate reader. Calibration standards of Trolox from 50 to 2500  $\mu\text{M}$  (corresponding to 0.75-37.6  $\mu\text{g}$  in 60  $\mu\text{L}$ ) in ethanol were used to prepare calibration curve.

### Determination of rosmarinic acid content

Rosmarinic acid content of methanol extracts was measured using a Waters HPLC system consisting of a 626 pump, a 600S controller, a 717 plus autosampler, a column oven operated at 25 °C, and a 996 DAD. The separation was carried out on a Symmetry C18, 5.0  $\mu\text{m}$  particle size, 4.6  $\times$  150 mm column. The mobile phase used was 1% acetic

acid/ acetonitrile 85:15 (solvent A) and methanol (solvent B). The analysis started with a solvent ratio of A/B of 9:1, and a linear gradient was performed to reach 100% B within 30 min. The flow rate was 1.5 mL/min and the injection volume, 20  $\mu\text{L}$ . The quantification of rosmarinic acid was done using the external standard method by preparing six calibration standards ranging from 1 to 500  $\mu\text{g/mL}$  and recording the calibration curve at 330 nm.

### Determination of total phenolics

Total phenolics content of samples in question was determined by using the Folin-Ciocalteu method. To do this, five microliters of sample and 5  $\mu\text{L}$  of Folin-Ciocalteu reagents (Merck) were mixed with 100  $\mu\text{L}$  of distilled water. The solution mixture was left for 3 min, after which 10  $\mu\text{L}$  of saturated  $\text{Na}_2\text{CO}_3$  and 125  $\mu\text{L}$  distilled water were added to it. Samples were left in the dark for a further 1 h and then the absorbance was measured at 730 nm against a blank by using a microplate reader (model BIORAD-iMark). A calibration curve with caffeic acid (0.1 mg/ml) was established, where 0-25  $\mu\text{L}$  of caffeic acid solution were used instead of the sample, and the total phenolic content was expressed in milligrams of caffeic acid per gram of dried plant material.

### Total flavonoid content

To determine total flavonoid content of studies samples, 40  $\mu\text{L}$  of each extract were added to 100  $\mu\text{L}$  of distilled water and mixed by 15  $\mu\text{L}$  of  $\text{NaNO}_2$  (2.5%) and 15  $\mu\text{L}$  of  $\text{AlCl}_3$  (10%). The solution was shaken for 15 min and then mixed by 50  $\mu\text{L}$  NaOH 1M. The solution was shaken again for 5 min and its absorbance against blank was measured at 490 nm using a microplate reader (model



BIORAD-iMark). To construct calibration curve, a range of catechin concentrations (0-20  $\mu$ l) was used instead of sample, and the total flavonoid content was expressed in milligrams of catechin per gram of dried plant material.

### Essential oil analysis procedure

GC-MS analysis of the oil was conducted using an Agilent instrument. The separation was done on a 30 m  $\times$  0.25 mm column coated with 0.25  $\mu$ m HP5-MS. The analytical conditions were as follows: carrier gas, He, 1.3 mL/min in the constant flow mode; injector temperature, 250  $^{\circ}$ C; injection volume, 1  $\mu$ L; split ratio, 15:1; temperature program, 2 min at 40  $^{\circ}$ C, raised at 3  $^{\circ}$ C/min to 180  $^{\circ}$ C, raised at 10  $^{\circ}$ C/min to 280  $^{\circ}$ C; transfer line to MSD, 280  $^{\circ}$ C; MSD, 170  $^{\circ}$ C. The ionization energy was 70 eV. The range  $m/z$  40-300 was scanned at a rate of 0.52 scans/s. The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for  $n$ -alkanes (C<sub>9</sub>-C<sub>30</sub>) and the oil under the same conditions. Identification of individual compounds was made by comparison of their mass spectra and their retention indices [21].

## Results

In this study, wild plants contained 0.9% v/w essential oil which is considerable lower than that of cultivated ones (1.8% v/w) (Table 1).

GC-MS analyses enabled us to characterize in total 24 volatile components in studied plants. Identified components along with their concentrations are listed in Table 2 according to their elution order on the HP column. Two samples tested were found to contain monoterpenes as the principal fraction, especially oxygenated ones (78.3 - 79.5%). In contrast, our samples were characterized by the trace amounts of sesquiterpenes. Components identified in the studied essential oils were differed considerably both qualitatively and quantitatively. Carvacrol (57.4 - 71.4%),  $p$ -cymene (8.6 - 12.5%) and thymol (2.6-8.4%), were identified as the most abundant components. Essential oils extracted from cultivated plants (74.0%) contained the higher amounts of phenolic compounds than wild growing ones (65.8%). Among other differences, the linalool content of wild growing plants was determined to be 5.4% in comparison to its level in the cultivated ones (2.7%).

**Table1- Volatile constituents of wild and cultivated *Satureja bachtiarica* plants of Yazd origin**

	Component	RI	Wild plants	Cultivated plants
1	$\alpha$ -Tujene	928	tr	0.35
2	$\alpha$ -pinene	936	0.98	0.79
3	Camphene	952	1.15	0.43
4	$\beta$ -Pinene (beta)	979	0.13	0.13
5	Myrcene	990	0.32	0.62
6	$\alpha$ -Phellandrene	1004	tr	tr
7	$\delta$ -3-Carene	1011	0.46	tr
8	$\alpha$ -Terpinene	1018	0.00	0.65
9	$p$ -Cymene	1028	12.51	8.62
10	$\gamma$ -Terpinene	1062	2.58	4.56

**Table1- continued**

Component	RI	Wild plants	Cultivated plants
11 <i>Cis</i> -Sabinene hydrate	1069	0.20	0.71
12 $\alpha$ -Terpinolene	1089	0.12	0.00
13 linalool	1098	5.35	2.65
14 Camphor	1150	0.10	0.00
15 Borneol	1170	5.51	1.76
16 4-Terpineol	1180	0.46	0.36
17 Thymol	1294	8.41	2.61
18 Carvacrol	1302	57.38	71.43
19 Carvacrol acetate	1374	0.93	0.00
20 $\beta$ -Caryophyllene E	1426	1.20	2.04
21 (+)-Aromadendrene	1446	0.12	tr
22 $\alpha$ -Humulene	1460	tr	0.09
23 Spathulenol	1585	0.36	0.19
24 (-)-Caryophyllene oxide	1591	1.68	0.84
Monoterpene hydrocarbons		18.26	16.17
Oxygenated monoterpenes		78.33	79.52
Sesquiterpene hydrocarbons		1.32	2.13
Oxygenated sesquiterpenes		2.04	1.03
Total		99.95	98.85
Essential oil content (v/w)		0.9%	1.8%

**Table 2- Total phenolics, total flavonoids and rosmarinic acid content and antioxidant activities of methanol extracts from studied plants**

Measured character	Wild plants	Cultivated plants
DPPH method (mg Trolox/g)	29.04	16.15
FRAP method (mg Trolox/g)	58.25	26.73
Total phenolics (mg Caffeic acid /g sample)	24.50	16.22
Total flavonoids (mg Catechin /g sample)	87.99	40.55
Rosmarinic Acid (mg/g dry weight)	3.73	1.73

In addition to essential oil, phytochemical features of methanol extracts of wild and cultivated plants were also considered in this study by measuring their total phenolics, total flavonoids and rosmarinic acid content. In all cases, wild growing plants were superior to cultivated ones. Total phenolics present in the methanol extract of wild growing plants were determined to be equivalent to 24.5 mg caffeic

acid /g sample. These plants contained 87.99 mg catechin /g sample in contrast to cultivated ones which contained 40.55 mg catechin /g sample. Similarly, rosmarinic acid content of wild and cultivated plants was 3.73 and 1.73 mg/g dry weight, respectively.

The antioxidant activity of methanol extracts is presented in Table 2 and expressed as milligrams of Trolox per gram of plant dry

matter. Studied samples exhibited various degrees of antioxidant activity in both tested systems. In DPPH method, the highest free radical scavenging activity was observed in the extract from wild growing plants (29.04 mg Trolox/g). However, cultivated plants of this population exhibited considerable a lower level of antioxidant activity in this system (16.15 mg Trolox/g). In the case of FRAP method, similarly, the highest activity was recorded in wild plants (58.25 mg Trolox/g) (Table 2).

## Discussion

It is well known that the chemical constituents of medicinal and aromatic plants, also their biological activities are influenced by genetic and environmental factors [6]. It has been reported that different *Satureja* species represent great variability in the phytochemical constituents and agromorphological traits [3, 6]. Several previous studies considered constituents of *S. bachtiarica* essential oils. In a sample collected from Ardabil province, thymol (20.6%), carvacrol (26.4%) and linalool (14.19%) were reported as the principal constituents [13]. In another study, Ahmadi et al. [14], analyzed essential oils of *S. bachtiarica* plants before and at the full flowering stages in field and provenance and reported carvacrol as the main constituent of studied samples (25.8-62.3%) except that from wild growing plants before flowering stage in which *p*-cymene (36.5%) was dominant. In a study by Hadian et al. [3], thymol (28.0%), caryophyllene oxide (17.0%) and carvacrol (13.2%) were identified as the major components of *S. bachtiarica* plants collected from Isfahan province. Also, Sefikon and Jamzad [22] reported thymol (44.5%) and  $\gamma$ -

terpinene (23.9%) as the major constituents of *S. bachtiarica* plants of Chahar Mahal-e-Bachtiari province origin. With collection of plant materials at the two different developmental stages (before and at the full flowering stage) from Shahre-Kurd province, Sefidkon et al. [6] identified *p*-cymene (36.5%), carvacrol (19.9%) and thymol (19.9%) as the main components of essential oil before flowering of *S. bachtiarica* plant, whereas at the full flowering stage *p*-cymene (25.8%) and carvacrol (25.2%) were dominant. Generally, variability in the essential oil composition of this plant may be related to parameters such as genetic factors, geographical origin, developmental stage, climatic conditions, etc.

In this study, the potential antioxidant activity of tested samples were documented using to different systems including DPPH radical scavenging activity and ferric reducing power (FRAP). Although the DPPH method usually is on the base of hydrogen atom transfer reaction, another mechanism including electron transfer has also been suggested [23]. In the second method, however, changes which may occur in the absorbance of solution as the result of formation of a blue colored  $\text{Fe}^{2+}$ -tripyridyltriazine compound from colorless oxidized  $\text{Fe}^{3+}$  form by the action of electron donating antioxidant compounds are measured [24]. In a previous study, antioxidant activity of *S. bachtiarica* essential oils has been reported [11]. Differences observed in the chemical profile and antioxidant activity of wild and cultivated plants, considering their similar origin, can be attributed, at least to the some extent, to geographical factors and climatic conditions.

Antioxidants are practical ingredients of foodstuffs and are widely used in food industry to protect them against deterioration



and lose of quality. In fact, oxidation of lipids is considered as one of the basic processes causing rancidity of food products, leading to their deterioration [24]. Due to their high antioxidant capacity, food industry at present widely uses synthetic preservers such as BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) to prevent oxidative rancidity in foodstuffs [24, 26]. However, there is a growing concern about potential side effects of synthetic preservers including carcinogenesis [25-27]. Therefore, there is at present a growing trend toward natural antioxidants to supplement or substitute synthetic counterparts, which are used in food and other related industries.

## Conclusion

In this study, essential oils and methanol extracts from wild growing and cultivated plants of *Satureja bachtiarica* Bunge of Yazd origin were investigated. There were significant differences among composition and

antioxidant activities of studied population and wild plants exhibited superior levels of antioxidant activity to cultivated ones. Antioxidant and antimicrobial activities of essential oil of *S. bachtiarica* have previously been reported [6-13]. The methanol extract of these plants also included the higher levels of total phenolics, total flavonoids and rosmarinic acid. Therefore, obtained results may be helpful for domestication of this plant, which is characterized by its phenolic essential oil, and development of natural antioxidants for use in food and other related industries. However, considering importance of phenolic compounds including flavonoids and rosmarinic acid in different industries, more studies should be conducted to explore agricultural practices favoring biosynthesis and accumulation of these components. They have exhibited, among others, health promoting effects and considerable antioxidant property which may be of importance in preservation of different products and the human health.

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