

Antioxidant Activities of *Satureja hortensis* L. Essential Oil during the Flowering Period

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ABSTRACT

Objective: This study was conducted to investigate the changes of total phenolic concentration and antioxidant activity of *Satureja hortensis* L. during the flowering stage cultivated under the ecological conditions of Rize, Turkey. **Material and Methods:** The plantation was established with natural cuttings of *Satureja hortensis* L. Cuttings were obtained at three stages (pre-flowering, full flowering, and post-flowering) and dried in an oven at 35°C. The analysis of essential oil in the leaves and flowers of the samples was carried out using a Clevenger apparatus for three hours. The total phenolics and antioxidant activity of essential oils was evaluated by Folin-Ciocalteu and DPPH free radical scavenging assays, respectively. **Results:** The total phenolic content varied between 746 ± 4 and $1087 \pm 44 \mu\text{M}$, and the highest value was obtained from the full-flowering stage. The SC_{50} values in the DPPH assay ranged from 6.65 ± 0.38 to $16.10 \pm 0.99 \mu\text{M}$ catechin equivalent and the highest activity was observed in the full flowering stage. Among all the samples, the essential oil of the cutting taken during the full flowering period showed the highest phenolic concentration and antioxidant activity. **Conclusion:** *Satureja hortensis* L. is a good natural antioxidant source with its high phenolic concentration and antioxidant activity.

Keywords: *Satureja hortensis* L, antioxidant activity, essential oil, polyphenol content, harvest times

INTRODUCTION

Phenolic compounds exhibit a wide range of physiological activities, such as antioxidant, anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, anti-thrombotic, cardioprotective, vasodilatory effects, and anti-carcinogenic.¹⁻³ Natural antioxidants can protect the human body from free radicals and are reported to slow the progress of many chronic diseases as well as lipid oxidative rancidity in foods.⁴ *Satureja hortensis* L. (*S. hortensis*) belongs to the family Lamiaceae. In Turkey, it is known as “yaz zahteri, çorba kekiği” and used as a culinary herb and for medicinal purposes. *S. hortensis* grows on serpentine hills, spread on dry, rocky and sunny hillsides, from the lowland to the mountains.⁵ The essential oil of *S. hortensis* cultivated in summer has been found to be rich in γ -terpinene and carvacrol⁶ and have a

variety of antibacterial, antifungal, antioxidant, antispasmodic, antidiarrheal and sedative properties.⁷⁻⁹ Many studies have reported that the essential oil of *S. hortensis* and their main phenolic constituents such as carvacrol and γ -terpinene show remarkable antifungal, antibacterial and antimicrobial activities.^{10,11} In Turkey, dried herbs of savory are used as herbal tea and condiment in addition to being utilized in folk medicine to treat asthma, colic, bronchitis, and coughs and in food industry as flavoring, aromatic and preservative agents. The aim of this study was to evaluate the influences of total phenolic concentration and antioxidant activity of *S. hortensis* essential oil collected from savory at three different stages in an experimental field.

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MATERIALS AND METHODS

Plant material and growth conditions

The seedling used in the study was collected from the Şavşat district of Artvin province, Turkey and cultivated in May 2010 under the ecological conditions of Rize (41°-20' N, 41°-28' E) at a research and application area belonging to Pazar Vocational School of Recep Tayyip Erdoğan University. The soil of the field area was of a sandy clay loam texture with medium acidity (pH = 5.2), low lime content (2.02%), medium nitrogen (0.14%), salt (0.56%) and phosphorus (1.86 ppm), little organic material (1.25%) and high level of potassium (250 ppm). During the vegetation period (May–August), the experimental area had an average temperature of 19.5°C and humidity of 78.8%, and the total precipitation 651.2 mm. A total of 40 plants were cultivated in each plot at a planting distance of 45 × 15 cm. After planting of the seedlings, 12 kg/da calcium ammonium nitrate (26% N) was applied as upper fertilizer. The study was carried out in three different harvest times as follows: pre-flowering (June 10, 2010), full flowering (July 3, 2010) and post-flowering (July 30, 2010).

Essential oil extraction

Plant materials were dried in an oven at 35°C and the leaves were separated from the other plant parts. Essential oils from each harvest of dried leaves (20 g) were isolated by a Clevenger-type apparatus for 3 h.

Determination of total phenolic content and antioxidant activity

The phenolic content in the essential oils of *S. hortensis* was spectrophotometrically determined at 700 nm by Folin-Ciocalteu's colorimetric method and expressed as catechin (mM) equivalents.¹² The antioxidant activity of the essential oils was measured by utilizing the diphenyl picrylhydrazil (DPPH) method and expressed as SC₅₀ (mg/mL); i.e., the concentration necessary for 50 % reduction of the DPPH.¹³

Statistical analysis

The data were subjected to an analysis of variance (ANOVA) using Statistical Analysis System (SAS) computer software at P < 0.05 and the means were compared with Duncan's new multiple range test (DNMRT).

RESULTS AND DISCUSSION

In this study, the total phenolic content of the essential oils ranged from 746 ± 4 to 1087 ± 44 µM. The antioxidant activities of these essential oils during the pre-, full and post-flowering stages were recorded as 6.65 ± 0.38, 16.10 ± 0.99 and 6.87 ± 0.27 µM catechin equivalent, respectively (Table 1). The highest phenolic concentration and antiradical activity were obtained from the essential oil extracted from the plant during the full flowering period (Figure 1, Figure 2).

In a similar study, Alizadeh *et al.*¹⁴ investigated the antioxidant activity and total phenolic content of *S. hortensis* extracts using a DPPH free radical scavenging assay and the Folin-Ciocalteu method. The authors reported that the total phenolic content varied between 23.58 and 24.52 (mg gallic acid equivalent/g dw) and the IC₅₀ values ranged from 8.45 to 8.6 µg/ml. Furthermore, the highest values for both phenolic content and IC₅₀ were obtained from the 1000 mg/plant complete fertilizer treatment. Dorman and Hiltunen¹⁵ used the same method and estimated the total extractable phenolic contents of the crude extract of savory and hexane, ethyl acetate, n-butanol and water fractions as 166 ± 2.7, 37.1 ± 1.1, 500 ± 47.3, 27.0 ± 9.1, and 67.2 ± 25.2 mg, respectively. The researchers also evaluated the free radical scavenging activity and the components within both the n-BuOH and H₂O fractions, and found the order of DPPH with IC₅₀ as 3.43 ± 0.082 and 2.16 ± 0.040 mg/ml, respectively. The antioxidant properties of thymol, carvacrol, and γ-terpinene have also been previously reported.¹⁶⁻¹⁸ Therefore, it is considered that the activity of essential oil is related to high contents

Table 1: Total phenolic substances (TPS) amount and DPPH free radical-scavenging values of *Satureja hortensis* L. essential oils during flowering period

Samples	Total Polyphenol (µM, n:6) [§]	DPPH Activity (SC ₅₀ , µM, n:6) [‡]
Standart (Catechin)	-	9.0 ± 1,0
Pre-flowering period	746 ± 4a	6.65 ± 10,38
Full-flowering period	1087 ± 44b	16.10 ± 0,99
Post-flowering period	1022 ± 24b	6.87 ± 0,27

§: Molar concentrations of total polyphenols in catechin molecular weight equivalent.

‡: Total phenolic concentration necessary for 50 % reduction DPPH.

a: Indicates a significant difference at P < 0.005

b: In the same column with different superscripts differ significantly at P < 0.005

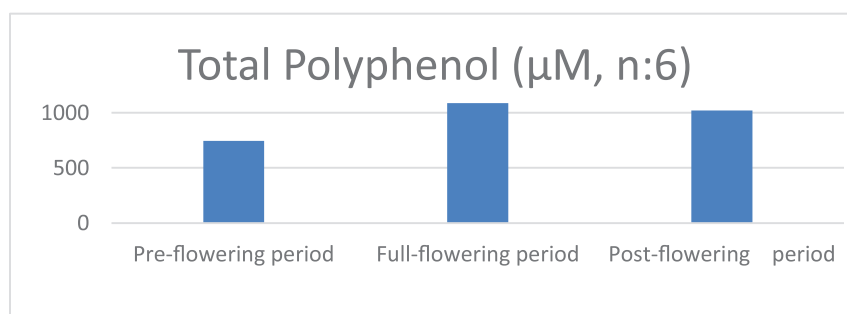


Figure 1: Total phenolics of *Satureja hortensis* L. essential oils during flowering period

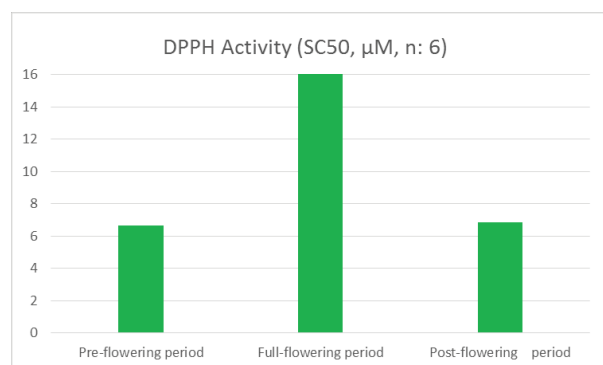


Figure 2: DPPH scavenging activity of *Satureja hortensis* L. essential oils during flowering period

of these components in savory oil. The presence of phenolic acids such as rosmarinic acid derivatives in *S. hortensis* has been demonstrated,¹⁹ and these polyphenols has been shown to possess an antioxidant activity in different test systems.²⁰ Thus, the activity observed in the DPPH assay can be related to the presence of rosmarinic acid derivatives in the extract.

The differences between the results of the current study and those of earlier studies may be due to the use of different extracts for analyses, environmental and genetic factors, chemo-types and the nutritional status of the plants as well as other factors that may have an influence on the antioxidant activity and total phenolic content. Several studies have suggested a relationship between antioxidant activity and phenolic contents of plants.^{15, 21-23} Similarly, in the current study, we found a positive correlation between the total phenolic content and antioxidant activity in all plant extracts.

CONCLUSION

Based on the results of this study, we determined that the antioxidant activity and phenolic content were significantly affected by harvesting times. The essential oils extracted from *S. hortensis* during the full flowering period showed the highest phenolic concentration and antioxidant activity. It is concluded that *Satureja hortensis*

L. is a good natural antioxidant source with its high phenolic content and antioxidant activity.

ACKNOWLEDGEMENT

None

CONFLICT OF INTEREST

None

ABBREVIATIONS USED

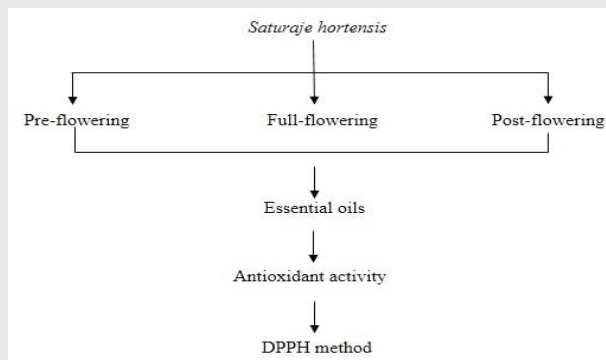
DPPH: 2,2-diphenylpicrylhydrazyl; DNMRT: Duncan's new multiple range test; ANOVA: Analysis of variance; SAS: Statistical Analysis System ;SC50: scavenging concentration 50%.

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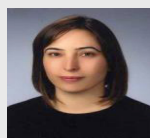
PICTORIAL ABSTRACT



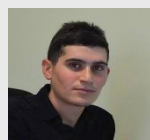
SUMMARY

- The antioxidant activity and phenolic content were significantly affected by harvesting times
- All essential oils have shown high and different DPPH scavenging activity
- The highest phenolic concentration and antioxidant activity were obtained from the essential oil extracted from the plant during the full flowering period
- There was a positive correlation between the total phenolic content and antioxidant activity in all essential oils

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