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Araştırma Makalesi

Satureja thymbra'nın Farklı Kısımlarının Toplam Fenol, Toplam Flavonoid ve IC50 İçerikleri ve Besin Elementleri Arasındaki İlişki

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ÖZ

Bu çalışmanın amacı, Antalya'nın Kaş ilçesinde doğal olarak yetişen *Satureja thymbra* bitkisinin çiçek, yapraklar ve saplar gibi farklı organlarının içermiş olduğu toplam antioksidan aktivitesi toplam fenol ve toplam flavonoid içerikleri gibi bazı kalite parametrelerindeki değişimin araştırılmasıdır. Aynı zamanda bu kalite parametrelerinin bitkinin besin elementi içerikleri ile arasındaki korelasyon incelenmiştir. Serbest radikal olan (DPPH) ile bitkinin farklı kısımlarından hazırlanan ektraktların antiradikal süpürücü aktiviteleri belirlenmiştir. DPPH serbest radikal aktivitesinde %50 kayba neden olabilen antioksidan bileşiğin konsantrasyonu IC_{50} değeri ile ifade edilmiştir. *S. thymbra*'nın yaprak ekstraktlarında antiradikal süpürücü aktivite (IC_{50} = 0.11 mg dw/mg DPPH) toplam fenol (38.79 mg GAE/g) ve toplam flavonoid (29.59 mg/g) içeriği bitkinin diğer kısımlardan oluşan ekstraktlara göre daha yüksek olduğu ortaya çıkmıştır.

Anahtar kelimeler: *Satureja thymbra,* fenol, flavonoid, antioksidan aktivitesi, besin elementleri

The Relationship Between Total Phenol, Total Flavonoid, and IC50 Contents of Different Parts of *Satureja thymbra* and Nutrient Elements

ABSTRACT

The goal of this study is to investigate the variations in certain quality parameters, including total antioxidant activity, total phenol and total flavonoid contents, of various organs, including inflorescences, leaves and stems, of the *Satureja thymbra* plant, which grows naturally in the Kaş district of Antalya. Also, the relationship between these quality parameters and the nutrient content of the plant was revealed. The free radical (DPPH) was used to determine the antiradical scavenging activities of extracts prepared from various parts of the plant. The concentration of the antioxidant compound that can cause a 50 % loss in DPPH free radical activity is expressed by the IC50 value. *S. thymbra* leaf extracts have higher antiradical scavenging activity (IC50 = 0.11 mg dw/mg^{-}DPPH), total phenol (38.79 mg GAE/g), and total flavonoid (29.0 mg/g) content than extracts of other plant components.

Key words: *Satureja thymbra,* phenol, flavonoid, antioxidant activity, nutrient elements.

INTRODUCTION

There are over two hundred species in the genus Satureja, which is widespread in the Mediterranean region and is a plant that belongs to the family *Lamiaceae* (Abdelshafeek et al., 2023). Turkey is home to 16 different species that belong to this genus, 5 of which are endemic (Selvi et al., 2022). *Satureja thymbra* L. grows in rocky terrain in the eastern Mediterranean Basin (between Italy and Jordan) and has many pink or red-purple flowers.

In addition to that, it is an aromatic plant that has hairs that are a grayish color, is woody, and grows in bush form (Baytop, 1999; Pinna et al., 2021). In Turkey, the *S. thymbra* plant, which is native to the coastal

regions that are controlled by the Mediterranean climate zone, is popularly known as thyme, pointed thyme, black thyme, and cheese thyme. Local communities collect it for their own usage as well as for commercial purposes, to be used as a spice, tea, and essential oil (Gürdal and Kültür, 2013).

In prior research on the *S. thymbra* plant, it was proven that the plant is rich in phenolic acids and flavonoid compounds and possesses potent antioxidant activity (Öztürk, 2012; Choulitoudi et al., 2021; Caliskan, 2023). The presence of these compounds provides *S. thymbra* L. with antibacterial, antifungal, analgesic, anti-inflammatory, antinociceptive, and antimicrobial effects (Momtaz and Abdollahi 2010; Giweli et al., 2012; Tepe and Çilkiz 2016; Caliskan, 2023).

Some quality factors, such as antioxidants, phenols, flavonoids, and essential oil components, have an effect on the commercial values of medicinal and aromatic plants, which have multiple applications in food, medicine, and cosmetics. It is important to know what factors affect the chemical variation and production of secondary metabolites like the essential oil, phenol, and flavonoid components of *S. thymbra*, which is sold in Turkey and is a good source of antioxidants in the form of phenolic substances.

The process of synthesis of secondary metabolites varies depending on the genotype of the plant as well as other environmental conditions. Depending on their function and stage of development in the life cycle, the accumulation and concentration of polyphenolic compounds with strong antioxidant activity change greatly in different parts and organs of plants (Wegiera et al., 2011; Chang et al., 2019). There hasn't been any published research on the polyphenolic components of the *S. thymbra* plant's various parts or the connection between the nutrients found in the leaves.

The aim of this study is to reveal how different parts of the *Satureja thymbra* plant, such as the inflorescence, leaf, and stem, change in terms of quality parameters like total antioxidant activity, total phenol, and total flavonoid content. At the same time, to determine whether or not there is a relationship between the total antioxidant activity, total phenol, and total flavonoid levels of the plant and the nutrient content of the plant.

MATERIAL and METHODS

Plant material

Plant samples of *Satureja thymbra* L. were collected from the Kaş district of Antalya in June 2012, during the blossoming season, at a height of 0–600 m (Figure 1). Prof. Dr. Gokhan Deniz, an academic from the Faculty of Education at Akdeniz University, identified the plant material on a taxonomic level. All analyses were performed on plant samples obtained from 15 different locations in the Kaş district.

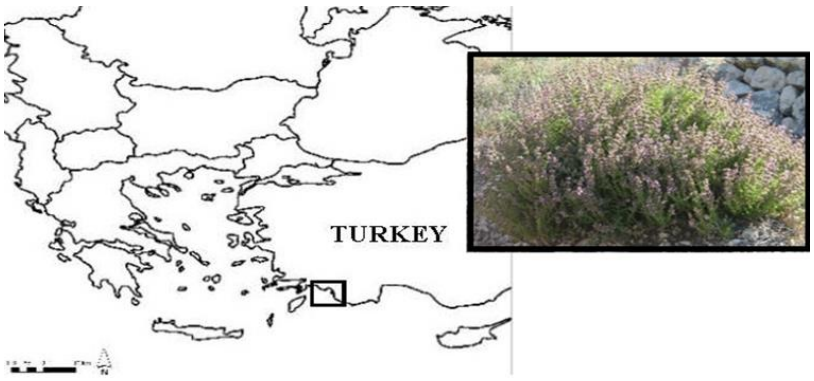

Figure 1. *Satureja thymbra* L. plant growing in its native habitat in Kaş

The processing of plant extracts

The plant samples, which were dried and crushed at room temperature, were weighed with a precision of 0.5± 0.001g and placed in falcon tubes. 9.5 ml of 80% (80:20, v/v) methanol was added to these samples, the tubes were sealed, and they were extracted at 150 rpm for one hour in an orbital shaker. The sample tubes were then centrifuged for 10 minutes at 7.000 rpm. With the aid of white-banded filter paper, the liquid portion at the top of the tube was filtered into a 50-ml flask. This process was repeated three times, and the extraction was ready for analysis after the extracts were gathered in a 50-ml (Sf:100) measuring balloon and filled to the volume of the balloon (Cemeroğlu, 2010). These extracts were used to determine the IC50 value and the total amount of phenolic and flavonoid substances.

Quantification of total phenolic content

The spectrophotometric method was used to figure out the total amount of phenolics. After adding 900 µl of distilled water to the test tube containing 100 µl of sample, 5 ml of 0.2 N folin-ciocalteau solution and 4 ml of sodium carbonate (7.5%) solution were added, respectively. After a two-hour incubation period at room temperature and in the dark, absorbance measurements were made against the blank at a wavelength of 765 nm using a spectrophotometer (Shimadzu UV-vis 160A). The total amount of phenolic compounds in the sample was given in milligrams of gallic acid per gram of dry weight (Spanos and Wrolstad, 1990).

Quantification of total flavonoid content

After adding 0.5 ml of sample, 2.5 ml of distilled water, and 150 µl of a 5% $NaNO_2$ solution to the test tube, the contents were stirred for 30 seconds in a vortex. Every five minutes, a solution was added to the final solution. 300 µl of a 10% $AlCl_3$ solution, 1 ml of a 1M NaOH solution, and 550 µl of distilled water, respectively, are the additional solutions. The spectrophotometer reading for the absorption value of the solution was determined at 510 nm. The obtained absorbance values were converted to mg (+)-catechin equivalent/g dry sample weight using a curve generated using (+)-catechin (Chang et al., 2006).

Quantifying antioxidant activity

To measure antioxidants, a method based on DPPH (1,1-diphenyl-2-picryl hydrazil) radical inhibition was used (Cemeroğlu, 2010). Increasing volumes (0, 20, 40, 60, 80, and 100 µl) were taken from each sample extract prepared in a tube; 600 µl of DPPH radical solution was added; and the total volume was completed with 6 ml of methanol. After 30 minutes in a dark atmosphere at the temperature of the room, the absorption value of the solutions was determined in a spectrophotometer at a wavelength of 517 nm. The blank sample's absorbance, which was prepared using 600 l of DPPH radical solution and 5.4 ml of methanol, was also read in the spectrophotometer at a wavelength of 517 nm. The percentage of DPPH scavenged (%DPPHsc) was computed as follows:

%DPPHsc = (Acont -Asamp/ Acont) * 100

Acont: the control's absorbance

Asamp: the sample's absorbance

The calculated IC50 values represent the sample concentration required to reduce the absorbance at 517 nm by 50%.

Plant analysis procedures

Nature-collected *Satureja thymbra* plant samples were rinsed with distilled water and dried at 65 ^{o}C in drying cabinets, after which the inflorescences, leaves, and stems of the plant were all mixed and ground and prepared for analysis (Kacar and İnal, 2008).

Nitrogen: The modified Kjeldahl method was used for nitrogen determination in dried and ground plant samples. The data was reported in milligrams per kilogram of dry materials. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Ca), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu): nutrient amounts in the extract obtained by the wet burning method were detected using the ICP-OES Perkin Elmer 7000 DV device. Results for the amount of nutrition in the dry matter were represented as mg/kg (Kacar and İnal, 2008).

Statistic evaluation

The Tukey test was used to compare the amounts of total phenol, total flavonoid, and IC50 in the plant's inflorescences, leaves, and stems. The correlation between total flavonoids, total phenolics, IC50 values, and some nutrient contents found in Inflorescences, leaves, and stems of the plant were calculated. In all data, the licensed SPSS 23 statistical application was used.

RESULTS and DISCUSSION

Total flavonoid, total phenolic contents, and antioxidant activity

It is generally known that medicinal and aromatic plants, such as *Satureja thymbra*, have a considerable amount of phenolic compounds; as a result, they also have high antioxidant properties. In terms of quality parameters such as antioxidant activity and total phenolic and flavonoid material content, it was determined that there were statistically significant variations between various organs of the *S. thymbra* plant ($p<0.01$).

It was revealed that the antioxidant impact of the plant's leaves was stronger (IC50 value: 0.11 mg dw/mg-DPPH). The inflorescence extracts showed only a moderate level of scavenging activity, while the leaf extracts showed a very high level of scavenging ability against the DPPH radical (IC50: 0.15 mg dw/mg-DPPH). However, the stem extracts of the plant were found to have the least DPPH scavenging activity and lowest capacity (IC50: 0.25 mg dw/mg-DPPH) (Table 1).

Table 1. Total flavonoids, total phenolics, and IC50 values of the plant's inflorescences, leaves, and stems

	EC50 [a]	Phenol [b]	Flavonoid [c]
Inflorescences	0.15±0.03 b	27.86±7.88b	20.86±14.24b
Leaves	0.11±0.04c	38.79±10.92a	29.59±6.57a
Stems	0. 25±0.07a	18.32±5.61c	7.96±2.09c
Significance	p<0.01	p<0.01	p<0.01

[a] The EC50 value is computed as mg dry weight of the plant, and the findings are expressed as mean ± standard deviation.
[b] : The amount of total phenols is measured in mg of gallic acid equivalents per gram of dry weight (dw), and the results are shown as the mean ± standard deviation.
[C] : Total flavonoids are calculated as mg of catechin equivalents per gram of dry weight, and results are presented as mean ± standard deviation (dw).

The total phenolic content of S.thymbra samples was determined by applying the folin-ciocalteu reagent to methanolic plant extracts. The results of the total phenol contents in different parts of the plant were presented in Table 1 as mg gallic acid equivalent per g dry weight (dw). Total phenol contents showed statistically ($p<0.01$) significant variation among different parts of *S. thymbra.* The total phenolic content of *S. thymbra* leaf extracts were the highest (38.79 mg GAE/g), followed by *S. thymbra* inflorescence extracts (27.86 mg GAE/g). When compared to other parts of the plant, stem extracts had the least amount of total phenol (18.32 mg GAE/g). Özkan (2007) repoted that the total phenolic content in extracts of a mixture of flowers and leaves of the *S. thymbra* plant ranged between 82.97 and 115.09 mg GAE per gram in her investigation utilizing several extraction techniques. In comparison to the plant extracts obtained in our investigation, the total phenolic content was higher in Özkan's (2007) study.

The total flavonoid concentration varied from 5.03 to 51.15 mg of catechin equivalents per gram of plant. The total flavonoid contents of *S. thymbra* varied among inflorescences, leaves, and stems, which was statistically significant ($p<0.01$). The leaves had the highest concentration (29.59 mg/g), whereas the stems had the lowest (7.96 mg/g). The total flavonoid content of a dried *S. thymbra* sample was calculated to be 3.26 mg/g by Skolua et al., (2005). Compared to the study by Skolua et al., (2005), we found that the total flavonoid content in our study was higher.

It has been determined that the findings of polyphenol contents such as phenol, flavonoid, and antioxidant obtained from the study differ from the findings in similar studies. The reason for this is thought to be caused by a number of things, such as the parts of the plant that were examined in the study, the time of harvest, the way the extract was made, as well as genetic and environmental variables.

The correlation between the nutrient content of *S. thymbra* leaves and their total flavonoid, total phenolic, and antioxidant activities.

The leaves had an average amount of 12436 mg/kg of nitrogen, 1155 mg/kg of phosphorus, 15459 mg/kg of potassium, 14281 mg/kg of calcium, and 1884 mg/kg of magnesium. When the microelement contents were examined, the average zinc, copper, iron, and manganese contents of the leaves were determined to be 27.93 mg/kg, 9.84 mg/kg, 187.26 mg/kg, and 19.26 mg/kg, respectively. Some of what we found about the nutritional values of *S. thymbra* was different from what other researchers had found (Özcan, 2004; Arslan and Özcan, 2012). These differences are considered to be mostly caused by environmental influences, genetic factors, geographic variations, and analytical processes (Lessmann et al., 2001; Sawicka et al., 2021; Subramanian et al., 2022).

In our research on whether there is a correlation between the total antioxidant activity, total phenol, and total flavonoid contents in different parts of the *Satureja thymbra* plant, such as inflorescences, leaves, and stems, and the nutrients detected only from the leaves of the plant, a negative correlation was observed between the total flavonoid contents of the inflorescences, leaves, and stems of the *S. thymbra* plant and some nutrients in the leaves. (Table 2).

Table 2: Correlation between total flavonoids, total phenolics, IC50 values, and some nutrient contents in Inflorescences, leaves, and stems of the plant.

	EC50			Phenol			Flavonoid		
	I	**L**	**S**	**I**	**L**	**S**	**I**	**L**	**S**
N	0.139	0.289	-0.375	-0.278	-0.322	-0.096	-0.573*	0.134	-0.431
P	-0.249	-0.067	0.059	0.177	-0.088	0.131	0.219	-0.026	-0.094
K	0.341	0.248	-0.068	-0.340	-0.181	0.350	-0.107	0.070	-0.068
Ca	-0.087	0.492	0.489	-0.134	-0.153	0.077	-0.230	-0.426	-0.170
Mg	-0.020	0.338	0.116	0.005	-0.025	0.263	-0.039	-0.126	-0.167
Zn	-0.094	0.512	0.216	-0.255	-0.245	-0.045	-0.540*	-0.485	-0.552*
Cu	-0.347	-0.003	-0.169	0.195	0.010	0.142	-0.162	-0.129	-0.444
Mn	-0.277	0.361	0.495	-0.017	0.005	-0.132	-0.414	-0.561*	-0.402
Fe	-0.134	0.255	0.209	-0.177	-0.134	0.151	-0.371	-0.475	-0.471

Significance: * : p< 0.05, **: p< 0.01
I: Inflorescences**, L:** Leaf, S: Stem

The correlation between the total flavonoid contents in the inflorescences and the N and Zn contents in the leaves, between the total flavonoid and Mn contents in the leaves, and between the total flavonoid contents in the stem and the Zn contents in the leaves were found to be statistically negative at the p<0.05 level. The flavonoid content of plants is known to rise in reaction to environmental stresses such as cold, salinity, and drought (Ma et al., 2014). Additionally, an increase in flavonoid content may occur as a result of plant stress caused by nutritional deficiencies. For instance, in a study on citrus trees, a rise in flavonoid levels was found only in trees with a zinc deficiency (Manthey et al., 2000). Additionally, Zn applications have been determined to increase the flavonoid content of basil (Abbasafir et al., 2020) and Spanish lavender plants (Mehrabani et al., 2017).

CONCLUSION

This paper contains an original study in which total phenol, flavonoid, and antioxidant content amounts were determined in different parts of the *S. thymbra* plant. In the study, the amount of some polyphenols and nutrients in different parts of the *S. thymbra* plant were detected, and their relationship was determined.

The values of antiradical scavenging activity, total phenol, and total flavonoid content were determined to be the highest in leaves, inflorescences, and stem extracts, respectively, in the analyses. The correlation study revealed a negative relationship between the total flavonoid content in different parts of the plant and the N, Zn, and Mn contents of the leaves. The goal of this study is to enhance the cultivation of plants such as *S. thymbra,* which currently has a low production rate but an economic value in our country, in a manner that is in conformity with standards and of high quality. The plant in issue is typically harvested from the natural environment and then exported. The implementation of production procedures that adhere to established standards is crucial in this process. There is almost no scientific research on the quality content of different parts of *S. thymbra.* This situation creates a significant lack of information about the potential of the plant. Further scientific investigation is warranted in this regard to ascertain the quality profiles of the *S. thymbra* parts.

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