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ANTIOXIDANT, PHENOLIC AND FLAVONOID PROPERTIES OF THYME (*Thymbra spicata*) JUICE AND EXTRACTION

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ABSTRACT

The aim of this study, chemical analyzes of extract and juice of *Thymbra Spicata* species (Tyme) were to investigate. The thyme juice was performed to obtain by microwave assisted extraction method (MAE). And also, maceration method was used for the thyme extraction. Percent inhibition values against DPPH and ABTS radicals were measured for antioxidant analysis. In addition, total phenolic compound and total flavonoid analyzes were also performed. Inhibition values of thyme juice and extract against DPPH radical were found to be 85.28% and 74.85%, respectively. Inhibition values against ABTS radical were determined as 98.85% for thyme juice and 83.90% for thyme extract. Total phenolic content values were found as 628.99 mg GAE/kg and 766.98 mg GAE/kg in thyme juice and extract, respectively. Accordingly, it was observed that the thyme extract value was higher. In total flavonoid substance analysis, it was found as 6945.63 mg QCE/kg in thyme extract, but it was determined that thyme juice did not contain flavonoid substances.

Introduction

Plants have been used for nutritional and medicinal purposes since ancient times. With

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the development of technology, it has been observed that plants show antioxidant properties. It is also stated that these properties are effective against human health and aging. (Dorman ,2004)

The Lamiaceae family is aromatic plants with more than 200 species and more than 3000 genera in the world. These plants occur especially in temperate and tropical regions. Turkiye has a rich species diversity in terms of these plants. There are 45 genera, 545 species and 730 taxa in the Lamiaceae family in Turkiye. the most common species are Thymus, Satureja, Thymbra origanum, and Coridothymus (Anon , 2003; Kocabaş and Karaman, 2001).

Thyme plant has an important place in Turkiye in terms of production and export. Therefore, it is used in fields such as pharmaceutical industry, food production, textile products, paint industry and cosmetics (Bozdemir, (2019). In addition, due to its antioxidant, antimicrobial and antibiotic properties, its use has become more important in terms of nutrition and health.

Thymbra spicata (Labiatae) is a species of thyme in the Lamiaceae family. This species is known as zahter, black thyme and mountain thyme in Turkiye (Barakat , 2013) Phenolic compounds such as carvacrol, γ-terpinene, thymol and p-cymene increase the importance of this species.

Therefore, the aim of this study, a series of analyzes were conducted to investigate the chemical properties of Thymbra spicata. For these analyses, thyme extract and thyme juice were used.

Material and Methods

Collection of thyme plants

The samples used in the study were collected from Mardin, Turkey in 2019. The identification of thyme plant samples was made by Maruf BALOS, a PhD from Harran University, Department of Biology. Thymbra spicata ssp. has been determined to be a Spicat breed.The samples were collected from the 41° 43′ 20.1072" east meridian and 37° 39′ 34.1604" north parallels in Altınoluk village of Dargeçit district of Mardin.

Extraction of thyme plant

For extraction, the thyme samples were ground using a Lavion Brand HC-100 model grinding machine for analysis and were stored at room temperature. The maceration method was preferred for the extraction process of the thyme plant. For this method, 25 g of the ground thyme plant was weighed and prepared by adding 200 ml of distilled water. The mixture was stored for 24 hours in the dark and at room temperature. As a result, the samples were filtered and used for analysis.

Obtaining thyme juice

Microwave-assisted essential oil for obtaining thyme juice device has been used. For this, 25 g of ground thyme was weighed and 200 ml of distilled water was added and thyme juice was extracted in the microwave for 1 hour at 200 Watts. Preliminary trials were made for optimum production.

Determination of antioxidant activity

DPPH radical scavenging activity

The scavenging activity of thyme juice and extracts against DPPH radical was determined by partial modification of the method (Brand-Williams et al., 1995). For analysis, three replications were prepared from thyme juice and extract into tubes as 0.1 ml sample - 2.9 ml

distilled water, 0.2 ml sample - 2.8 ml distilled water, 0.3 ml sample - 2.7 ml distilled water. 1 ml of DPPH radical was added to the prepared mixtures and mixed in a vortex. It was then incubated for 30 min in the dark and at room temperature. The absorbance at the end of the waiting period was measured in a SHIMADZU UV-1280 UV-VIS model spectrophotometer at a wavelength of 518 nm. The trolox standard was measured at different concentrations and a calibration curve was created. The results are also given in terms of troloxs. Trolox calibration curve is given in Figure 1.

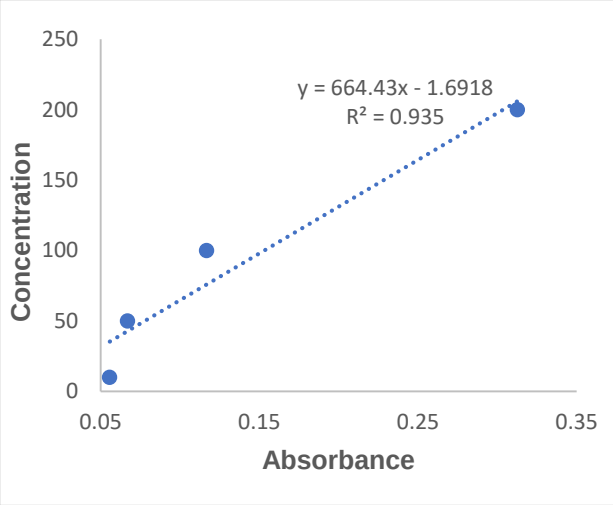

Figure 1. Trolox calibration curve generated for ABTS and DPPH

ABTS radical scavenging activity

Scavenging activity against ABTS+ radical, It was made according to (Re et al. 1999). accordingly, 7 mM ABTS+ cation aqueous solution was mixed with 0.00245 M potassium persulfate (K2S2O8) dissolved in water. The prepared solution was kept for 12 hours at room temperature and in the dark. Before using ABTS+ solution for analysis, it was diluted with distilled water until its absorbance was 0.708±0.025 at 734 nm in the spectrophotometer. For analysis, thyme juice and extracts were taken into 0.8 ml tubes and completed with ABTS prepared to 4 ml. After the mixtures were kept in the dark and room temperature for 10 minutes, absorbance values were measured at 734 nm wavelength with Shimadzu UV-1800 UV-VIS brand Spectrophotometer. The trolox standard was measured at different concentrations and a calibration curve was created. The results are also given in terms of troloxs. Trolox calibration curve is given in Figure 1.

Determination of total phenols

The method developed by phenolic analysis (Singleton and Rossı, 1965) was modified and used for thyme juice and thyme extract. According to this method, 1:9 folin reagent was prepared with distilled water. Then, 7.5% Na2CO3 was prepared with distilled water. After the solutions were prepared, 0.4 ml of thyme juice and extract were added to the tubes, 2 ml of folin reagent and finally Na2CO3 solution were added and mixed with vortex. The mixture was incubated for 1 hour at room temperature and in the dark. At the end of the waiting period, measurements were made with a SHIMADZU UV-1280 UV-VIS model spectrophotometer at a wavelength of 765 nm. Different concentrations of gallic acid standard were used to draw the calibration curve. Results are given in mg(GAE) kg. The gallic acid calibration curve created for total phenolic analysis is given in Figure 2.

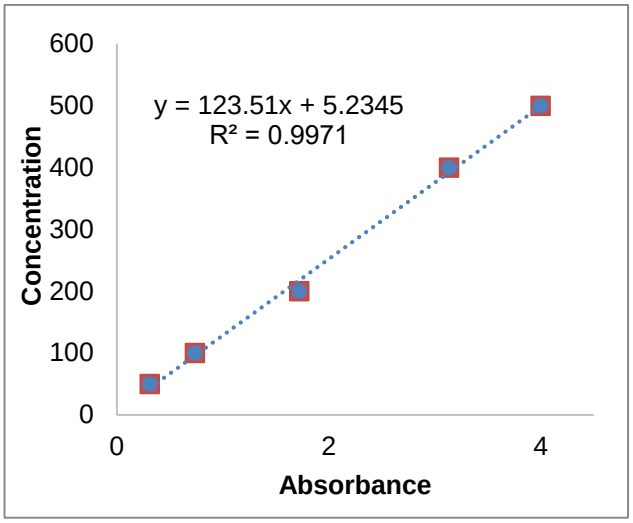

Figure 2. Gallic acid calibration curve generated for total phenolic analysis

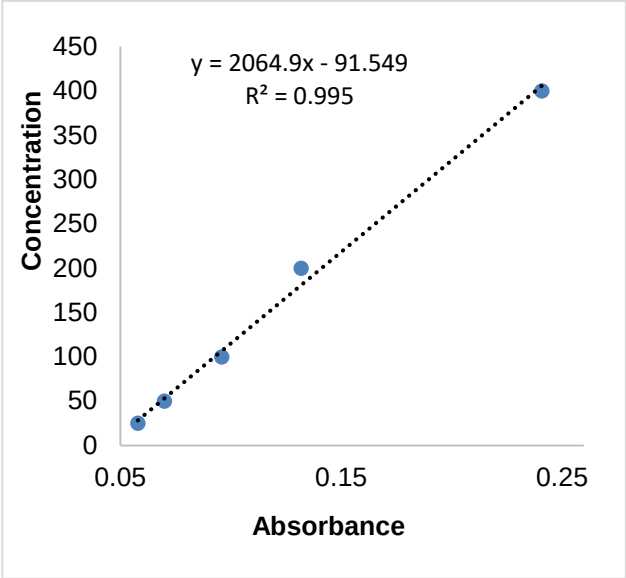

Figure 3. Quercetin calibration curve generated for total flavonoids

Determination of total flavonoids

Total flavonoid analysis in thyme juice and extract were made according to the method (Zhinsen et al, 1999). Accordingly, as solutions prepared for analysis, 5% $NaNO_2$ solution, 10% $AlCl_3$ solution, and 1 M NaOH solution were prepared with distilled water. After the solutions were prepared, 1 ml of thyme juice and extracts were put into the tubes. 4 ml of solvent (distilled water) was added to the tube and dissolved by vortexing. Then, 0.3 ml of 5% $NaNO_2$ solution and 0.3 ml of 10% $AlCl_3$ solution were added. It was mixed and kept in the dark for 5 min. After waiting, 2 ml of 1 M NaOH solution was added and left for 6 minutes. The volume of the tubes was then made up to 10 ml with solvent. The absorbance of the prepared mixture was measured at 510 nm wavelength in the SHIMADZU UV-1280 UV-VIS brand model spectrophotometer. In flavonoid analysis, 25, 50, 100, and 200 concentrations of quercetin standard were used to draw the calibration curve.

Result and Discussion

Antioxidant Activity Results

The percent (%) scavenging values of thyme juice obtained by microwave assisted extraction method and thyme extract obtained by maceration method against DPPH free radical are shown in Table 1 and Figure 4. When the results were examined, the inhibition values of thyme juice and extract were found to be 85.28% and 74.85%, respectively. When the literature studies were examined, it was observed that T. spicata essential oil of thyme inhibited DPPH radical by 92.34% (Kizil et al. 2014). It was stated that the essential oil of T.spicata species scavenged 90.9% against the DPPH radical (Iriti et al. 2014). In another study, Sengun et al. (2021) determined that T. spicata essential oil of thyme inhibited DPPH radical as 93.61%. In this study, it was observed that the % inhibition values of thyme juice containing essential oil against DPPH radical were close to the literature. It was observed that the inhibition values were lower in the thyme extract sample.

Table 1. DPPH free radical scavenging activity in thyme juice and extract

Sample type	Con.	DPPH inhibition Percent amount (%)	Trolox equivalent (mg TEAC/kg)
Thyme juice	50 µl/ml	85.28±1.09	122.99±5.72
Thyme extract	50 µl/ml	74.85±1.43	177.48±5.57
BHT			73.61±7.31

* Mean ± standard deviation of three parallel analyzes, Con., Concentration

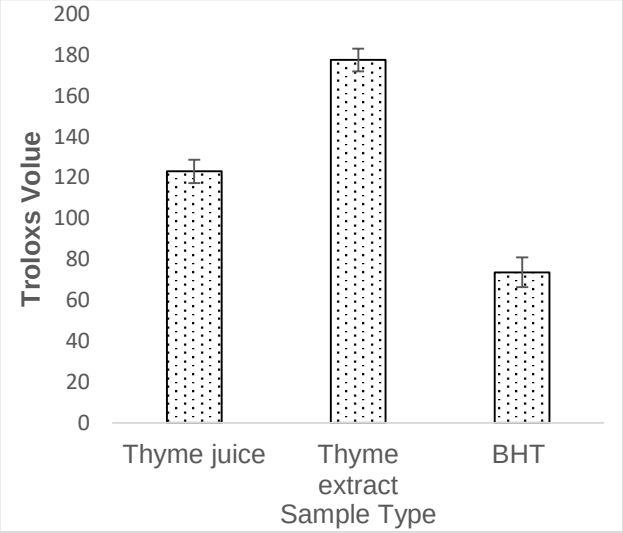

Figure 4. Graph of change of DPPH inhibition values in troloxs at 50 µl/ml concentration of thyme juice and extracts

Inhibition values of thyme juice and extract against ABTS radical are shown in Table 1 and Figure 5. According to these results, the inhibition values of thyme juice and extract were found to be 98.85% and 83.90%, respectively. Sengun et al. (2021) also found that the inhibition value of essential oil of T. spicata thyme species against ABTS radical was found to be 98.28%. In this study, inhibition values of thyme juice containing essential oil against ABTS radical were found so similar to the literature.

Table 2. Antioxidant activity against ABTS radical in thyme juice and extract

Sample type	Con.*	ABTS inhibition Percent amount (%)	Trolox equivalent (mg TEAC/kg)
Thyme juice	200 µl/ml	98.85±0.08	31.30±0.38
Thyme extract	200 µl/ml	83.90±2.21	97.75±9.86
BHT	-	-	73.61±7.31

* Mean ± standard deviation of three parallel analyzes Con., Concentration

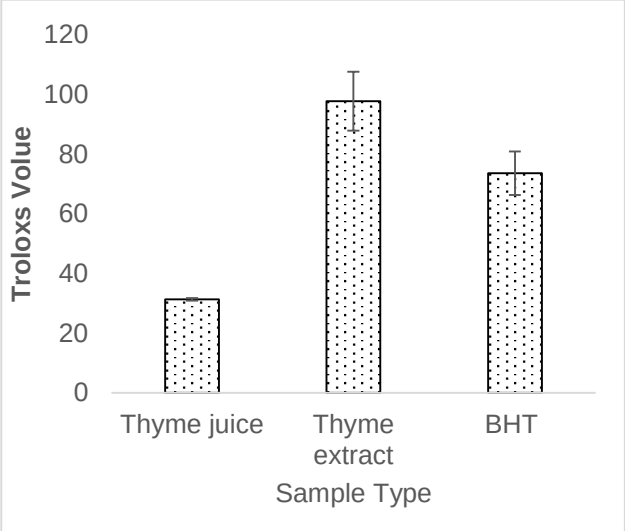

Figure 5. Graph of change of ABTS inhibition values of thyme juice and extract at 200 µl/ml concentration in troloxs

Total Phenolic Content and Total Flavonoid Content Results

Total phenolic content and total flavonoid analyzes of thyme juice obtained by microwave assistence extraction method and thyme extracts obtained by maceration method were investigated and the results are given in Table 3 and Figure 6. The phenolic contents of thyme juice and extract were found to be 628.99 mg GAE/kg and 766.98 mg GAE/kg, respectively. Sengun et al. (2020) found the TPC values of thyme extract and essential oil as 350 mg GAE/kg and 3440 mg GAE /kg, respectively.

Similarly, Tanrıkulu et al. (2017) stated that phenolic content of the methanol extract was 9630 mg GAE/ kg. In another study, Gümüş et al. (2011) reported that the phenolic content of T. spicata L. extract was in the range of 55580–75190 mg GAE /kg. The difference in the analysis results in the literature is thought to be due to the application and content of the samples. In addition, phenolic content of thyme extract was found higher than thyme juice in our study.

Table 3. Analysis of total phenolic content (TPC), and total flavonoid content (TFC) in thyme juice and extract

Sample type	TPC mg GAE/kg	TFC mg QCE/kg
Thyme juice	*628.99±16.45	0
Thyme extract	766.98±23.95	*6945.63±18.92

* Mean ± standard deviation of three parallel analyzes
* TPC- Total phenolic content
* TFC- total flavonoid content

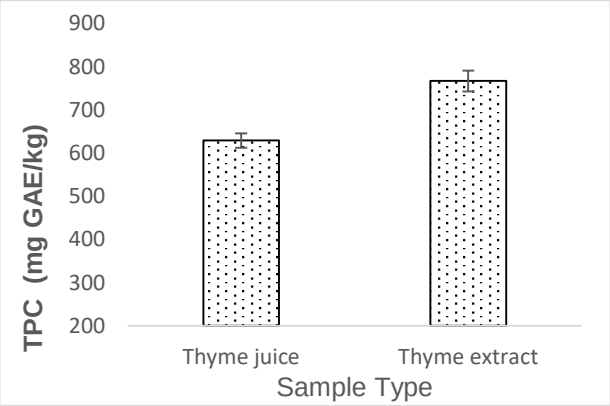

Figure 6. Graph of change of TPC of thyme juice and extracts

When the total flavonoid content results were examined, no flavonoid content was observed in thyme juice. In the thyme extract, 6945.63 mg QCE/kg was obtained. In their study, Bayan et al.(2017) stated that the total flavonoid content of the methanol extract of T. spicata thyme species was 60150 mg QCE/kg. Compared with the literature, the results of the study were found to be lower. This result is thought to be related to the applied method.

Conclusion

In this study, antioxidant, total phenolic and total flavonoid contents of thyme juice and extract were determined. It is known that thyme juice obtained by microwave method contains essential oils. Thymol and carvacrol essential oils are the most commonly known ones in the thyme species. In the scope of the study, it was observed that the antioxidant, phenolic and flavonoid amount of thyme extracts were a good level. According to results, it could be showed as good potential that thyme extract and juice has functional food because of phenolic, flavonoid and their antioxidant properties.

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Information

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