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Determination of Antioxidant Capacity and Phenolic Content of Tunceli Garlic Extracts (*Allium Tuncelianum*) by Different Solvents

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Abstract

Free radicals are wastes that come into play when using oxygen in the body. These materials have high destructive ability and could break the structure of molecules in contact. Free radicals are primarily eliminated or destroyed by the natural antioxidant defense systems in the body. When the activity of free radicals is more intense than the body's antioxidant activity, metabolic imbalance and oxidative stress in the cells. As a result, diseases such as premature ageing, heart disease and cancer can be observed. Many carcinogens, such as chemicals in the air, additives in the kitchen, drug remnants, sunlight, exhaust fumes, increase the need for antioxidants. The amount of antioxidant produced by the body can be enough. Therefore, it is necessary to consume antioxidant-rich foods in the diet. In this study, total phenolic content, DPPH and ABTS radical reduction percentage and total flavonoid content were determined of extracts obtained by different solvents (water, ethanol and methanol) with ultrasound-assisted from Tunceli garlic. In addition, the total antioxidant capacity was determined with CUPRAC and DPPH method. While TPC was found 25.09-30.08 mg GAE/100 g, TFC was found 18.92-25.54 mg QE/100 g. DPPH, ABTS were found 48.09-90.23%. CUPRAC was determined 44.58-111.12 mg CAE/100 g.

Keywords: *Allium tuncelianum*, antioxidant activity, total phenolic content, ultrasound

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INTRODUCTION

Free radicals are essential for every biochemical process and are an essential part of aerobic life and metabolism. Free radicals are constantly produced by oxygen metabolism. There is a dynamic balance between the number of free radicals that naturally occur in the body and the antioxidants that remove or destroy them and protect their bodies from their harmful effects. It is known that many diseases can occur if the free radical-antioxidant balance is disturbed in the body.

Antioxidants interact with free radicals and neutralize them. This way eliminates the harmful effects of free radicals. So antioxidants are also known as 'free radical scavengers' (Galano & Raúl Alvarez-Idaboy, 2019). Antioxidants are either produced naturally by the body (endogenous antioxidants) or taken externally (exogenous antioxidants). Exogenous antioxidants are also commonly referred to as dietary antioxidants. Fruits, vegetables and grains have high dietary antioxidants (Diplock, 1998; Karabulut and Gülay, 2016).

The adverse effects of free radicals on human life and the role of antioxidants in eliminating these harmful effects are better understood day by day. Plant-based antioxidant-rich foods traditionally composed of the bulk of the human diet. Descriptive epidemiological studies show that people with high fruit and vegetable intake have a low risk of epithelial cancer, especially in the upper gastrointestinal regions and in the respiratory tract (Møller & Loft, 2006). Studies on the protective effects of fruits and vegetables rich in natural antioxidants against these diseases are ongoing.

In our country, some of the endemic plant species grow in the Tunceli region surrounding the Munzur mountains, and one of the most important is Tunceli garlic (Allium tuncelianum). Tunceli garlic is a plant with a single leaf with white and purple flowers and creamy-white onions. It is endemic in eastern (Sarikamiş et al., 2010). Tunceli garlic carry fertile black seeds that can be used for propagation (Kiralan et al., 2013). Bulbs are propagated aseptically. However, their propagation and fertilization are relatively slow (Kızıl et al., 2009).

Tunceli garlic is an endemic species and is widely found around Tunceli province, especially Ovacık and Pülümür districts and on the skirts of Munzur mountains and is called mountain garlic (Firat, 2015; Koyuncu & Güvenç, 1994). Since the 1980s, the plant has been removed and traded, with an estimated annual figure of 15-20 tons (Firat, 2015). A. tuncelianum, which was defined as a subspecies of A. macrochaetum by (Kollman, 1983) was later defined as a different species (Özhatay et al., 1997). A. tuncelianum, also known as Tunceli garlic, is in the genus of 2n=16 chromosomes, the Monocotyledonea (monocotyledons) class, the Liliflore order, and the Allium genus of the Liaceae family (Özhatay, 2002).

Garlic has a wide range of uses, especially in the herbal therapy and food industry, and its benefits have been shown in many studies. It protects to human against heart disease by lowering high blood pressure, high cholesterol and triglyceride levels. They can destroy carcinogens that cause tumor formation by activating the immune system with the help of allinase and some other substances in their structure. Some of the sulfur-containing compounds of garlic have been found to have a positive effect on both high and low blood sugar cases, as they can specially regulate sugar metabolism. It is a strong and natural antiseptic because it destroys microbes (Gün, 2018). In addition, it has been determined that Tunceli garlic has an anti-parasitic effect (Aykur et al., 2020). In this study, Tunceli garlic was extracted with different solvents and the antioxidant capacity of the extracts was determined.

MATERIAL and METHOD

Material

In this study, Tunceli garlic was purchased from local markets in Tunceli province of Turkey in 2016. The provided garlic was washed, sorted and stored in plastic bags at -20 °C until analysis. All chemicals used were provided by Merck (Darmstadt, Germany) and Sigma (St. Louis, USA). Ultrapure water was used to prepare chemicals for analysis.

Extraction of Samples

5 g of the samples were weighed, then 25 mL of solvent (water, ethanol, methanol) was added. The mixture homogenized with ultraturrax (IKA, T18, Staufen,Germany)It was treated in an ultrasonic water bath (Lab Companion, UC 10, Boston, USA) for 30 minutes.

It was centrifuged at 8500 rpm for 20 minutes at 4 °C in a homogenized refrigerated centrifuge (Centurion Scientific K3 Series, Chichester, UK).). And then, the supernatant was collected and filtered with 0.45 µm filters. These extracts prepared for total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; ABTS) radical scavenging capacity and cupric reducing antioxidant capacity (CUPRAC).

Total Phenolic Content (TPC)

Total phenolic compounds were determined using the Folin-Ciocalteu reagent Singleton et al., (1999) method with modification. The Folin-Ciocalteu method is based on the measurement of absorbance according to the color intensity formed by the reagent that gives. Briefly, 1 mL of garlic samples and 45 mL water was added to a 100 mL flask. 1 mL of Folin-Ciocalteu reagent was added to it. After 3 minutes, 3 mL of 3% Na2CO2 solution was added. The resulting mixture was shaken at room temperature in the dark for 2 hours. Finally, the absorbance of the samples against pure water at 720 nm was measured in the UV spectrophotometer (Shimadzu, Japan). Gallic acid was used as the standard phenolic compound. The results are given as Gallic acid equivalent.

Total Flavonoid Content (TFC)

TFC was detected Woisky & Salatino (1998) method with minor modification. 1 g of garlic extract was completed to 10 mL with the solvents used in the study. 1 mL of this solution was taken, and 4 mL of 2% ethanolic AlCl3 solution was added thereto. After 1-hour incubation at room temperature, the absorbance was read at 420 nm. All values were expressed as mg quercetin equivalents (QE) per 100 g fresh matter of garlic sample.

DPPH Free Radical Removal Capacity

Determination of DPPH free radical scavenging capacity (DPPH) of garlic samples was performed by modifying the method of Mercan et al., (2018). 1 mL of garlic extracts was taken, and 2 mL of DPPH solution was mixed. After the mixture was incubated in the dark for 30 minutes at room temperature, its absorbance against the solvent was determined at 517 nm in the UV spectrometer. Results are given as inhibition (%).

ABTS Radical Scavenging Capacity

In this method, solutions of 2.45 mM K2S2O8 and 7 mM ABTS (2,2'-azino-bis (3-ethylbenzthioazoline-6-sulfonic acid) were mixed at a ratio of 1:1 and incubated for 16 hours at room temperature in the dark. The absorbance of prepared ABTS radical solution was diluted with ethanol until 1.850 ±0.05 abs in 734 nm. This absorbance was used as the control absorbance. Then 4 mL of this radical solution was taken into test tubes. 100 µL of plant extracts were added onto these tubes and incubated for 2 hours at room temperature and in the dark. At the end of this period, the absorbance of the samples was recorded at 734 nm against the curd consisting of PBS (Phosphate Buffer, pH = 7.4) (Wu et al., 2009) Decreasing absorbance gives the amount of ABTS radicals removed from the medium (Keser et al., 2013). Results are given as inhibition (%).

Cupric Reducing Antioxidant Capacity (CUPRAC)

CUPRAC values of Tunceli garlic extracts were determined by modifying the method of (Apak et al., 2004). First, 1 mL of 10 mM Cu (II), 7.5 mM neocuprine and 1 mL NH4Ac (1 M, pH 7) were added to the test tubes. Then 1 mL of garlic extract was added. After the mixture was kept in the dark for 30 minutes, its absorbance at 450 nm was measured. Cafeic acid was used as standard.

Statistical Analysis

All analyzes in the study were done in triplicate. One-way variance analysis was used to examine the statistical differences between the groups. Duncan multiple comparison test was used to determine among which groups the differences were. Results were analyzed statistically by SPSS 24.0 package program.

RESULTS and DISCUSSION

TPC were found in water extract (35.08 ± 1.95 mg GAE/100 g), methanol and ethanol extracts was determined 20.58 ±1.44 mg GAE/100 g and 27.42 ± 1.12 mg GAE/100 g, respectively. The differences between the TPC amount of all extracts are statistically significant ($P < 0.05$). The graph of gallic acid used as a standard and equation are given in Figure 1.

Ağbaş et al., (2013) compared the chemical properties of Tunceli garlic and commercial garlic. In the study, the TPC amount of the ethanol extract of Tunceli garlic was 16.21 mg GAE/g and the water extract was 54.25 mg GAE/g, while the TPC amount of the ethanol and water extracts of commercial garlic was 49.47 mg GAE/g and 4.01 mg GAE/g, respectively. Karaaslan et al., (2019) investigated the TPC amount of Tunceli garlic using acidified solvents. This study, they determined the TPC amount of acidified water, acetonitrile, methanol and ethanol extracts between 0.206±0.012-0.537±0.027 mg GAE/g Fresh weigh Gün (2018) determined the TPC amount of ethanol extract of Tunceli garlic as 16.72 µg GAE/ml.

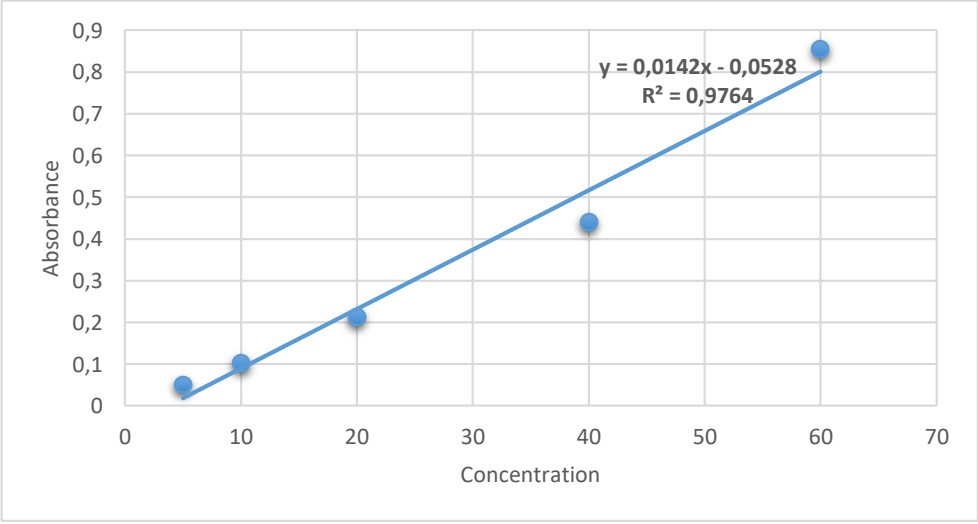

Figure 1. Gallic acid standard curve and equation

TFC amount of Tunceli garlic is shown in Table 1. TFC detected methanol (25.54±1.83 mg QE/100 g), water (21.59 ± 1.95 mg QE/100 g) and ethanol extracts (18.92±1.44 mg QE/100 g) It has been detected in extracts. While the difference between methanol and ethanol extracts is statistically significant ($P < 0.05$), the differences with water extract are not statistically significant ($P > 0.05$). Gün (2018), the TFC amount of the ethanol extract of Tunceli garlic was determined as 6.73±0.11 µg QE/ml. Bozin et al. (2008) reported that 80% methanol extracts of the garlics they produced were detected in TFC between 4.16-6.99 11 µg QE/g.

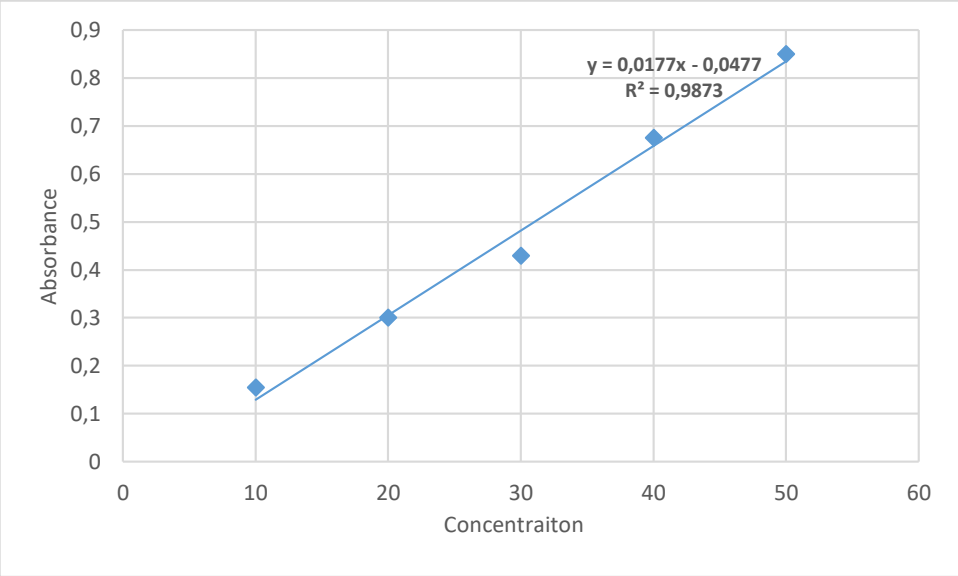

Figure 2. Quercetin standard curve and equation

Table 1. TPC, TFC, DPPH *, ABTS * and CUPRAC values of different solvent extracts of Tunceli garlic (*Allium tuncelianum*)

	Water	Ethanol	Methanol	BHT
TPC (mg GAE/100g)	35.08a	20.58c	25.09b	
TFC (mg QE/100g)	21.59ab	18.92b	25.54a	
DPPH (500 ml/L	48.09c	74.75b	90.23a	75.02b
ABTS (100 ml/L)	63.03c	86.98a	70.45b	69.89b
CUPRAC (mg CAE/g)	44.58c	30.97b	111.12a	

DPPH was the lowest inhibition in water extract (48.09±1.26%), followed by ethanol (74.75±2.54%) and BHT (75.02 ± 2.6%) extracts. The highest inhibition was determined in the methanol extract (90.23±2.79%). Ağbaş et al., (2013) compared the chemical properties of Tunceli garlic and commercial garlic in their study. In the study, they determined the DPPH activity of the ethanol extract of Tunceli garlic as 83.69% and the water extract as 59.44%, while the DPPH activity of the ethanol and water extracts of commercial garlic was 80.35% and 45.70%, respectively. Gün (2018) determined the DPHH capacity of the ethanol extract of Tunceli garlic between 19.4 ±0.7-89.7 ±0.3% in study.

ABTS capacity was determined as the lowest inhibition in water extract (63.03±1.50%), followed by methanol (70.45±1.50%) and BHT (69.89±0.93%) extracts. The highest inhibition was determined in the methanol extract (86.98±1.60%). ABTS test results in boiled garlic samples were determined as 5.5±2.5, 37.2±3.6, 57.3±5.2, 66.3±6.3 and 17.6±1.7, 30.8 ± 3.3, 47.5±5.1, 56.2±5.9 percent inhibition (Gorinstein et al., 2006). Kang et al., (2012) optimized the extraction conditions of black garlic in their study and determined the ABTS value of black garlic as 75.02 inhibition percentage.

Karaaslan et al., (2019) determined ABTS activities of acidified water, acetonitrile, methanol and ethanol extracts as 0.202, 0.255, 0.260 and 0.203 mg TEAC/g, respectively. Ağbaş et al. (2013) determined the ABTS activity of the ethanol extract of Tunceli garlic as 90.47% and the water extract as 48.54%, while the ABTS of the ethanol and water extracts of commercial garlic was 40.84% and 46.75%, respectively.

The standard curve of cupric reducing antioxidant capacity (CUPRAC) is shown in Figure 4. CUPRAC capacity was determined in the lowest ethanol extract (30.97±0.89 mg CAE/100 g), while the highest was determined in the methanol extract (111.12±3.06 mg CAE/100 g) (Table 1). Karaaslan et al., (2019) found ABTS activities of acidified water, acetonitrile, methanol and ethanol extracts as 33, 53, 88 and 45 mg CAE/g respectively.

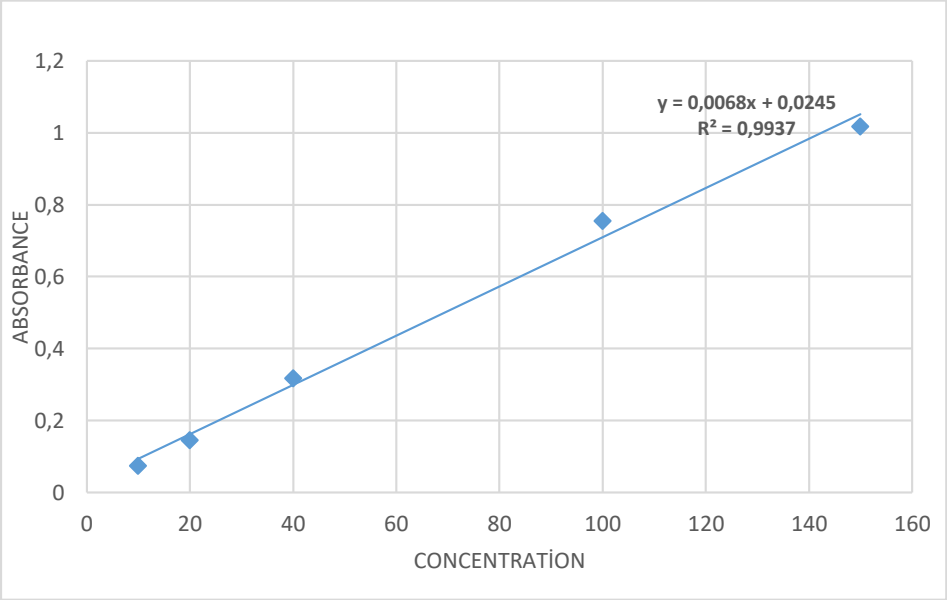

Figure 3. Caffeic acid standard curve and equation

CONCLUSION

In the study, the antioxidant capacities of the extracts of Tunceli garlic in different solvents and ultrasonic water bath, which is an endemic species for Tunceli, were determined using TPC, TFC, ABTS, DPHH and CUPRAC methods. According to the results obtained, the change of extraction solvent had a statistically significant effect on the results.

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