Bartın Orman Fakültesi Dergisi | Journal of Bartin Faculty of Forestry
2023, 25(2): 308 – 313 | DOI: 10.24011/barofd.1259611

The determination of total antioxidant activity, phenolic content and reducing power amounts of hazelnut (Corylus avellana L.) leaves grown in Giresun

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Article History
Received: 03.03.2023
Accepted: 05.07.2023
Published: 15.08.2023

Research Article

Abstract – In the present study, total antioxidant activity (TAC), phenolic compound amounts (TPC) and reducing power (RP) of hazelnut leaves collected from Giresun were investigated. Antioxidant levels were determined in the water extracts of hazelnut leaves by using methods according to the appropriate literature. In the results obtained from water extracts at three different doses (1, 2.5 and 5 mg/kg), it was determined that the total antioxidant capacity was high depending on the dose and phenolic content. The high antioxidant capacity of hazelnuts produced in humid regions in our country also depends on the phenolic substance content it has.

Keywords – *Hazelnut leaves, total antioxidant activity, reducing power, phenolic compound amounts*

Giresun'da Yetiştirilen Fındık (Corylus avellana L.) Yapraklarında Toplam Antioksidan Aktivite, Fenolik Madde ve İndirgeyici Güç Miktarlarının Belirlenmesi

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Makale Tarihçesi
Gönderim: 03.03.2023
Kabul: 05.07.2023
Yayım: 15.08.2023

Araştırma Makalesi

Öz – Bu çalışmada, Giresun'dan toplanan fındık yapraklarının toplam antioksidan aktivitesi (TAC), fenolik bileşik miktarları (TPC) ve indirgeme gücü (RP) incelenmiştir. Fındık yapraklarının su ekstraktlarında antioksidan seviyeleri literatüre uygun yöntemler kullanılarak belirlenmiştir. Su ekstraktlarından üç farklı dozda (1, 2.5 ve 5 mg/kg) elde edilen sonuçlarda doza ve fenolik içeriğe bağlı olarak toplam antioksidan kapasitenin yüksek olduğu belirlenmiştir. Ülkemizde nemli bölgelerde üretilen fındığın antioksidan kapasitesinin yüksek olması sahip olduğu fenolik madde içeriğine de bağlıdır. Sonuçlardan da görülebileceği gibi fenolik içerik de doza bağlı olarak artış göstermiştir.

Anahtar Kelimeler – *Fındık yaprağı, toplam antioksidan aktivite, indirgeme gücü, fenolik bileşik miktarları*

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1. Introduction

Free radicals containing one or more unpaired electrons in their atomic or molecular structure are high-energy, unstable, short-lived and low-weight molecular compounds. Unpaired electrons in their structure cause the production of free radicals and damage cell membranes, lipids, proteins, nucleic acids and DNA. They form the basis of many diseases such as cancer, cardiovascular diseases, nervous system degenerative diseases and diabetes. To eliminate of these damages, antioxidant systems are used to inhibit reactive oxygen species caused by endogenous and exogenous sources. These system components prevent the progression of peroxidation (Odabasoglu et al., 2005, 2006).

The antioxidant molecules; it repairs damaged lipids, proteins and DNA molecules in the cell structure, neutralizes free radicals, suppresses reactions that produce free radicals, and increases the synthesis of other antioxidant enzymes. Therefore, a high antioxidant level in the organism is more advantageous. To maintain this advantage, organisms can choose to increase their own antioxidants or outsource their antioxidant needs (Yucel et al., 2007) The most remarkable parameters in the effective determination of antioxidant potential are the reducing power capacity and the amounts of phenolic compounds. The antioxidant activity in many plants varies depending on the phenolic substance it has (Lee, 2001).

The phenolic substances, which are included in secondary metabolites, are the main parameters in the investigation of antioxidant activity in many plants. Phenolic substances are found in various parts of plants and fulfill many physiological, biochemical and physiological functions. It plays a very important role in eliminating oxidative damage caused by reactive oxygen species, protecting against UV radiation, resisting pathogens, and wound healing by joining the cell wall structure (Miller & Ruiz-Larrea, 2002; Yao et al., 2004; Pereira et al., 2006; Shahidi et al., 2007). It has been determined that flavonoids, which constitute the majority of phenolic substances, are also the subject of research and have many biological activities (anti-allergic, anti-inflammatory, antiviral, anticarcinogenic, antithrombotic, antihepatotoxic). (Shi and Noguchi, 2001; Yao et al., 2004).

Corylus avellana L., native to Europe and Asia, constitutes 80% of the world crop. Although there are many species, Corylus avellana L. and its hybrids are characterized as deciduous, rounded, 6-12 cm long and diagonal, double-knurled trees or shrubs with soft hairs on both surfaces, which can grow up to 6 m in height (Oliveira et al., 2007; Masullo et al., 2015). It is known to have a wide geographical distribution in Europe. For economic reasons, its production is carried out in a controlled manner in temperate and humid winters. It is grown mostly in the Black Sea Region in our country and is one of the important resources with economic value for the public (Altunpala and Bozoğlu, 2018). Both the hazelnut itself and its leaf are seen as natural food product. (Amaral et al., 2005).

In the present study, it was aimed to determine the phenolic substance content of hazelnut, which is widely used among foods, and accordingly the amount of antioxidant capacity.

2. Material and Method

2.1 Chemicals

All chemicals used in the experiment were obtained from Sigma-Aldrich.

2.2 Collection of Leaf Samples and Preparation of Extracts

The hazelnut leaves used in the study were collected from hazelnut trees in Yağlıdere district of Giresun province in August 2021-2022. The collected leaves were dried by laying them in a dry and cool place. Dried hazelnut leaves were ground into powder after mixing with liquid nitrogen in a mortar. The system was created with using the soxhlet apparatus and 100 g of the leaves were extracted in a shaker water bath for four days.

Water (50 ºC, 500 ml) was used as solvent. After filtering the extract, it was lyophilized and the water was evaporated. Antioxidant capacity, reducing power and phenolic compound amounts were determined from thoroughly dried leaf samples.

2.3 Determination of Antioxidant Activity

The thiocyanate technique was used to determine the antioxidant activity of extracts from hazelnuts, as described by Mitsuda et al. (1996). In a test tube, 1 mg of extract was dissolved in 1 ml of distilled water, 4 ml of phosphate buffer (0.2M, PH= 7.0) and 5 ml of linoleic acid solution were added and incubated at 37 ºC. After incubation, 0.1 ml of the incubation mixture was vortexed and applied to solutions of 75 percent ethanol and 30 percent ammonium thiocyanate at 4-hour intervals. The mixture was treated with a 0.02 M solution of $FeCl_2$ in 35 percent HCl and absorbances were measured at 500 nm versus blank. Incubation was stopped when the control group reached its maximum absorbance (Mitsuda et al., 1996).

2.4 Determination of Total Phenolic Contents

The Folin-Ciocalteu solution was used as described in the protocol to measure the total amount of phenolic compounds (Slinkard & Singleton, 1977). In a test tube, 0.5 mg of lyophilisate was dissolved in 0.5 ml of distilled water, 2.5 ml of Folin-Coicalteu solution was added and incubated at 30 ºC for 5 minutes. 2 ml of Na_2CO_3 was then added to the mixture and incubated for an additional 90 minutes at 30 ºC. At the end of the period, absorbances were measured at 765 nm. Gallic acid equivalents (GAE) per gram of lyophilisates were calculated.

2.5 Determination of Reduction Force

The reducing forces of the leaf samples were calculated using a technique developed by Yen and Chen (1995) (Yen and Chen, 1995). 0.5 mg of extract was dissolved in 0.5 ml of distilled water, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution were added into the test tube and incubated at 50 ºC for 30 minutes. Next, 2.5 mL of 10% TCA solution was added and centrifuged at 3000 rpm for 10 minutes. After removing 2.5 ml of supernatant from the mixture and adding 2.5 ml of 0.1 percent FeCl3 and 2.5 ml of distilled water, absorbance at 700 nm was measured. A high absorbance reading indicates high reducing power.

2.6 Statistical Analysis

Statistical analyses were performed using the appropriate SPSS program. Statistical differences and significance levels were determined by ANOVA test, and results at the $p<0.05$ level were considered significant.

3. Results and Discussion

Plants are the main source of natural antioxidant compounds. Because of this, herbs are known as super antioxidants. One of the most important parameters in determining the antioxidant potential is the amount of phenolic compounds. Phenolics found in various parts of plants are polyphenolic components. The most widely available plant phenolics are flavonoids, particularly cinnamic acid derivatives, coumarins, tocopherols, and phenolic acids. There is a good correlation between antioxidant capacity and these compounds (Harborne and Williams, 2000; Silva et al., 2000; Merken et al., 2001).

Hazelnut plant (*Corylus avellana L.*), belonging to the Betulaceae family, is a tree native to Europe and Asia. It grows in temperate climates such as Turkey, Spain and Italy (Bottone et al. 2019). Although Turkey and Italy are the main producing countries for hazelnut cultivation (80% of the world crop), it has also spread to the southern hemisphere in recent years (Alaşalvar et al., 2006). It is the most important species in terms of

hazelnut production as deciduous, round, 6-12 cm long and cross, soft hairy and double-toothed trees or shrubs that can grow up to 6 m in height (Oliveira et al., 2007; Masullo et al. , 2015). Hazelnut is mostly grown in the Black Sea Region in Turkey and is one of the important resources with economic value for the public (Altunpala & Bozoğlu, 2018). Hazelnuts are grown and processed in this region and offered to domestic and international markets. Hazelnut tree leaf, which is a by-product of hazelnut harvest, is also seen as a potential natural food source today (Amaral et al., 2005).

The damage caused by reactive oxygen species is kept under control by the existing antioxidant systems in the organism. However, in pathological conditions, the oxidant and antioxidant balance changes. Studies have shown that certain phenolic antioxidants prevent cell death caused by oxidative stress (Youim ve Joseph, 2001; Parihar ve Hemnani, 2003). In our current research; the results of activity analyzes of hazelnut leaf water extracts after 60 hours of incubation with linoleic acid emulsion are summarized in Table 1 as inhibition (%). Trolox and ascorbic acid were used as positive controls for hydrophilic antioxidants. Compared to the control, it was found that the three different doses of hazelnut leaf extract prevented the formation of peroxide the rates of 41.6%, 57.2%, 61.1%, while the positive controls ascorbic acid and trolox prevented the formation of peroxide at a rate of 49.4% and 78.2% ($p<0.05$).

Table 1

The comparison of antioxidant activity (TAA), reducing power (IG) and total phenol compounds (TFB) amount of hazelnut leaf water extract

Samples	Dose (mg/ml)	Total antioxidant activity		Reducing power	Phenolic compound amount
		Mean Absorbance (60. hour, 500 nm)	Inhibition %	Mean Absorbance (700nm)	(mgGAE/glyophilisate)
Giresun hazel-nut leaf water extract	1	1.50±0.06e	41.6	0.15±0.01a	0.90±0.02a
	2.5	1.10±0.001c	57.2	0.26±0.02b	1.25±0.01b
	5	1.00±0.01b	61.1	0.31±0.01c	1.58±0.02c
Ascorbic acid	1	1.30±0.001d	49.4	-	-
Trolox	1	0.56±0.02a	78.2	-	-
Control	-	2.57±0.04f	-	-	-

The values are presented as mean ± SD. Significant at $p < 0.05$. Values with the same letter are not different according to Duncan test for statistical purposes.

As can be seen in Table 1 and Figure 1 phenolic content was determined as 0.90±0.02a, 1.25±0.01b and 1.58±0.02c, respectively, depending on the doses. The phenol content also increases in direct proportion to the total antioxidant capacity.

The antioxidant effects of plant phenolics are mainly due to their redox properties. Therefore, they act as reducing agents, hydrogen donors, singlet oxygen inhibitors and metal chelators (Packer et al., 1999; Summanen et al., 2001). Reducing power is defined as the ability to donate electrons or donate electrons to free radicals and is considered another important parameter for a molecule an antioxidant effect. Plants with high antioxidant activity are expected to have higher reducing power capabilities. In our research, the data obtained in accordance with the literature show parallelism. The reducing powers were 0.15±0.01a, 0.26±0.02b, and 0.31±0.01c, depending on the dose, respectively.

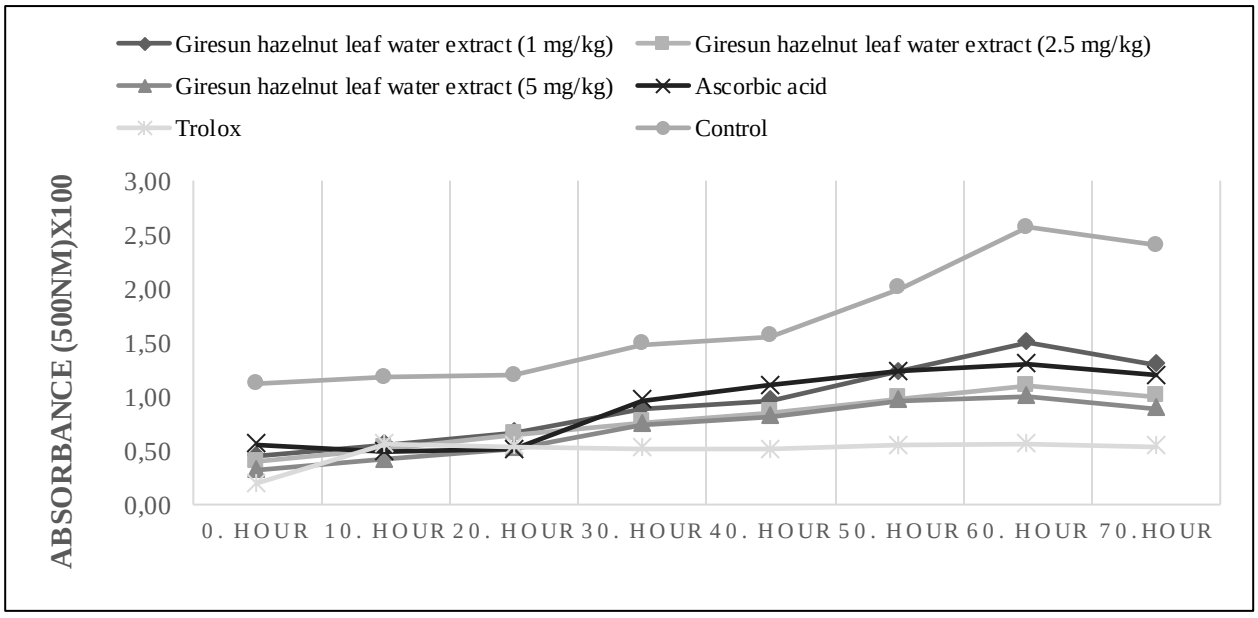

Figure 1. The comparison of antioxidant activity (TAA), reducing power (IG) and total phenol compounds (TFB) amount of hazelnut leaf water extract

The polyacetyls, phenolic acids, flavonoids, coumarins and terpenes, which are active components in plants, are reported as powerful antioxidants. The different phytochemicals available greatly affect the biological activities of plants. In line with all these data, we can say that hazelnut leaf has high antioxidant activity like many other plants and this is due to flavonoids. We believe that it will pave the way for the continuity of some biological studies.

4. Conclusion and Recommendations

In line with the results obtained from the present study, it is observed that the leaves of C. avellana are a significant source of antioxidants and phenolics. In addition, hazelnut extracts can be a guide for detailed pharmacological studies in terms of developing new natural antioxidant products for the treatment of various oxidative stress-related diseases. Examination of plants with high antioxidant content can be a new gain in alternative treatment.

Conflict of Interest

The authors declared no conflict of interest.

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