In vitro Investigation of Antioxidant Properties of Turnip (*Brassica Rapa Subsp. Rapa)*

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Abstract

Turnip (Brassica rapa subsp. Rapa), which grows in many regions of Western Asia and Europe, is a seasonal and herbaceous plant belonging to the cruciferous family. As a result of the studies, it has been revealed that the turnip juice has antioxidant properties. However, no previous study has been carried out on the antioxidant activity of turnip root as a source. In this study, the activities of turnip, which were subjected to Soxhlet extraction with solvents with different polarities, were compared with standards known to have antioxidant properties and thus, antioxidant activity of the extracts was tried to be determined. The radical scavenging effects, total antioxidant activities, reducing power, metal chelating capacity, β-carotene bleaching power of water, ethanol and ethyl acetate extracts in different concentrations were studied. In addition, proline determination, total phenolic compound amount, total anthocyanin and total carotenoid amounts were measured in all extracts. As a result of the studies, it was found that the antioxidant activity of the turnip plant studied in the form of root was higher than the standard antioxidants in some parameters studied, and the activity values were interpreted by comparing with other plants in the same species.

Keywords: Turnip, antioxidant activity, free radicals, Soxhlet extraction, Brassica, natural product

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INTRODUCTION

Today, it has been revealed by many studies that free radicals and the oxidative degradation they cause play a significant role in a significant part of the illnesses whose mechanism is illuminated (Koca and Karadeniz, 2003; Lushcha, 2014). Structures that act in response to oxidative degradation on biomolecules such as DNA, protein, carbohydrate, and lipid, which play key functions for the continuity of life, are antioxidants. Antioxidants that prevent oxidative stress caused by free radicals over other molecules also significantly reduce the damage capacity of free radicals. By utilizing this radical cleansing activity of antioxidants, cellular damage caused by free radicals is either inhibited or delayed (Lobo et. al., 2010).

The importance of antioxidants with antitumor activity, preventing cardiovascular diseases, producing remarkable results against aging, and resistance to inflammation are increasing day by day (Podsędek, 2007). The activities and antioxidant effects of antioxidants, which can be taken into the body with many different consumable foods, increase with the consumption of foods with high polyphenol composition (Belguith-Hadriche et. al., 2013).

Turnip radish (Brassica rapa subsp. rapa) whose adaptation ability is very high, is a plant of the Brassicaceae family (cruciferous or cabbage) in the genus Brassica and have a large growing area. Turnip, which loves warm and cool seasons, is also resistant to cold despite this structure. Due to be flexible in the tolerance to cold and warm temperatures, can be grown in many parts of western Asia and Europe but in Turkey, Adana, Osmaniye, Mersin is especially where it is cultivated. However, it is also possible to grow in colder regions (Padilla et. al., 2005).

To the authors' knowledge, there are no published reports on the antioxidant activity of the turnip root. The studies on the antioxidant activity of natural resources have gained speed in recent years, and in this study, antioxidant activity of the extracts of turnip root, which has not been used as a direct material in a similar study, with the help of solvents with different polarity; radical scavenging effects, total antioxidant activities, reduction power, metal chelating capacity, β-carotene bleaching power were studied and proline determination in all extracts, total phenolic compound amount, total anthocyanin and total carotenoid amount were also measured. Antioxidant activity results obtained and compared with standard antioxidants BHA, BHT, Trolox, ascorbic acid, α-tocopherol, catechin and epicatechin.

MATERIAL and METHOD

Source Plant and Extraction Process

In this study, the root part of the turnip plant, which is obtained from a local street market in Küçükçekmece district of Istanbul province and can be used both in leaves and root, was preferred for research purposes. In order to obtain the extracts, 50 grams samples taken from the turnip root were subjected to Soxhlet extraction for 4 hours with water, ethanol and ethyl acetate solvents each of which is 100 mL. Then, solvents were removed from the extracts using an oven, fume hood and the rotary evaporator. Extracts which obtained from turnip and the turnip itself was stored at +4 °C throughout the study for determination of its antioxidant activity.

Measurement of Antioxidant Activity

Lambda 25 UV / VIS Spectrophotometer (Perkin Elmer) was used to measure the antioxidant activity and percent inhibition values. PB-11 pH meter (Sartorius) was used for pH control of the prepared solutions. In the centrifuged solutions, 3K30 model (Sigma) centrifuge device was used. A 1086 model (GFL) shaking incubator was used for the incubation procedures. For distilled water used in solutions, LA620 (ELGA) was used.

DPPH• Radical-Scavenging Assay

The Brand-Williams method was used to determine this parameter (Min and Boff, 1998). During the application of this method, 2.5 mg DPPH• was dissolved in 125 mL ethanol. 0.75 mL taken from BHA, α-tocopherol and catechin samples prepared as standard with plant extracts diluted in different concentrations as 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL from stock added over 1.5 mL of DPPH• and the samples were mixed thoroughly using vortex. The absorbance values of the samples kept in the dark for 30 minutes at room temperature against ethanol at 517 nm were measured.

The control solution used during the study was chosen as the DPPH• radical with added water. For the % scavenging activity of the DPPH• radical, the following equation was used where A_0 corresponds to the absorbance of the control solution and A_1 as standard or samples.

$$\text{DPPH}^{\bullet} \text{ radical scavenging effect (\%): } [(A_0 - A_1) / A_0 \times 100] \quad (1)$$

Reducing Power

The basis of this parameter, which is based on Oyaizu method, is the measurement of absorbance values at 700 nm of colored complexes formed by $K_3Fe(CN)_6$, TCA and $FeCl_3$ compounds with molecules that are antioxidant sources (Oyaizu, 1986). In the application of the method, water, ethanol, ethyl acetate extracts and BHA, catechin and α- tocopherol standards were prepared and diluted to different concentrations between 100-250 µg/mL.. 2.5 mL of phosphate buffer (0.2 M and 6.6 pH) and 2.5 mL of 1% (w/v) $K_3Fe(CN)_6$ were added on 1 mL of each sample, respectively. The well mixed samples were incubated for 30 minutes at 50 °C. Then, 2.5 mL of 10% (w/v) TCA solution was added to each sample solution and the samples were centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2.5 mL distilled water and 0.5 mL 0.1% (w/v) $FeCI_3$ solution were added to the 2.5 mL samples taken from the filtrates. The absorbances of the colored solutions formed at 700 nm were read. The green color present at the beginning of the reaction turned blue at the end of the application, indicating the reducing power of the standards and samples.

Metal Chelating Activity

Decker and Welch (1990) method was used to determine the metal chelating activity (Gülçin, 2012). In the implementation of the experiment, 3.7 mL of distilled water and 0.1 mL of $FeCl_2$ (2 mM) were added to 1 mL each of the samples diluted to different concentrations between 100-250 µg/mL. The mixed samples were left in the dark for 10 minutes at room temperature. In this method, in which EDTA, Trolox, BHA and α-tocopherol are used as standard, the absorbance at 562 nm of the colored solutions formed as a result of the complexes was measured. Using the absorbance values of the resulting colored solutions and the equation given below, the percentage of inhibition of the complex formed between Ferrozin and Fe^{2+} was calculated. In this equation, A_0 corresponds to the absorbance of EDTA, which is the control solution, while A_1 refers to the absorbance of samples prepared from extracts and standard antioxidants.

$$\text{Metal chelating activity (\%): } [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

Proline Assay

For proline analysis, a method derived from very little change was used based on the application developed by Bates (Bates et. al., 1973). First of all, the proline sample to be used as standard was prepared and diluted at concentrations of 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL and 25 µg/mL. Then, 1 mL each of the acid ninhydrin solution prepared with 1.25 g of ninhydrin, 30 mL of glacial acetic acid and 20 mL (6 M) of phosphoric acid was added onto 1 mL of proline samples. To the resulting solution, 1 mL of glacial acetic acid was added and the mixed solution was incubated at 100 °C for 1 hour. To the samples cooled in an ice bath after incubation, 2 mL of toluene was added to the samples and proline standard samples were obtained. The resulting colored toluene phase was separated from the underlying water phase and the absorbance of the separated phase at 520 nm against toluene was measured. The same procedure was followed for the samples prepared from the extracts. 15 mg samples taken from plant extracts were dissolved in 3 mL and 3% sulfosalicylic acid solution. Samples diluted to concentrations between 100-250 µg/mL were centrifuged.

After centrifugation, 1 mL each was taken from the filtered samples and firstly, 1 mL of acid ninhydrin solution and then 1 mL of glacial acetic acid were added to them. The resulting solutions were left to incubate at 100 °C for 1 hour. After the incubation, 2 mL of toluene was added to the samples cooled in an ice bath, and the absorbance of the colored phases of the samples against toluene at 520 nm was measured. The proline content of the extracts prepared from the turnip root was calculated in terms of proline by using the standard curve drawn.

Determination of Total Flavonoid

In the determination of the total flavonoid content, the colorimetric method was developed by Zhishen et al. (1999) preferred (Zhishen et. al., 1999). While the concentration of (+)-catechin used as standard varies between 20-100 µg/mL, the concentrations of solutions prepared from turnip root extracts were chosen between 100-250 µg/mL. 1.25 mL distilled water was added to each 0.25 mL taken from the samples. Firstly, 75 µL and 5% (w/v) sodium nitrite was added to each sample and waited for 6 minutes. Then, 150 µL and 10% (w/v) aluminum chloride was added to each sample, and another 5 minutes was waited. At the end of the specified time, 0.5 mL and 1 M NaOH were added to each test tube. Then, each sample was completed to a volume of 2.5 mL with the addition of 275 µL of distilled water. The absorbance of the samples, which were mixed thoroughly, against ethanol at 510 nm was measured. The antioxidant activities of the samples were expressed in terms of (+)-catechin used as standard in the method.

Total Antioxidant Activity Assay

Thiocyanate method was used for the determination of total antioxidant activity. In the thiocyanate method, the regulated version of the method developed by Osawa and Namiki in 1981, was used (Osawa and Namiki, 1980). Samples were prepared from each turnip root extract and BHA and α-tocopherol standard solutions in concentrations ranging from 100-250 µg/mL, and the samples were diluted by adding phosphate buffer solution (0.04 M and pH 7.0) to a total volume of 2.5 mL. On the solutions in the tubes and the stock solutions containing only distilled water instead of sample, 2.5 mL of linoleic acid emulsion obtained by combining 1750 µg Tween 20, 1550 µL linoleic acid and 496.7 mL of phosphate buffer solution was added and at 37 °C left for incubation in the dark. By taking 0.1 mL each of the samples at 24 hour intervals, 4.7 mL of 75% ethanol, 0.1 mL of 30% (w/v) ammonium thiocyanate solution and 0.1 mL of 0.02 M $FeCl_2$ solution prepared using 3.5% HCl were added. The % inhibition values of the samples whose absorbance was measured against ethanol at 500 nm by waiting for 3 minutes were calculated according to the following equation, where A_0 corresponds to the absorbance values of the control solution and A_1 corresponds to the absorbance extracts and standards:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A0] \times 100 \qquad (3)$$

β-Carotene Bleaching Test

In the method, first, 5 mg of trans-β-carotene was dissolved in 5 mL of chloroform. 0.2 mL of trans-β-carotene solution was added to the solution consisting of 20 mg linoleic acid and 200 mg Tween 40. After the chloroform in the mixture was evaporated in fume hood, 50 mL of distilled water was added to the emulsion to reach its final form. While studying this parameter in which the samples were prepared in a single concentration, 0.2 mL of the plant extracts prepared at a concentration of 2 mg/2 mL and the BHA solution used as a standard were taken, and 5 mL of the prepared emulsion was added to each of them. Then, samples were incubated for one hour at 50 °C.

The absorbance values of the samples taken at 0th, 60th and 120th minutes were read at 470 nm. Distilled water was used as a blank solution. In the β-carotene bleaching method, two different control solutions were used: BHA solution was the positive control and distilled water was the negative control. By considering the absorbance values of BHA accepted as 1, the Relative Antioxidant Activity (RAA) calculation determined for bleaching power tests is given in the equation below (Peksel et. al., 2013).

$$RAA = \text{Absorbance of the sample} / \text{Absorbance of the BHA solution} \quad (4)$$

$ABTS^{\bullet+}$ Assay

To determine $ABTS^{\bullet+}$ radical scavenging activity, the method developed by Arnao et al. (2001) was applied (Arnao et. al., 2001). First, 7.4 mM ABTS solution and 2.6 mM $K_2S_2O_8$ solution were prepared and mixed in equal amounts of volume and kept in the dark at room temperature for 12 hours to obtain the $ABTS^{\bullet+}$ radical solution. 20 mL of methanol was added on 1 mL of $ABTS^{\bullet+}$ solution, and dilution with methanol was continued until the absorbance of this radical solution reached 1.1 ± 0.02 at 734 nm. A solution of 2850 µL $ABTS^{\bullet+}$ was added to 150 µL of extract solutions and Trolox, BHA, epicatechin and α-tocopherol standard solutions which have different concentrations as 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL. The resulting solutions were kept in the dark for 2 hours. At the end of the determined period, the absorbance values of the prepared samples were measured against distilled water at 734 nm. The antioxidant activities of each extract and BHA, epicatechin and α-tocopherol standards were expressed in Trolox equivalent, which is another standard used in the method.

$DMPD^{\bullet+}$ Radical Scavenging Assay

For determination of $DMPD^{\bullet+}$ radical scavenging activity, method was developed by Fogliano et. al. was applied (Fogliano et. al., 1999). To obtain the $DMPD^{\bullet+}$ radical, 100 mL acetate buffer solution (at 0.1 M and 5.3 pH) and 0.2 mL $FeCl_3$ (0.05 M) solution were added onto 1 mL of DMPD solution (100 mM). 1 mL each of the prepared $DMPD^{\bullet+}$ radical solution was taken and extracts with BHA and α-tocopherol standard solutions which diluted to concentrations between 100-250 µg/mL were added on the $DMPD^{\bullet+}$ radical solution. After waiting for 10 minutes due to the formation steps of $DMPD^{\bullet+}$ radicals, measurement was taken at 505 nm in spectrophotometer. As the control solution, samples containing only distilled water were used instead of extract or standard solution. The following equation was used to calculate the $DMPD^{\bullet+}$ radical scavenging activity in the parameter using buffer solution as blank solution. In equation, A_0 is the absorbance value of the control solution and A_1 is the absorbance values of the standard solution or the extracts.

$$DMPD^{\bullet+} \text{ radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (5)$$

Determination of Total Chlorophyll and Total Carotenoid

The total chlorophyll and carotenoid content determined according to the method developed by Kocaçalışkan and Kadıoğlu in 1990 is based on measuring the absorbance values at 450, 645 and 663 nm of the solutions obtained by dissolving 2.5 mg plant extracts in 2.5 mL distilled water (Kocaçalışkan and Kadıoğlu, 1990). The following equations were used to determine the total chlorophyll and carotenoid content in the plant extracts with the absorbance values obtained, respectively:

$$\text{Chlorophyll a} = 12.7A_{663} - 2.69A_{645} \quad (6)$$

$$\text{Chlorophyll b} = 22.9A_{645} - 4.68A_{663} \quad (7)$$

$$\text{Total Chlorophyll} = 20.2A_{645} + 8.02A_{663} \quad (8)$$

$$\text{Total Carotenoid} = 4.07A_{450} - [(0.0435 \times \text{Chlorophyll a}) + (0.367 \times \text{Chlorophyll b})] \quad (9)$$

Nitric Oxide Radical Scavenging Assay

For nitric oxide radical scavenging activity, Marcocci et al.'s work was taken as reference (Marcocci et. al., 1994). 2 mL each of extract solutions prepared in four different concentrations, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL, were taken and 0.5 mL of sodium nitroprussiate solution prepared in phosphate salt buffer (pH 7,4) was added onto it. The mixed solutions were incubated for 3 hours in a 37 °C water bath. After the incubation process, 0.5 mL of the prepared solutions was taken and 0.5 mL of Griess reagent containing 1% sulfanilamide prepared in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride was added to them. The absorbance values of the solutions obtained were read at 570 nm and against the phosphate salt buffer. In this experiment, $NaNO_2$ solution at concentrations 2.5 µg/mL, 5 µg/mL, 7.5 µg/mL and 10 µg/mL was used as a standard. As a result of the absorbance values read, the $NO^{\bullet}$ radical scavenging activity of the extracts was expressed in terms of nitrite concentration.

Anthocyanin Assay

On the basis of this parameter, where the plant itself is used instead of the plant extracts, Padmavati et al.'s work is included (Padmavati et. al., 1997). According to the method, 250 mg samples were taken from both the colored and colorless part of the turnip root. The samples taken were treated with methanol acidified with 1% HCl and kept at +4 °C and in the dark. At the end of the 24-hour period, the solutions were stirred at +4 °C for 1 hour and in the dark. The solutions obtained were first filtered with filter paper, then centrifuged at 1000 rpm for 15 minutes. To calculate the anthocyanin content, the absorbance values of the filtrate were read at 530 and 657 nm. In order to reach the anthocyanin content from the absorbance values obtained, an extinction coefficient of 31.6 $M^{-1}cm^{-1}$ was used and with the help of the equation below, the anthocyanin content in the plant sample was calculated.

$$\text{Concentration of anthocyanin (µmol/g)} = [(A_{530} - 0{,}33 \times A_{657}) / 31{,}6] \times [V(mL) / m(g)] \quad (10)$$

Total Phenolic Material Amount Assay

Slinkard-Singleton method was used to determine the total phenolic content of the turnip root (Slinkard and Singleton, 1977). Folin-Ciocalteau reagent which diluted with 22.5 mL distilled water to obtain 1:2 ratio was added to the pyrocatechol standard and extracts prepared at a concentration of 1 mg/mL. After the addition, the well mixed samples were kept for 3 minutes and 1.5 mL of 2% (w/v) Na_2CO_3 was added to each. The prepared solutions were left to incubate for two hours at room temperature with intermittent shaking. After two hours of incubation, the absorbance values of the extracts and the pyrocatchol standard at 760 nm were measured. Distilled water was used as the blank solution during the measurement. The total phenolic substance amount of the extracts obtained from the turnip root was stated as the equivalent of the sample in µg in terms of mg of pyrocatechole.

RESULTS and DISCUSSION

DPPH• Radical-Scavenging Assay

The graphic of DPPH• radical scavenging activities of standard solutions and extracts is given in Figure 1 and Table 1. The best activity among the extracts was in ethanol extract, with a value of 23.04%. While the activity in the water extract was 11.60%, the activity value in the ethyl acetate extract was observed as maximum 5.88% at most.

While the activity of BHA from standard solutions increased to 86.85%, this value was 84.35% for catechin and 86.22% for α-tocopherol.

Reducing Power

As a result of the application of this Oyaizu method, the Fe^{3+} reduction capacity of the turnip root extracts obtained from the turnip plant with the help of different solvents was determined by comparing with the BHA, Catechin and α-tocopherol standards. The results obtained are shown in the graphic in Figure 2 and Table 2. The increase in the absorbance values of each sample indicates the amount of Fe^{2+} released and this amount is directly proportional to the antioxidant activity. The reducing power of water extract and ethanol extract at 100 μg/mL sample concentration was obtained as close to the α-tocopherol standard. However, when the absorbance values of all extracts are compared with the BHA, catechin and α-tocopherol standards, it was seen that the reducing power of the extracts is not concentration dependent.

Metal Chelating Activity

In this parameter, metal chelating capacity of EDTA, standard antioxidants and extracts is summarized with the graphic in Figure 3 and Table 3. According to this graph, it was observed that the metal chelating activity of water, ethanol and ethyl acetate extracts remained at 40% values, whereas the formation of Ferrozin-Fe^{2+} complex reached almost 100% values on EDTA. While the metal chelating activity of all three extracts was higher than the BHA, Trolox and α-tocopherol standards, it was observed that the water extract had the lowest metal chelating activity among the extracts with 39.10%, and the ethanol extract had the highest metal chelating activity with 41.39%.

Proline Assay

Proline, one of the twenty essential amino acids, is responsible for stimulating the pentose phosphate pathway in plants. Synthesis of cytosolic proline helps regulate the pentose phosphate pathway by oxidizing NADPH. According to this embodiment, with increasing proline concentration, the pentose phosphate pathway is induced, thus inducing the synthesis of phenolic compounds. Plants with high proline content also have high amounts of phenolic compounds. This high amount of phenolic compound also expresses the antioxidant capacity of the plants that can be consumed (Peksel et. al., 2013). The proline content of the extracts obtained from the turnip root with the help of water, ethanol and ethyl acetate solvents is given in Table 4 as μg proline/mg extract.

Determination of Total Flavonoid

Flavonoids, which are the most important group of polyphenol compounds in plants, are known as the source of radical scavenging effect with high antioxidant activity. The total flavonoid content in the plants provides high levels of antioxidant activity. The total flavonoid content of the extracts obtained from the turnip root, obtained as a result of the experimental studies of this parameter, is given in Table 5 in terms of μg catechin/mg extract equivalent.

According to the values gathered as a result of the studies, the highest value was found in the ethyl acetate extract and the lowest flavonoid content was reached in the water extract.

Total Antioxidant Activity Assay

Thiocyanate method was used to determine the total antioxidant activity. During the application of the method, the amount of peroxide released in the first steps of lipid oxidation in linoleic acid emulsion was measured for 3 days at 24-hour intervals. The first measurements were carried out before the prepared solutions were left to incubation in the water bath and the values given in the graphic in Figure 4 were obtained.

The second measurements were carried out after 24 hours and the values obtained are shown in Figure 5. The parameter was terminated as a result of the measurements made on the third day, as the gradually decreasing % activity value of the extracts was completely finished at the 48th hour for water extract and ethanol extract and shown in the graphic in Figure 6. The values of each measurement are summarized in Table 6.

The highest activity % as a result of the experiments carried out in the parameter; 40.52% for water extract, 39.51% for ethanol extract and 37.31% for ethyl acetate extract. As a result of the measurements at the 24th hour and repeated once again at the 48th hour, the activities of the extracts decreased at the end point and therefore the highest values for the extracts were obtained and recorded at the 0th hour. The activities of BHA and α-tocopherol standard solutions decreased gradually as from the 24th hour, as in the same extracts. Despite the activity still available at the 48th hour, the highest activity of the standards was determined at the 0th hour as 58.39% and 79.48%, respectively.

β-Carotene Bleaching Test

The results obtained by applying β-carotene bleaching method with the values shown in Table 7 are given. As a result of this parameter, the absorbance values of the extracts and therefore the antioxidant activities determined with these absorbance values were calculated by accepting the BHA standard used as a positive control in the method, and as a result of this parameter, the relative antioxidant activity values of all extracts were obtained very close to the BHA standard solution with the elapsed time.

$ABTS^{\bullet+}$ Assay

The inhibition % achieved in this method is given in Trolox equivalent, which is used as a standard and is shown in the graph in Figure 7 and Table 8 with μg Trolox values per mg extract. According to this method, in which all three extracts have an average activity according to Trolox standart, higher than Epicatechin standard and lower activity than α-tocopherol and BHA standard. Ethyl acetate extract was the most able to remove $ABTS^{\bullet+}$ radical from the environment. In the method, ethanol extract had the least activity in removing the $ABTS^{\bullet+}$ radical from the environment, as can be seen from the values given in the graph. In addition, it has been observed that increasing the concentration does not have much effect on the % activity value.

$DMPD^{\bullet+}$ Radical-Scavenging Assay

The highest activity for the extracts was seen at the concentration value of 250 mg/μL, and the highest activity value was found in the ethyl acetate extract at this concentration with 27.94%. The activity of BHA showed a concentration-dependent increase and was obtained as 53.32% in 250 mg/μL. It can be said that % radical scavenging activity values obtained in this parameter are more affected by concentration than other methods.

Determination of Total Chlorophyll and Total Carotenoid

It has been proven by previous experimental studies that chlorophyll a, chlorophyll b and carotenoid compounds, which are not polyphenolic and have an important place in the pigments class, have antioxidant activity. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid contents obtained from three different extracts obtained from turnip root are shown in Table 10, and it was observed that all values appeared differently in different extracts.

Nitric Oxide Radical Scavenging Assay

Nitric oxide, which emerged during the incubation process performed during the application of the method, was released by sodium nitroprusside and enabled the radical scavenging activity of the extracts to be determined. According to the results, the nitric oxide radical scavenging activity, which was obtained very high regardless of the concentration, is shown in the graph given in Figure 9 and Table 11, corresponding to the nitrite concentration that enables the determination of the antioxidant activity of the extracts during the reaction.

Anthocyanin Assay

Anthocyanins, a very common type of flavonoids with antioxidant properties, are the structures that give colour to many plants. Anthocyanins taken into the body by the foods that can be consumed both protect the body against oxidative damage and minimize the risks of diseases caused by oxidative stress. As a result of the experiments repeated separately for the purple and white colored parts of the turnip root, the anthocyanin content of this plant was calculated as 0.5479 μmol/g plant for the coloured part and 0.0048 μmol/g plant for the white part.

Total Phenolic Material Amount Assay

The total phenolic material amounts of the extracts in terms of μg pyrocatechol/mg extract calculated as a result of the values obtained after the experiments are given in Table 5. Accordingly, the highest phenolic material composition was found in the ethanol extract, the lowest phenolic material composition was found in the water extract. As the results show, the total phenolic content of the turnip root is quite low compared to the pyrocatecol standard.

LIST OF TABLES

Table 1. DPPH• radical scavenging activity (%) of extracts and standards

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	5.32%	7.66%	10.34%	11.60%
Ethanol extract	9.95%	14.80%	17.27%	23.04%
Ethyl acetate extract	4.96%	5.43%	5.53%	5.88%
BHA	83.92%	84.86%	85.52%	86.85%
Catechin	82.41%	83.81%	84.32%	84.35%
α-tocopherol	85.52%	85.63%	85.86%	86.22%

Table 2. Reducing power of the extracts and standards (as an absorbance)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	0.0336	0.0289	0.0273	0.0240
Ethanol extract	0.0272	0.0233	0.0222	0.0173
Ethyl acetate extract	0.0101	0.0110	0.0126	0.0174
BHA	0.1296	0.1505	0.1513	0.1630
Catechin	0.0499	0.0624	0.0657	0.0717
α-tocopherol	0.0305	0.0346	0.0547	0.0823

Table 3. Fe^{2+} chelating capacity of the extracts and standards (%)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	30.72%	32.69%	36.71%	39.10%
Ethanol extract	36.31%	38.05%	40.51%	41.39%
Ethyl acetate extract	35.06%	36.47%	39.49%	41.15%
EDTA	92.86%	97.09%	98.55%	99.29%
BHA	0.68%	1.07%	1.36%	2.97%
Trolox	2.11%	5.29%	7.78%	8.96%
α-tocopherol	0.54%	1.15%	4.03%	5.39%

Table 4. Absorbance values and proline amounts of extracts

Extract	Absorbance	Concentration (µg proline/mg extract)
Distilled water extract	0.4248	4.7411
Ethanol extract	0.2093	2.3359
Ethyl acetate extract	0.1112	1.2411

Table 5. Absorbance values and Catechin amounts of extracts

Extract	Absorbance	Concentration (µg catechin equivalent/mg extract
Distilled water extract	0.93525	259.7325
Ethanol extract	1.406025	390.5
Ethyl acetate extract	1.8373	510.2975

Table 6. Total antioxidant activities (%) of the extracts and standards measured in three trials

Sample (0th Hour| 24th Hour | 48th Hour) \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	32.95 - 12.88 - 0	35.63 - 16.48 - 0	39.47 - 17.68 - 0	40.52 - 20.3 - 0
Ethanol extract	37.72 – 11.53 - 0	38.18 – 15.31 - 0	38.65 – 17.4 - 0	39.51 – 17.88 - 0
Ethyl acetate extract	33.1 – 15.53 - 0	33.96 – 22.22 – 2.31	36.19 – 24.88 – 6.84	37.31 – 25.7 – 11.21
BHA	53.47 – 41.22 – 36.71	55.24 – 43.05 – 37.03	57.05 – 43.35 – 41.19	58.39 – 46.12 – 42.44
α-tocopherol	72.75 – 42.67 – 27.62	75.55 – 46.34 – 33.20	78.51 – 50.42 – 35.82	79.48 – 53.65 – 36.45

Table 7. Antioxidant activity in terms of RAA value

Sample	RAA value (60th minute)	RAA value (120th minute)
Distilled water extract	0.82	0.75
Ethanol extract	0.81	0.74
Ethyl acetate extract	0.80	0.73
BHA	1	1
Control solution	0.76	0.64

Table 8. $ABTS^{\bullet+}$ radical scavenging activity of extracts and standards in µg Trolox per mg extract

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	137	142.25	143.73	151.42
Ethanol extract	100.17	114.83	124.81	137.6
Ethyl acetate extract	139.94	148.81	155.94	158.65
BHA	195.67	226.17	228.96	234.08
Epicatechin	35.52	47.46	61.31	83.33
α-tocopherol	234.44	234.67	234.77	234.90

Table 9. $DMPD^{\bullet+}$ radical scavenging activity (%) of extracts and standards

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	8.95%	10.89%	15.91%	22.06%
Ethanol extract	18.08%	21.34%	24.23%	26.76%
Ethyl acetate extract	21.01%	22.43%	26.23%	27.94%
BHA	35.51%	41.68%	47.33%	53.32%
α-tocopherol	2.73%	6.23%	10.81%	12.83%

Table 10. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid values of the extracts

Sample	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Total Carotenoid
Distilled water extract	0.0241	0.0634	0.0875	0.0931
Ethanol extract	0.0139	0.0058	0.1972	0.1536
Ethyl acetate extract	0.0844	0.1508	0.2350	0.0476

Table 11. The value of the nitric oxide radical scavenging activity of the extracts in terms of nitrite concentration (%)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	96.80	96.46	96.22	95.16
Ethanol extract	94.49	94.09	93.35	92.84
Ethyl acetate extract	96.29	96.01	95.64	95.09

Table 12. Absorbance values and total amount of phenolic material in terms of pyrocatechol

Sample	Absorbance	Concentration (µg pyrocatechol equivalent/mg extract)
Distilled water extract	0.0104	2.4186
Ethanol extract	0.0178	4.1395
Ethyl acetate extract	0.0143	3.3256

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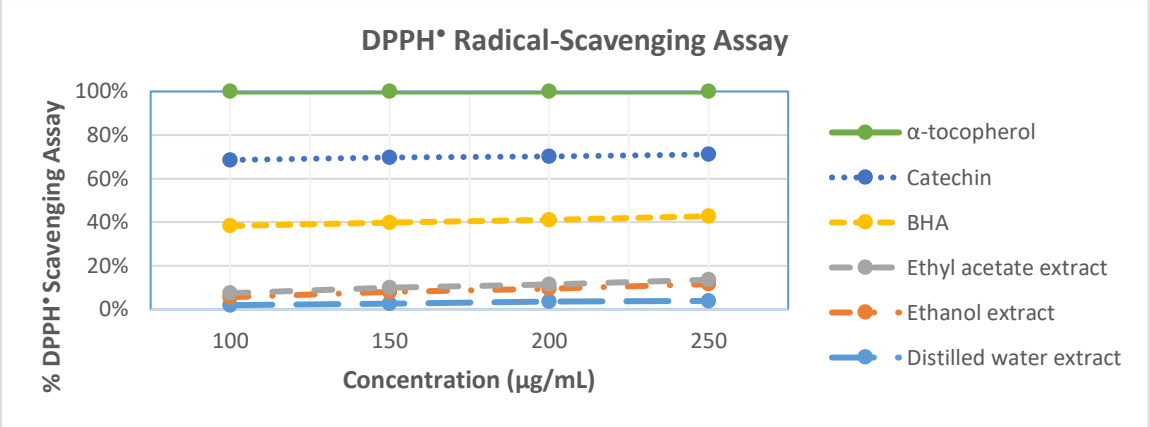

Figure 1. DPPH• radical scavenging activity of extracts and standards

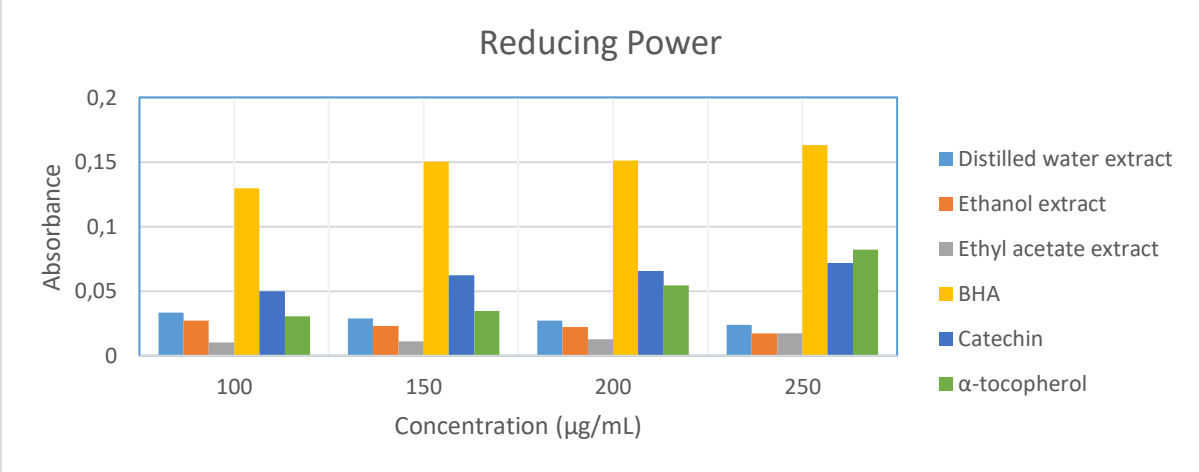

Figure 2. Reducing power of extracts and standards

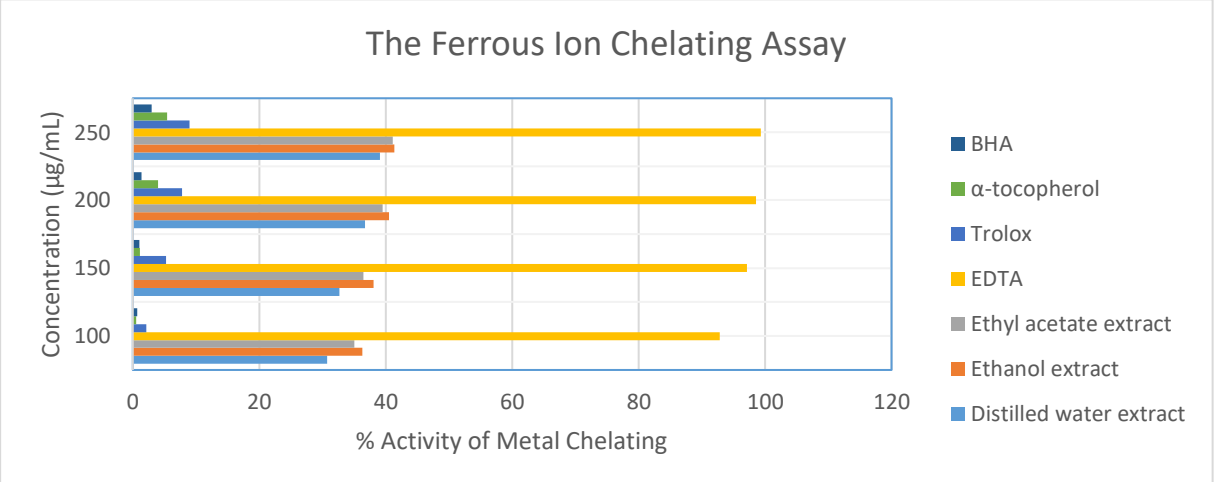

Figure 3. Metal chelating activity of extracts and standards

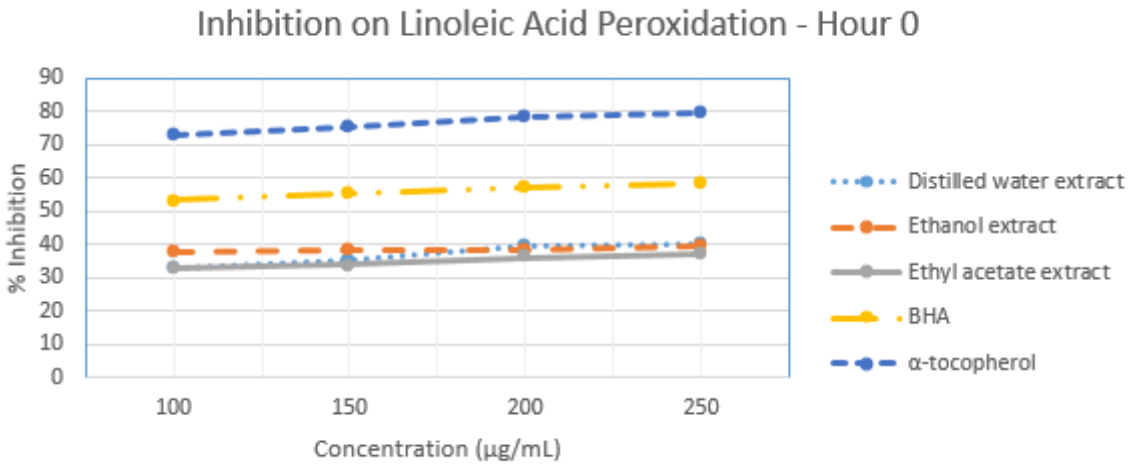

Figure 4. Total antioxidant activities of extracts and standards at 0th hour

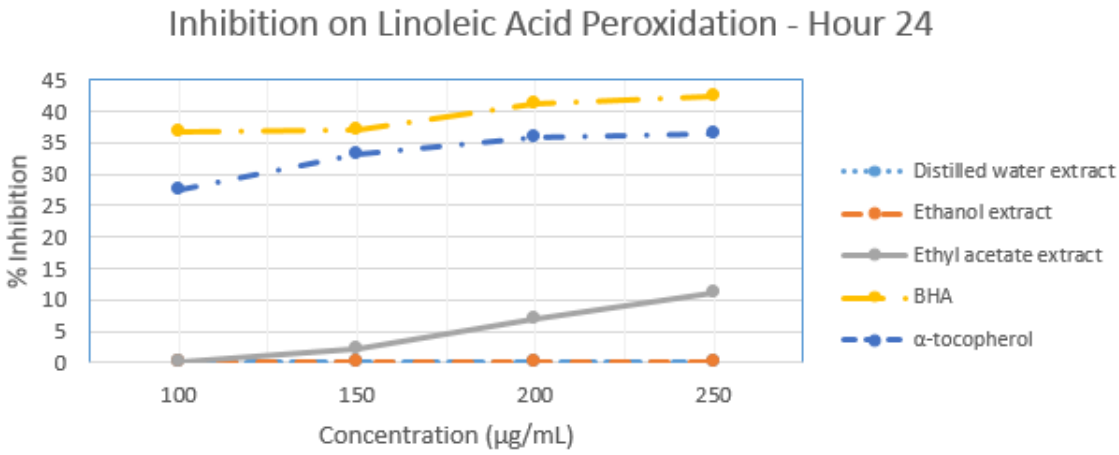

Figure 5. Total antioxidant activities of extracts and standards at 24th hour

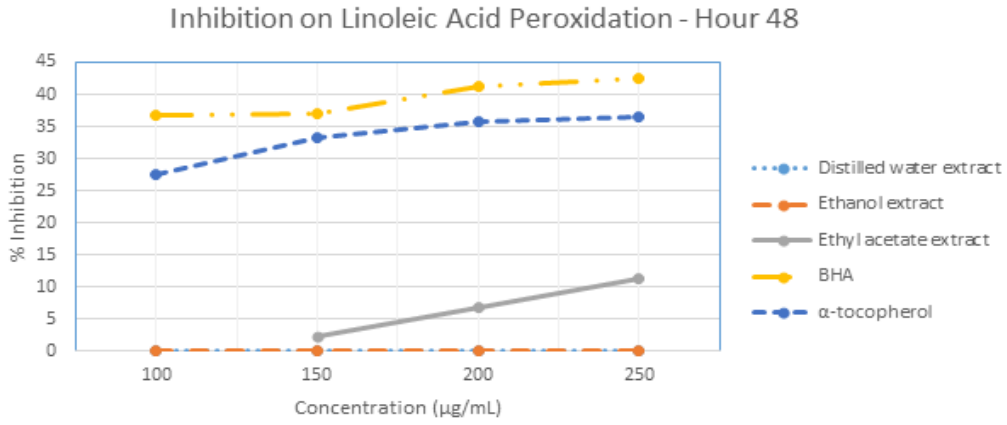

Figure 6. Total antioxidant activities of extracts and standards at 48th hour

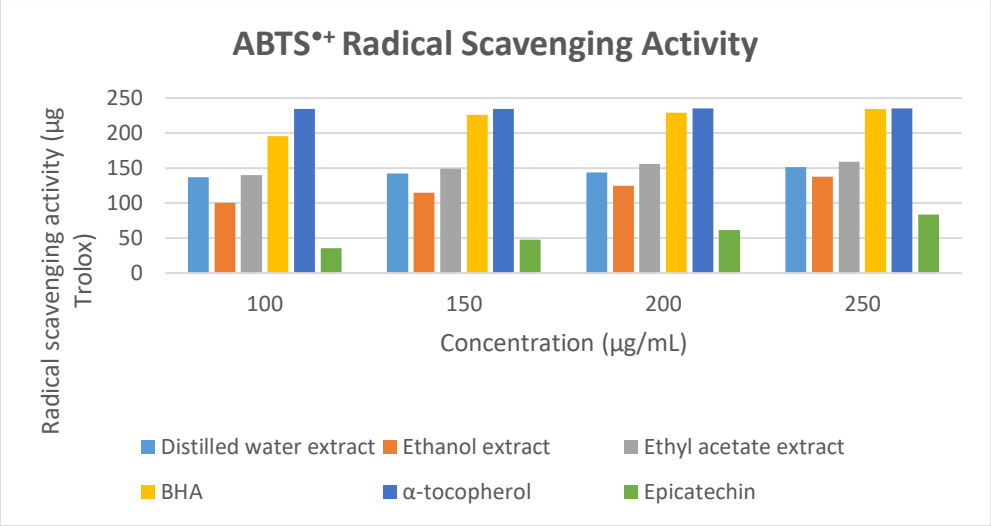

Figure 7. ABTS$^{\bullet+}$ radical scavenging activity of extracts and standards in µg Trolox per mg extract

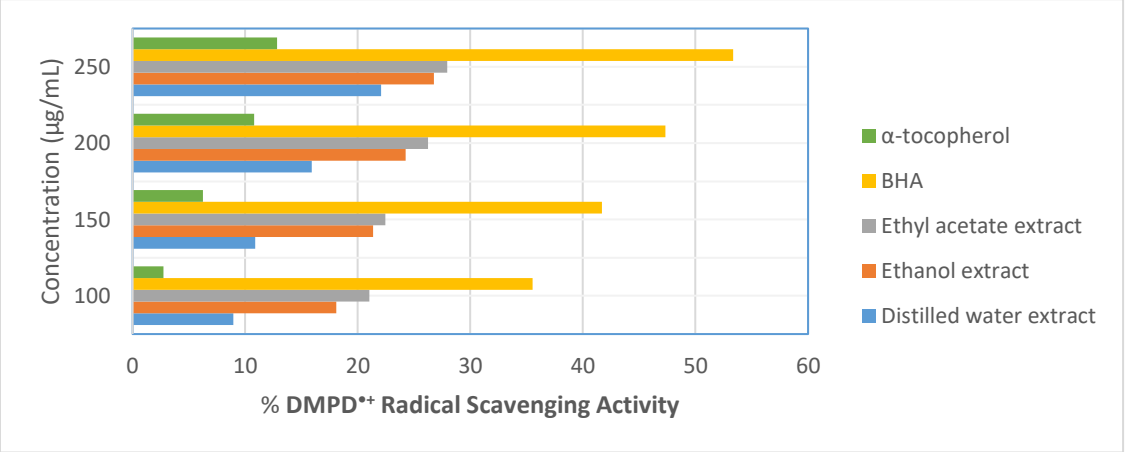

Figure 8. DMPD$^{\bullet+}$ radical scavenging activity of extracts and standards

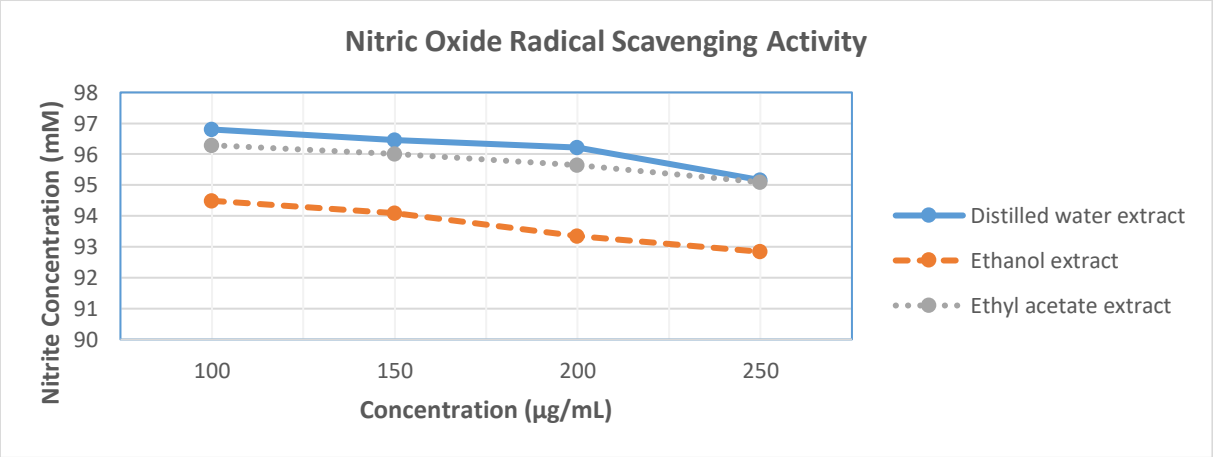

Figure 9. Values of nitric oxide radical scavenging activity of extracts in nitrite concentration

CONCLUSION

Synthetic antioxidants such as BHA, BHT, Trolox and α-tocopherol have been used for a long time for the purposes of extending the shelf life of foods, ensuring that they can be stored for a longer time, and increasing their durability. However, the possibility that synthetic antioxidants have toxic properties and cause carcinogenic effects, it is necessary to use natural antioxidants. In this study, the antioxidant activity of turnip root (*Brassica rapa subsp. Rapa*) was compared with the standard antioxidants.

Regarding the antioxidant activity, many natural and synthetic sources are already used as antioxidant molecules and provide ease of interpretation by providing comparison with the literature. However, since turnip, whose antioxidant activity we examined, had not been included in such a study before as a root form, the results were interpreted by comparing them with other plant species in its family. For $DPPH^{\bullet}$ radical scavenging activity, which is the most studied activity in Brassica species, cauliflower (Brassica oleracea var. Botrytis) with an activity of 23% and cabbage (Brassica oleracea L var. Capitata) with an activity of 9% stand out (Gülçin and Köksal, 2008; Singh et. al., 2006). The highest value in this parameter belongs to rapeseed with 73% scavenging activity (Soengas et. al., 2018). The scavenging activity of cabbage, cauliflower and pyramid cauliflower plants (Brassica oleracea var. Romanesco) compared for $ABTS^{\bullet+}$ radical scavenging activity varies between 1.91 and 3.10 in μg Trolox equivalent (Volden et. al., 2008). In a study using cauliflower, Brussels sprouts, and broccoli, total phenolic contents varied between 746.1 μg and 2306.6 μg in μg of catechin, while $DMPD^{\bullet+}$ radical scavenging activity was obtained as 70% for cauliflower (Florkiewicz et. al., 2018; Nawaz et. al., 2018)

The results obtained in similar experiments show that turnip root has higher antioxidant activity in some parameters.

In conclusion, we subjected a material whose antioxidant activity had not been studied before, to some experiments for this purpose. Although the results we reached were promising for some parameters, they were not obtained as high as expected for some parameters. The fact that it is a plant that grows under the ground is the only reason turnip root lacks some of biochemical components. In future studies, the components of turnip root able to be analyzed as every biomolecule level and their effects on each parameter can be examined in more detail.

REFERENCES

Arnao M. B., Cano A. & Acosta M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity, *Food Chemistry*, 73 (2): 239-244. DOI: doi.org/10.1016/S0308-8146(00)00324-1

Bates L.S., Waldren R. P. & Teare I. D. 1973. Rapid determination of free proline for water-stress studies, *Plant and Soil*, 39: 205–207. DOI: doi.org/10.1007/BF00018060

Belguith-Hadriche O., Bouaziz M., Jamoussi K., Simmonds M., El Feki A. & Makni-Ayedi F. 2013. Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds, *Food Chemistry*, 138: 1448-1453. DOI: doi.org/10.1016/j.foodchem.2012.11.003

Florkiewicz A., Socha R., Filipiak-Florkiewicz A. & Topolska K. 2018. Sous-vide technique as an alternative to traditional cooking methods in the context of antioxidant properties of Brassica vegetables, *Journal of the Science of Food and Agriculture.* 99 (1): 173-182. DOI: doi.org/10.1002/jsfa.9158

Fogliano V., Verde V., Randazzo G. & Ritieni A. 1999. Method for Measuring Antioxidant Activity and Its Application to Monitoring the Antioxidant Capacity of Wines, J. Agric., *Food Chem.* 47 (3): 1035–1040. DOI: doi.org/10.1021/jf980496s

Lushcha V.I. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification, *Chem Biol Interact.,* 224: 164-75. DOI: doi.org/10.1016/j.cbi.2014.10.016

Gülçin İ. 2012. Antioxidant Activity of Food Constituents: on Overview, *Arch Toxicol,* 86: 345-391. DOI: doi.org/10.1007/s00204-011-0774-2

Gülçin, İ. & Köksal, E., (2008). "Antioxidant Activity of Cauliflower (Brassica oleracea L.)", *Turkish Journal of Agriculture and Forestry,* 32: 65-78. DOI: Not available.

Koca N. & Karadeniz F. 2003. Serbest Radikal Oluşum Mekanizmaları ve Vücuttaki Antioksidan Savunma Sistemleri, *Gıda Mühendisliği Dergisi,* 16: 27-32. DOI: Not available.

Kocaçalışkan İ. & Kadıoğlu A., 1990. Bitki fizyolojisi laboratuvar kılavuzu. *Atatürk Üniversitesi Fen-Edebiyat Fakültesi Yayınları*, 1: 119. DOI: Not available.

Lobo V., Patil A., Phatak A. & Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacogn Rev*, 4(8): 118–126. DOI: 10.4103/0973-7847.70902

Marcocci L., Maguire J.J., Droylefaix M.T. & Packer L. 1994. The Nitric Oxide-Scavenging Properties of Ginkgo Biloba Extract EGb 761, *Biochemical and Biophysical Research Communications*, 201 (2): 748-755. DOI: doi.org/10.1006/bbrc.1994.1764

Min D.B. & Boff J.M. 1998. Food Lipids: Chemistry, Nutrition, and Biotechnology. CRC Press, New York, pp. 335-347.

Nawaz H., Shad M.A. & Rauf A., 2018. Optimization of extraction yield and antioxidant properties of Brassica oleracea Convar Capitata Var L. leaf extracts., *Food Chemistry,* 242: 182-187. DOI: doi.org/10.1016/j.foodchem.2017.09.041

Osawa T. & Namiki M. 1980. A Novel Type of Antioxidant Isolated from Leaf Wax of Eucalyptus leaves, *Journal Agricultural and Biological Chemistry*, 45 (3): 735-739. DOI: doi.org/10.1080/00021369.1981.10864583

Oyaizu M., 1986. Studies on products of browning reaction prepared from glucose amine, *Japanese Journal of Nutrition*, 44: 307–315. DOI: Not available.

Padilla G., Cartea M. E., Rodríguez V. M. & Ordás, A. 2005. Genetic diversity in a germplasm collection of Brassica rapa subsp rapa L. from northwestern Spain, *Euphytica*, 145 (1-2): 171–180. DOI: doi.org/10.1007/s10681-005-0895-x

Padmavati M., Sakthivel N., Thara K. V. & Reddy A. R., 1997. Differential sensitivity of rice pathogens to growth inhibition by flavonoids, *Phytochemistry*, 46 (3): Pages 499-502. DOI: doi.org/10.1016/S0031-9422(97)00325-7

Peksel A., Imamoglu S., Kıymaz N. & Orhan N., 2013. Antioxidant and radical scavenging activities of Asphodelus aestivus Brot. extracts, *Internatıonal Journal of Food Properties*, 16 (6): 1339-1350. DOI: doi.org/10.1080/10942912.2011.587622

Podsędek A., 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review, LWT - *Food Science and Technology*, 40: 1-11. DOI: doi.org/10.1016/j.lwt.2005.07.023

Singh J., Upadhyay A. K., Bahadur A., Singh B., Singh K. P. & Rai, M. 2006. Antioxidant phytochemicals in cabbage (Brassica oleracea L. var. capitata), *Scientia Horticulturae*, 108 (3): 233-237. DOI: doi.org/10.1016/j.scienta.2006.01.017

Slinkard K. & Singleton V. L. 1977. Total Phenol Analysis: Automation and Comparison with Manual Methods, *Am J Enol Vitic*., 28: 49-55. DOI: Not available.

Soengas P., Cartea M. E., Velasco P. & Francisco M., 2018. Endogenous Circadian Rhythms in Polyphenolic Composition Induce Changes in Antioxidant Properties in Brassica Cultivars, *Journal of Agricultural and Food Chemistry*, 66 (24): 5984–5991. DOI: doi.org/10.1021/acs.jafc.8b01732

Volden J., Borge G. I. A., Bengtsson G. B., Hansen M., Thygesen I. E. & Wicklund T. 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (Brassica oleracea L. ssp. capitata f. rubra), *Food Chemistry*, 109(3): 595–605. DOI: doi.org/10.1016/j.foodchem.2008.01.010

Zhishen J., Mengcheng T. & Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chemistry*, 64 (4): 555-559. DOI: doi.org/10.1016/S0308-8146(98)00102-2.