International Journal of Nature and Life Sciences

IJNLS

https://dergipark.org.tr/tr/pub/ijnls

e-ISSN: 2602-2397 https://doi.org/10.47947/ijnls.1338186

Article

Determination of Antioxidant Activities in Raw and Boiled Extractions of *Raphanus raphanistrum* L. Species Naturally Growing in Edremit Gulf (Balıkesir/Turkey)

Hatice İnci Aladi[1], Tülin Aşkun[2], & Selami Selvi[3]*

[1] Balıkesir University, Institute of Science, Biology Department of Presidency, Balıkesir, Türkiye; haticealadi10@gmail.com.tr; https://orcid.org/0000-0003-2605-4018

[2] Balıkesir University, Faculty of Arts and Sciences, Department of Biology, Balıkesir, Türkiye; tulaskun@yahoo.com; https://orcid.org/0000-0002-2700-1965;

[3] Department of Plant and Animal Production Medical and Aromatic Plants Programme, Altınoluk Vocational School, Balıkesir University, 10870, Balıkesir, Türkiye; https://orcid.org/0000-0002-9959-6945

* Corresponding autor: sselvi2000@yahoo.com

Abstract: The plants that naturally spread in Edremit Gulf are consumed by the local people in various ways either cooked or raw. In this study, *Raphanus raphanistrum* species from the Brassicaceae family, which is also known to be used as food, was used. DPPH and CUPRAC antioxidant activity analyses were carried out on the extracts obtained from the aerial parts of the raw and boiled fresh plant. The total phenolic contents were determined and the phenolic compounds contained and their interactions with the boiling process were determined by HPLC method. The extracts were stored in a deep freezer at -20 °C to be used in further studies. In terms of the total amount of phenolic compounds, 12.65 µg GAE/mg extract was detected in the boiled extract of *R. raphanistrum* species, and it has a higher value compared to the raw extract. In the boiled extract, which has a higher value in terms of total flavonoid content than raw, was found 54 µg Rutin Equivalent /mg fresh extract. Compared to the CUPRAC method, the boiled extract showed higher (68.58 µgTE/mg extract) activity. The antioxidant activity of the boiled extract was found to be higher than the DPPH method, and the EC 50 value was measured as 1975.25 µg/mL. According to HPLC method, resveratrol, rosmarinic acid, quersetin, kaempferol and naringenin substances were determined as in the raw extract. It was determined that while naringenin was destroyed in the boiled extract, were changed the amounts of other phenolic compounds.

Keywords: Antioxidant activity, Edremit Gulf, Flavonoid, Phenolic, Raw and boiled extract

Citation: İnci Aladi, H., Aşkun, T., & Selvi, S. (2023). Determination of antioxidant activities in raw and boiled extractions of *Raphanus raphanistrum* L. species naturally growing in Edremit Gulf (Balıkesir/Turkey). International Journal of Nature and Life Sciences, 7 (2), 65-78.

Received: Agust 05, 2023
Accepted: September 09, 2023
Online Published: September 19, 2023

1. Introduction

Turkey is one of the countries with very rich flora in the world with around 12000 plant taxa due to its geographical location, geomorphological structure, various soil types and being under the influence of different climate types (Polat and Selvi, 2011). Edremit Gulf, geographically forming the north of the Aegean Region, is an important region located on the shores of Çanakkale and Balıkesir provinces, including various tourism centres and Kazdağları (Polat and Selvi, 2011).

The genus *Raphanus* L. is represented in the world by 4 species (5 taxa); It is represented by 2 species (5 taxa) in Turkey (The Plant List 2010; Güner et al. 2012). One of the important species of the genus *Raphanus* is *R. raphanistrum* L. The roots and leaves of this species, known as Wild Radish (English) or Yabani turp (Turkish), are consumed in some countries, including Turkey, for food purposes (Baytop 1999; Aladı et al., 2022).

The use of natural plant species in the diet has increased as they are rich in minerals, fibre, vitamins and antioxidants. According to the research, the medicinal values of these natural plants are increasing day by day due to their antibacterial, anticarcinogenic, antidiabetic, and protective properties of the liver, and cardiovascular system (Bolkent et al., 2001; Yıldırım et al., 2001; Onar-Çelik et al., 2012).

The effects of some secondary compounds found in natural plants on free radicals, have attracted the attention of scientists and has led to an increase in research on these subjects. Reactive oxygen species, which are among the most important free radicals, are produced naturally during the vital functions of cells. Cells have developed various defence

mechanisms to protect themselves from the harmful effects of these free radicals. These defense mechanisms, which are briefly called antioxidants, are generally examined in two parts enzymatic and non-enzymatic.

Enzymatic antioxidants consist of enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Benzie, 2000). Non-enzymatic antioxidants are mainly composed of compounds such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene, phenolic compounds, glutathione, cysteine, lipoic acid and uric acid (Battin and Brumaghim, 2009).

In epidemiological studies, it has been determined that herbal components are quite beneficial against reactive oxygen species; It has been stated that the protective effects of fruits and vegetables are caused by natural compounds such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoids, sulfur compounds (glutathione, cysteine, methionine), flavonoids and phenolic acids (Halvorsen et al., 2002).

There are about twenty analytical methods developed for the determination of the capacity of antioxidants in natural plants. Literature studies show that the antioxidant capacity of an antioxidant compound measured by one method may differ from that measured by another method (Huang et al., 2005).

This study covers a part of the doctoral thesis, and it aimes to compare the antioxidant activity analyses and total phenolic and flavonoid substance contents in the raw and after-boiling extracts of the *R. raphanistrum*, as well as to determine the phenolic compounds it contains by HPLC method and to determine the interaction of these compounds with the boiling process.

2. Materials and Methods

2.1 Plant Materials

The *R. raphanistrum* species used in the study were obtained from the rural area of the Çamdibi district of Havran, located within the borders of Edremit Gulf, before blooming in autumn. Fresh leaves and above-ground parts were used for the plant. Identification of the plant sample was made using Flora of Turkey. Identification of the plant sample was made using one volume of Flora of Turkey (Davis, 1965). The flowering habitus of the plant and its basal leaves are shown in Figure 1.

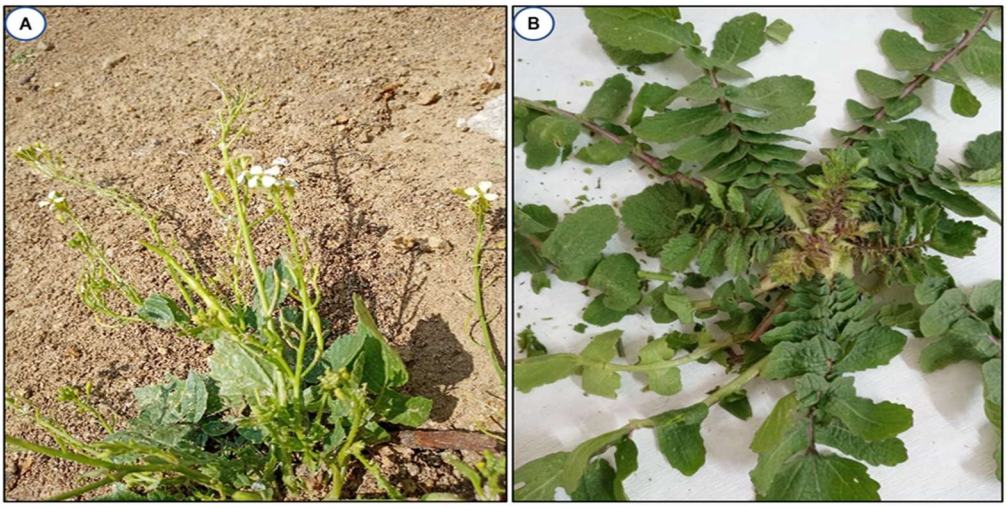

Figure 1. *R. raphanistrum*; A) Habitus, B) Basal leaves.

2.2.Chemicals and Reagents

1% Na_2CO_3 solution (Sigma-Aldric, S7795), 5% $NaNO_2$ ($NaNO_2$, Merck 6544) solution, 10% $AlCl_3$ ($AlCl_3$, Merck 1064), 1 M NaOH (NaOH, Merck 6462), Gallic acid (Sigma-Aldrich, G7384), Rutin (Merck, 153-18-4), Copper (II) Chloride Solution 10 mM(Merck), 1 M Ammonium acetate(Merck, 101116.1000), Neocuproin 7.5 mM (sigma-Aldrich, N1501), Trolox 1 mM (Cayman chemical), Folin-Ciocalteu reagent (Sigma-aldrich, 47641), DPPH (AB 211798, 2.2-Diphenyl-1-picrylhydrazl), Methanol (Sigma-aldrich 24229).

2.3.Preparation of Herbal Extracts

The basal leaves and aerial parts of the plants were used fresh. The plants were washed with tap water and dried by draining. It was cut into small pieces with the help of a plastic knife. For the raw extraction of the plants, 20 g of the fresh and chopped parts were weighed and placed in 200 ml of 96% ethanol. The plants were kept in the dark at room temperature for 17-20 days.

For boiled extractions, 50 g of chopped plant samples were boiled in 75 ml boiling water at 100 °C for 5 minutes in a closed pot (Oboh, 2004; Turkmen and Velioğlu, 2005). The plants, whose water was filtered with the help of a strainer, were quickly cooled on the ice tray. 20 grams of the plants were weighed and kept in 200 ml of 96% ethanol for 17-20 days in the dark at room temperature.

The solvents of the plant samples were removed using a rotary evaporator at 45-50 °C. The obtained extracts were kept in a fume hood for one or two nights until they were completely removed from their solvents. The obtained plant extracts were stored in a deep freezer at -20 °C for later use to determine the total amount of phenolic and flavonoid substances, to perform antioxidant activity analyzes (DPPH, CUPRAC) and to perform the HPLC analysis method.

2.4.Determination of Total Phenolic Compound Quantity

The total amount of phenolic substances in the plant extracts was determined according to the method developed by Slinkard and Sigleton (1977). In the study, gallic acid (25-200 µg/mL) solution prepared with methanol was used as a standard substance to draw a calibration curve.

The concentration of plant extracts was prepared to be 1 mg/mL. 250 µL of each of the plant extracts and gallic acid dilutions were taken into separate test tubes.

Then, 1 mL of Folin&Ciocalteu reagent (diluted 1:9) was added to each tube. Then, 750 µl of 1% Na_2CO_3 solution was added to each tube. After the mixtures were kept in the dark at room temperature for 2 hours, the absorbance against the blank was read at 765 nm in the spectrophotometer (Thermo Scientific Multiskan GO). Methanol was used as a blank. Experiments were done in triplicate repetitions. The phenolic content of the plants was given as gallic acid equivalent (µg GAE/mg fresh extract) (Slinkard and Singleton, 1977).

2.5.Determination of Total Flavonoid Amount

The total amount of flavonoids in plant extracts Zhishen et al. (1999) was determined according to the method developed. In the study, it was routinely used as a standard flavonoid compound. Dilutions of 25-300 µg/mL were prepared to draw the calibration chart.

0.25 mL was taken from these solutions and plant extracts at a concentration of 1 mg/mL. 1.25 mL of distilled water and 75 µL of 5 % $NaNO_2$ were added to it. It was kept at room temperature for 6 minutes. 150µL of 10 % $AlCl_3$ solution was added and left for five more minutes. After adding 0.5 mL of 1 M NaOH solution and 275 µL of distilled water, the tubes were thoroughly mixed by vortexing. Absorbance values at 510 nm were read against the blank. The flavonoid

content of the extracts was expressed as rutin equivalent (µg Rutin/mg fresh extract). Experiments were done in three repetitions.

2.6. Cu(II) ion reducing antioxidant capacity method (CUPRAC)

The method developed by Apak et al. (2004) was used to determine the antioxidant activity values of the extracts obtained from plant samples by the CUPRAC method. In this method, were used 10 mM Copper (II) Chloride Solution (in distilled water), $7.5x10^{-3}$ M Neocuproin Solution (in 96% ethanol), 1 M Ammonium Acetate (CH_3COONH_4) Buffer Solution (pH: 7 in deionized water).

1 mL of each solution was taken into the test tubes prepared for the analyses, 0.5 mL of sample extract and 0.6 mL of deionized water were added, and the final volume was adjusted to 4.1 mL. The mixtures were kept in the dark for 30 minutes and the absorbance values were measured at 450 nm in a spectrophotometer.

The same procedures were repeated using deionized water instead of the sample for the blank. Trolox solution prepared in the range of 25-250 µg was used for the calibration curve. The CUPRAC antioxidant capacity values of the extracts were calculated as µmol Trolox equivalent/mg sample using the calibration equation (Apak et al., 2006).

2.7. DPPH Activity Analysis

This study was carried out with some modifications to the antioxidant activity determination method with the DPPH radical capture method developed by Kumaran and Karunakaran (2007). Plant extract and standard substance (50 µL) were added to 450 µl of a 0.004% MeOH solution of DPPH. Absorbance at 519 nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard.

2.8. HPLC Conditions

HPLC was performed using a Shimadzu HPLC device, according to preparation techniques for phenolic compounds (Caponio, Alloggio, Gomes, 1999). The following equipment was used for reverse-phase chromatography: DAD detector (Imax = 278) and SIL–10AD vp auto-sampler. The system controller was SCL-10Avp, the pump was LC- 10ADvp and the degasser was DGU- 14A. The column oven was CTO-10Avp and the column was Agilent Zorbax EclipseXDB-C18 (250 x 4.60 mm) 5 µm. Mobil phases were A: 3% acetic acid and B: methanol and the flow speed was 0.8 mL/minute. The column temperature was 30 ºC and the injection volume was 20 µl.

Chromatogram standards were gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, vitexin, rutin, naringin, hesperidine, apigenin-7-glucoside, rosmarinic acid, eriodictyol, quercetin, naringenin, luteolin, apigenin, and carvacrol.

The standard chromatogram of phenolics according to their retention time is shown in Figure 1, and the list of standard phenolics and their retention times are shown in Table 1.

Table 1. The list of standard phenolics and retention times.

	Phenolics standard	Retention times
1.	Gallic Acid	9.00
2.	Chlorogenic Acid	27.52
3.	4hydroxybenzaldehide	29.36
4.	Vanilic Acid	33.00
5.	Cafeic Acid	33.66
6.	Epicatechin	34.44
7.	Syringic Acid	37.26
8.	p-Coumaric Acid	40.70
9.	Echinocoside	41.89
10.	Ferulic Acid	43.16
11.	Ursolic Acid	46.41
12.	Naringin	48.10
13.	Benzoic Acid	48.14
14.	2-Hydroxycinnamic Acid	48.73
15.	Hesperidin	49.55
16.	Rutin Hydrate	50.42
17.	Resveratrol	52.24
18.	Apigenin-7-Glukozit	52.45
19.	Rosmarinic Acid	52.89
20.	Juglon	57.86
21.	Transcinnamic Acid	59.12
22.	Quercetine	61.74
23.	Naringenin	62.92
24.	Kaempferol	68.25

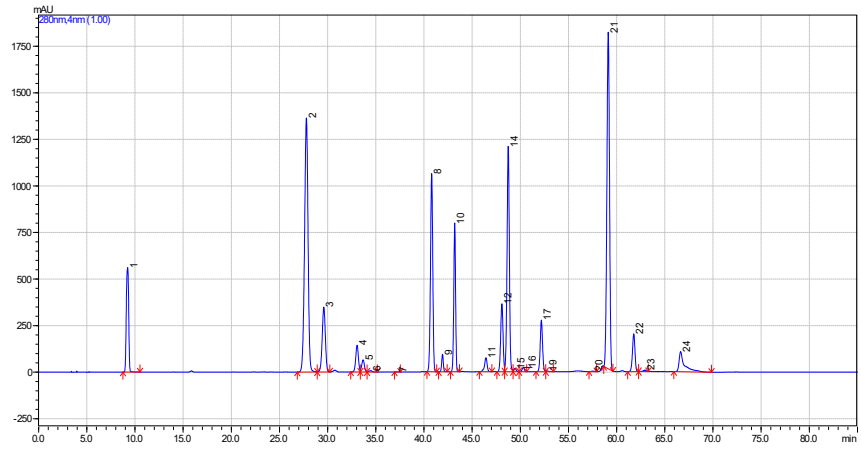

Figure 2. Standard chromatogram of the phenolics according to retention time.

3. Results

Antioxidant activity data were examined of raw and boiled extracts of *R. raphanistrum*, which we used in our study. At the end of the extraction processes, the percent extraction yields of the extracts obtained from the samples were calculated and given in Table 2. When the raw and boiled extracts of the plant were compared, it was determined that the % yield values changed. In addition, 50 g of the plant sample was boiled for 5 minutes; then, the water was filtered, dried and weighed again. According to the results obtained, a loss of 93.98% occurred in the weight of the boiled plant.

3.1. Total Phenolic Compound Analysis

In this study, the total amount of phenolic compounds in plant extracts that were raw and boiled for 5 minutes was determined (Slinkard and Sigleton, 1977). The total amount of phenolic compounds was calculated as µg gallic acid /mg fresh extract with the help of the standard graph (Figure 3) prepared by taking the gallic acid equivalent.

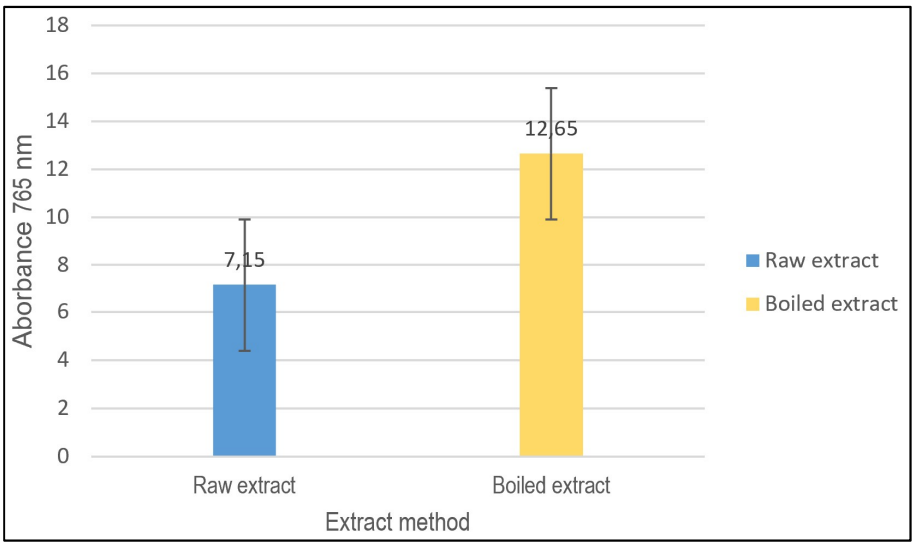

Figure 3. The phenolic content of plant extracts with gallic acid equivalent (µg/mg extract).

In the Figure 3, it is seen that the total amount of phenolic substances is higher in the boiled extract of the *R. raphanistrum.*

3.2. Total Flavonoid Substance Analysis

The total amount of flavonoids in plant extracts Zhishen et al. (1999) was determined according to the method developed. In the figure, it is seen that the total amount of flavonoid substances is higher in the boiled extract of the *R. raphanistrum* (Figure 4).

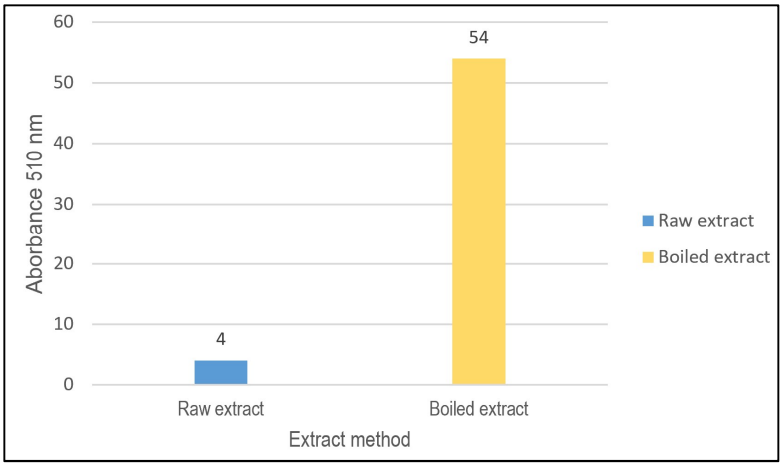

Figure 4. The total amount of flavonoid substances, which is the Rutin equivalent (µg /mg) of the plant extracts.

3.3. Antioxidant Activity Analysis by CUPRAC Method

The method developed by Apak et al (2004) was used to determine the antioxidant activity values of the extracts obtained from plant samples by the CUPRAC method.

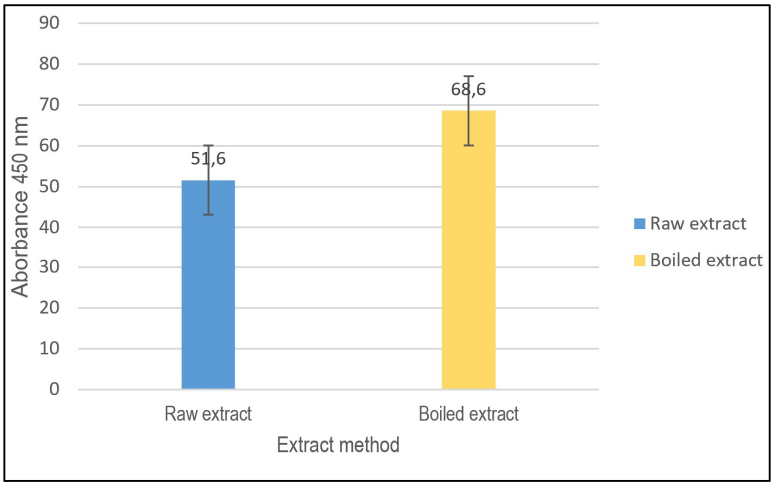

Figure 5. Trolox equivalents of plant extracts according to the CUPRAC method

In the Figure 5, it is seen that the antioxidant activity capacity of the raw extract of *R. raphanistrum* is higher according to the CUPRAC method, while the activity decreases after boiling.

3.4 DPPH Analysis Method

EC 50 values were calculated by drawing the Trolox inhibition graph according to the DPPH analysis method.

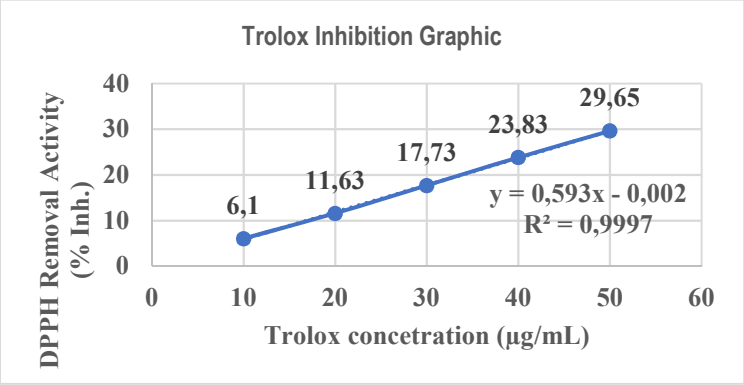

Figure 6. DPPH removal activity of Trolox dilutions.

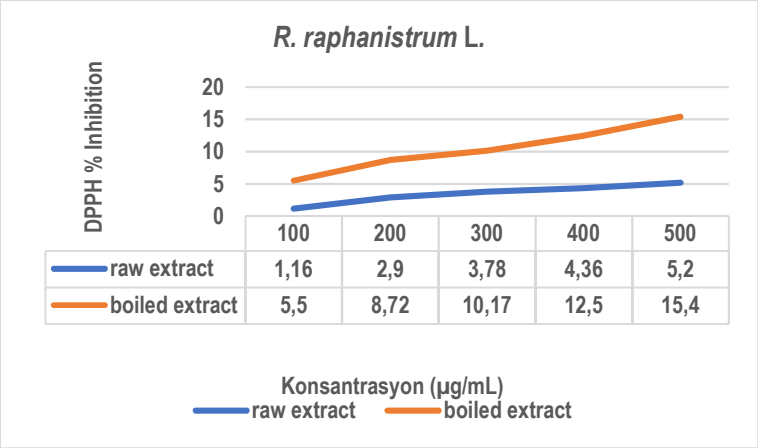

Figure 7. DPPH removal activity of plant extracts.

The EC 50 value of Trolox was calculated as 84.313 µg/mL from the Trolox inhibition graph shown in Figure 6. EC 50 values of raw and boiled extracts of *R. raphanistrum* are calculated from the graph in Figure 7 and shown in Table 2. The EC 50 value of the raw extract was calculated as 5198 µg/mL, and the EC 50 value of the boiled extract was calculated as 1975 µg/mL. As seen in the Figure 7, DPPH radical scavenging activity increased after was boiled the plant.

Table 2. Antioxidant capacity, extraction % yield of plant extracts.

Species name	Extract form	Total amount of phenolic substance µgGAE/mg extract	Total flavonoid substance content µgRE/mg extract.	Antioxidant capacity method (CUPRAC µgTE/mg extract	DPPH Analysis EC 50 value (µg/mL)	% yields of plant extracts
R. raphanistrum L.	Raw	7.15	4	51.6	5198.1	2.91
	Boiled	12.65	54	68.58	1975.25	2.29

3.5. HPLC (high performance liquid chromatography) analysis

3.5.1. R. raphanistrum L.

HPLC analysis was performed with raw and boiled extracts of *R. raphanistrum.* The measured values of phenolic compounds in raw and boiled extracts are given in Table 3.

Table 3. Raw and boiled extract of HPLC analysis of *R. raphanistrum* in 100 ppm.

No	Phenolic compounds	Raw extract 100 ppm (Units mg/mL)			Boiled extract 100 ppm (Units mg/mL)		
		Ret. Time	Area	Conc.	Ret. Time	Area	Conc.
1	Gallic Acid	0	0	0	0	0	0
2	Chlorogenic acid	0	0	0	0	0	0
3	4hydroxybenzaldeh	0	0	0	0	0	0
4	Vanilic Acid	0	0	0	0	0	0
5	Cafeic Acid	0	0	0	0	0	0
6	Epicatechin	0	0	0	0	0	0
7	Syringic acid	0	0	0	0	0	0
8	Echinocoside	0	0	0	0	0	0
9	p-coumaric Acid	0	0	0	0	0	0
10	Ferulic Acid	0	0	0	0	0	0
11	Benzoic Acid	0	0	0	0	0	0
12	2-Hydroxycinnamic	0	0	0	0	0	0
13	Hesperidine	0	0	0	0	0	0
14	Rutin hydrate	0	0	0	0	0	0
15	Resveratrol	52.213	13558	0.582	51.989	1510	0.532
16	Rosmarinic Acid	53.327	204977	48.288	54.113	50135	12.493
17	Transcinnamic acid	0	0	0	0	0	0
18	Quercetine	61.364	43122	0.583	61.963	142908	1.110
19	Naringenin	64.016	4764	2.942	0	0	0
20	Ursolic acid	0	0	0	0	0	0
21	Apigenin 7 glucosid	0	0	0	0	0	0
22	Juglon	0	0	0	0	0	0
23	Tyramine	0	0	0	0	0	0
24	Naringin	0	0	0	0	0	0
25	Kaempferol	67.613	1031312	1.567	68.178	666845	0.593

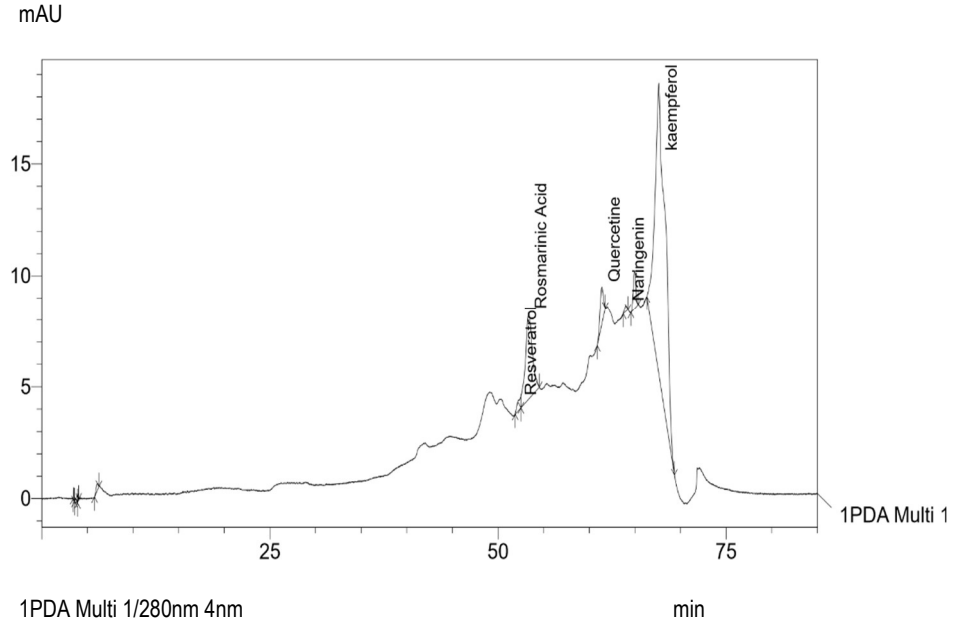

Figure 8. HPLC chromatogram obtained from 100 ppm raw extracts of *R. raphanistrum.*

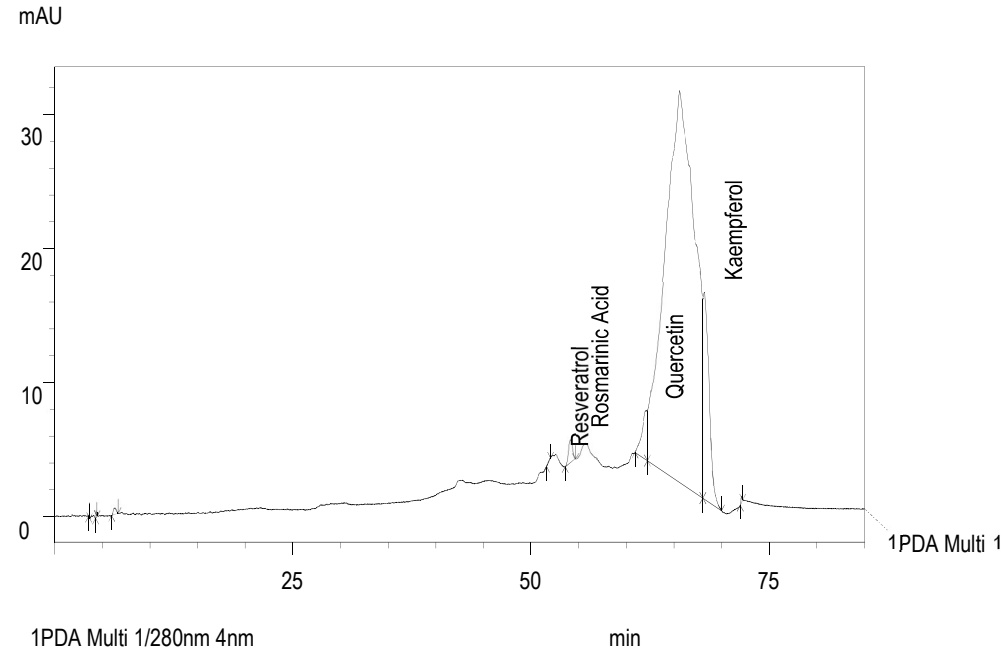

Figure 9. HPLC chromatogram obtained from 100 ppm boiled extracts of *R. raphanistrum.*

Resveratrol, rosmarinic acid, quersetin, kaempferol and naringenin substances were determined in *R. raphanistrum* 100 ppm raw extract (Figure 8). After boiling, naringenin was destroyed. The amount of quercetin slightly increased, while

decreased the amount of rosmarinic acid and kaempferol. There was a slight decrease in the amount of resveratrol (Figure 9). The measured values of phenolic compounds in raw and boiled extracts are given in Table 3.

4. Discussion and Conclusions

In recent years, interest in phenolic compounds, especially flavonoids, has increased considerably due to their strong antioxidant effects that protect plants from adverse environmental conditions. These polyphenolic phytochemicals, which are found in widely consumed plants, have many biological properties such as antibacterial, hepatoprotective, anti-inflammatory, anticancer and antiviral agents that support human and animal health and help reduce the risk of disease. Studies have shown that in addition to these properties, almost every flavonoid group has antioxidant activity capacity. Antioxidant mechanisms of action, on the other hand, provide the regulation or protection of antioxidant defences by chelating trace elements that function in the formation of free radicals or by inhibiting the formation of ROS and scavenging ROS (Kumar and Pandey, 2013, Kopustinskiene et al., 2020, Shi et al., 2021). Flavonoids in foods are generally responsible for color, taste, prevention of fat oxidation and preservation of vitamins and enzymes (Kumar and Pandey, 2013).

This study reveals the changes in total phenolic and flavonoid substance amounts in the raw and after-boiled extracts of the *R. raphanistrum* consumed as food. In addition, CUPRAC and DPPH methods were performed to detect changes in antioxidant activity. It was also determined how the phenolic compounds contained in it were affected by the boiling process by the HPLC analysis method.

According to the results obtained in our study, it was observed that the boiled extracts of the *R. raphanisrum* were higher in total phenolic (12.65 µgGAE/mg extract) and flavonoid (54 µgRE/mg extract) substances compared to the raw extract. The total phenolic content of the raw extracts was determined as 7.15 µgGAE/mg extract and the total flavonoid substance content was 4 µgRE/mg extract. According to the Cuprac antioxidant analysis results, the activity of the boiled extract (68.58 µgTE/mg extract) was found to be higher than the raw extract (51.6 µgTE/mg extract). It was determined that the boiled extract showed higher activity in terms of DPPH radical scavenging activity.

According to the studies, several complex events occur during heat treatment that change the quality characteristics of the food such as sensory properties and nutritional properties. The structure and composition of the food, the type of heat treatment applied and the temperature can cause an increase in the amount of phenolic compounds (Sakac et al., 2011). Depending on the degree and duration of the heat treatment applied, pro-oxidant and antioxidant molecules may be formed. It is stated that during heat treatment, antioxidants naturally found in food may deteriorate and new components with antioxidant activity may be formed. These new antioxidant compounds are formed during Maillard reactions, especially in the initial phase. The degree and duration of the applied heat treatment can change the antioxidant properties (Calligaris et al., 2004).

In a study by Volden et al (2008), it was revealed that there was a 59% decrease in the anthocyanin content of red cabbage after boiling. In a study, were investigated the effects of boiling and steaming carrots and cauliflower on phenolic compounds such as caffeic, sinapic, p-coumaric, chlorogenic and ferulic acids (Mazzeo et al., 2011). In this study, it was determined that the boiling process harmed phenolics other than p-coumaric acid in carrots. Boiling destroyed almost all of the phenolic compounds in carrots. In the same study, it was determined that phenolic components other than caffeic acid in carrots increased with the steaming process. As a result of the research, it was determined that there was an increase in the amount of phenolic components in the steamed cauliflower.

According to the HPLC analysis results in our study, resveratrol, rosmarinic acid, quercetin, kaempferol and naringenin substances were determined in *R. raphanistrum* 100 ppm raw plant extract. After boiling, naringenin was destroyed. The amount of quercetin slightly increased, while decreasing the amount of kaempferol and resveratrol. The

amount of rosmarinic acid decreased by almost four times with the boiling process and its concentration decreased from 48.288 to 12.493 mg/mL.

Studies on the cytotoxic effects of phenolic and flavonoid compounds on various types of cancer are increasing day by day. It has been stated that genistein, daidzein, resveratrol, baikalein, flavopridol, apigenin and catechins, which are plant-derived natural compounds whose anticarcinogenic effects have been widely studied, may be important therapeutic agents in the treatment and protection of pancreatic cancer (Roginsky et al. 2005).

It was observed that the plants we used in the study were rich in phenolic compounds and flavonoid substances, and this richness became more diversified with the formation of different new compounds when boiled. We can say that these natural plants may have protective effects against many diseases such as cancer, diabetes and heart diseases, due to the intake of different compounds when consumed both raw and cooked. Considering the bioavailability of phenolic and flavonoid substances, we can think that they can be used as an adjunct to treatment due to their anticancer effects.

An increase in the total phenolic and flavonoid substance amounts was observed by boiling the plant for five minutes. In addition, antioxidant activity (CUPRAC and DPPH) capacity also increased. According to the HPLC analysis results, the presence of new substances was not observed in response to the substances destroyed by the blanching process.

In line with the studies, it is possible to summarize the increase in antioxidant activity as the inactivation of oxidative enzymes that prevent antioxidant activity by heat treatment, the destruction of the cell wall by heat treatment, the release of insoluble phenolic compounds and the formation of new antioxidant compounds with heat treatment (Pinelo et al., 2005).

However, studies have also shown that temperatures above 200 ºC damage phenolic compounds (Kim et al., 2006). More studies are needed to explain the effect of heat treatment on these bioactive compounds.

As can be seen from the studies conducted, it has been revealed that there are differences between the consumption of raw foods and the consumption of them cooked in terms of the various compounds they contain. In terms of antioxidant activity, we can say that the plant we studied is more suitable to be consumed by boiling.

Conflict of Interest

The author have no conflict of interest to declare.

Financial Disclosure

Author declare no financial support.

Authors' Contributions

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

Acknowledgements: This study includes a part of the doctoral thesis titled "Determination of antioxidant activities in raw and boiled extractions of some natural edible plants in Edremit Gulf (Balikesir)". Funded by the BAP (Balıkesir University Scientific Research Projects Unit) project no 2022/087.

References

1. Aladı, H. İ., Satıl, F., & Selvi, S. (2022). Yenilebilir doğal bitkilerin etnobotanik ve gastronomik açıdan değerlendirilmesi: Edremit Körfezi (Balıkesir) örneği . Journal of the Institute of Science and Technology, 12 (3), 1375-1385. https://doi.org/10.21597/jist.1085847
2. Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel antioxidant capacity index for dietary polyphenols and vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry, 52, 7970-7981.
3. Apak, R., Güçlü, K., Özyürek, M., Karademir, S. E., & Erçag, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. International Journal of Food Sciences and Nutrition, 57, 292-304.
4. Battin, E. E., & Brumaghim, J. L. (2009). Antioxidant activity of sulfur and selenium: A review of reactive oxygen species scavenging, glutathione peroxidases, and metal-binding antioxidant mechanisms. Cell Biochemistry and Biophysics, 55, 1-23.

5. Baytop, T. (1999). *Türkiye' de Bitkiler ile Tedavi* (ilaveli ikinci baskı). İstanbul: Nobel Tıp Kitabevi, 193–194.
6. Benzie, I. F. F. (2000). Evaluation of antioxidant defense mechanisms. European Journal of Nutrition, 39, 53-61.
7. Bolkent, S., Yanardağ, R., & Karabulut-Bulan, O. (2001). The effects of *Melissa officinalis* on liver of hyperlipidaemic rats: A morphological and biochemical study. Proceedings 10th Scientific Conference Electron Microscopy Society of Malaysia, 8-10th November, Kuala Lumpur.
8. Calligaris, S., Manzocco, L., Anese, M., & Nicoli, M.C. (2004). Effect of heat treatment on the antioxidant and pro-oxidant activity of milk. International Dairy Journal, 14, 421-427.
9. Caponio, F., Alloggio, V., & Gomes T. (1999): Phenolic compounds of virgin olive oil: Influence of paste preparation techniques, Food Chemistry, 64, 203–204.
10. Davis, P. H (Ed.). (1965). *Flora of Turkey and the East Aegean Islands* (Vol. 7). Edinburgh UK.: Edinburgh University Press.
11. Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M. T. (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler).* İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını.
12. Halvorsen B. L., Holte K., Myhrstad M. C. W., Barikmo I., Hvattum E., Remberg S. F., Wold, A. B., Haffner, K., Baugerod, H., Andersen, L. F., Moskaug, Q., Jacobs, D. R., & Blomhoff, R. (2002). "A systematic screening of total antioxidants in dietary plants. The Journal of Nutrition, 132 (3), 461-471.
13. Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53 (6), 1841-1856.
14. Kim, S. Y., Jeong, S. M., Park, W. P., Nam, K. C., Ahna, D. U., & Lee, S. C. (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extract. Food Chemistry, 97, 472-479.
15. Kopustinskiene, D. M., Jakstas, V., Savickas, A., & Bernatoniene, J. (2020). Flavonoids as anticancer agents. Nutrients, 12 (2), 457. https://doi.org/10.3390/nu12020457.
16. Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal, 162750. https://doi.org/10.1155/2013/162750.
17. Kumaran, A., & Karunakaran, J.R. (2007). In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. Food Science and Technology, 40, 344–352.
18. Mazzeo, T., N Dri, D., Chiavaro, E., Visconti, A., Fogliano, V. (2011). Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetables. Food Chemistry, 128, 627-633.
19. Oboh, G. (2004). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. LWT - Food Science and Technology, 38 (5), 513–517.
20. Onar-Celik, H., Yusufoğlu, A., Türker, G., & Yanardağ, R. (2012). Elastase, tyrosinase and lipoxgenase inhibition and antioxidant activity of an aqueous extract from *Epilobium angustifolium* L. leaves. Journal of Medicinal Plants Research, 6, 716-726.
21. Pinelo, M., Rubilar, M., Sinerio, J., & Nunez, M.J. (2005). A thermal treatment to increase the antioxidant capacity of natural phenols: catechin, resveratrol and grape extract cases. European Food Research and Technology, 221, 284-290.
22. Polat, R., & Selvi, S. (2011). Edremit Körfezi'nin (Balıkesir) arı bitkileri üzerine bir araştırma. Harran Üniversitesi Ziraat Fakültesi Dergisi, 15(2), 27-32.
23. Roginsky A. B., Ujiki M.B, Ding X. Z., & Adrian T. E. (2005). On the potential use of flavonoids in the treatment and prevention of pancreatic cancer. In Vivo, 219, 61-67.
24. Sakac, M., Torbica, A., Sedej, I., & Hadnadev, M. (2011). Influence of breadmaking on antioxidant capacity of gluten free breads based on rice and buckwheat flours. Food Research International, 44, 2806- 2813.
25. Shi, S., Li, J., Zhao, X., Liu, Q., & Song, S. J. (2021). A comprehensive review: Biological activity, modification and synthetic methodologies of prenylated flavonoids. Phytochemistry, 191, 112895. https://doi.org/10.1016/ j.phytochem.2021.112895
26. Slinkard, K., & Singleton, V. L. (1977). Total phenol analyses: automation and comparison with manual methods. American Journal of Enology and Viticulture, 28, 49-55.
27. The Plant List (2010). Version 1. Published on the Internet; http://www.theplantlist.org/ (accessed 1 September 2023).

28. Turkmen, N., Sari, F., & Velioglu, Y.S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry, 93, 713-718.
29. 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, 595–605.
30. Yıldırım, E., Dursun, A., & Turan, M. (2001). Determination of the nutrition contents of the wild plants used as vegetables in upper Çoruh Valley, Turkish Journal of Botany, 25, 367-371.
31. Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559.