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COMPARISON OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITIES OF ETHANOL AND METHANOL EXTRACTS OF *Pistacia vera* L., *Prunus dulcis* AND *Rhus coriaria* L. LEAVES

Lale DUYSAK[1]*, Barış ÖZORMAN[1]

[1]*Atatürk University, Faculty of Pharmacy, Department of Biochemistry, 25030, Erzurum, Türkiye*

Abstract: *Pistacia vera* L., *Prunus dulcis*, and *Rhus coriaria* L. are among the plants frequently cultivated in the Gaziantep region of Turkey. The aim of this study is to determine the total phenolic content of ethanol and methanol extracts of the leaves of these three plants at different concentrations using DPPH (1,1-diphenyl-2-picrylhydrazyl), FRAP (Ferric Ion Reducing Antioxidant Power), and CUPRAC (Cu^{2+} Ion Reducing) methods. Ethanol and methanol extracts were obtained from the leaves of Pistacia vera L., Prunus dulcis, and Rhus coriaria L. The Folin-Ciocalteu Reagent (FCR) was used to determine the total phenolic component levels in these three plants. DPPH, FRAP, and CUPRAC techniques were used to evaluate antioxidant activities. To calculate the equivalent antioxidant capacity of the extracts, different reference sample concentrations in the range of 50, 125, and 250 g/mL were prepared. As a result, it was found that the antioxidant capacity increased with concentration. The FRAP test and total phenolic content were found to be highest in the methanol extract of the *P. vera* L. plant. It was found that the inhibition value of *P. vera* L. leaves in the ethanol extract was high. It has been determined that the ethanol extract of *R. coriaria* L. leaves has the highest reducing property for Cu^{2+} using the CUPRAC method.

Keywords: Antioxidant, *Pistacia vera* L., *Prunus dulcis*, *Rhus coriaria* L. Total phenolic content.

***Corresponding author:** Atatürk University, Faculty of Pharmacy, Department of Biochemistry, 25030, Erzurum, Türkiye
E mail: lgozcu@atauni.edu.tr (L. DUYSAK)
Lale DUYSAK https://orcid.org/0000-0001-7872-3880
Barış ÖZORMAN https://orcid.org/0009-0007-4163-1211

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1. Introduction

Pistacia vera L. (Pistachio, *P. vera*) belongs to the Anacardiaceae family (Torun et al., 2021). Pistachio is the only species in this genus that produces edible nuts with commercial potential (Ferguson et al., 2005). Syria, Iraq, Iran and some regions of Türkiye are among the production areas of *P. vera* (Ibrahım and Mayı, 2023; Nezami and Gallego, 2023). Berries are a rich source of antioxidants including magnesium, potassium, calcium, protein, carbohydrates, dietary fibers, fat, folic acid, vitamin K, gamma-tocopherols, phytochemicals and polyphenols and flavonoids (Dreher, 2012) The antioxidant, anti-inflammatory, antimicrobial, and antiviral potential of polyphenols in pistachio has been proven by in vitro and in vivo experiments. Studies have also shown that consumption of *P. vera* plays a role in cognitive function and has beneficial effects on human skin health (Landaverde-Mejia et al., 2024; Mandalari et al., 2021) The antioxidant amounts in *P. vera* are variable and can vary significantly depending on the genetic quality of the plant, its pre-harvest and post-harvest collection, storage conditions, and different measurement methods used to measure antioxidant activity (Bolling et al., 2010; Liu, et al., 2014). *P. vera* is one of the most important export products in the Southeastern Anatolia Region of Türkiye in terms of production and export (Şimşek, 2018). Data from 2023 shows that 473 thousand tons of *P. vera* were exported worldwide. Türkiye has 32 thousand tons, which puts it in second place among exporting nations. According to 2022 data of the Food and Agriculture Organization, Türkiye ranks second in the world with 34% of the area where *P. vera* is planted (FAO, 2024).

Prunus dulcis (almond, *P. dulcis*) is a plant belonging to the Rosaceae family. It is native to Asia. It can usually be consumed fresh, dried or roasted. It is also used in cooking and confectionery. It is rich in fat. It is a rich source of polyunsaturated Fatty Acids (PUFA), especially linolenic acid, which is known for its ability to reduce the level of Low Density Lipoproteins (LDL) in the blood, preventing atherosclerotic plaques and therefore the risk of heart infarction (Berryman et al., 2011). In addition, , *P. dulcis* contain minerals such as calcium, potassium, phosphorus, and magnesium, and antioxidants, especially tocopherols. In addition to their nutritional properties, , *P. dulcis* have been reported to reduce body weight, have an effect on glucose levels, oxidative stress and inflammation, and reduce the risk of colon cancer in rats (Kamil and Chen, 2012). It has also been reported to be effective in the treatment of various skin diseases such as constipation,

and eczema, acne, as well as diseases such as gastro-enteritis (Kamil and Chen, 2012). Extracts of whole , *P. dulcis* kernel, brown hull, shell, green hull cover (stem), and leaves have strong free radical scavenging capacities due to the presence of flavonoids and other phenolic compounds (Esfahlan et al., 2010). Additionally, ethanolic extracts of leaves, flowers, and seeds (250 and 500 mg/kg) showed antidiabetic activity against normal and streptozotocin-induced diabetic mice (Rao, 2012). Bottone et al. reported in their study that, *P. dulcis* leaves can be considered a rich source for the extraction of bioactives with antioxidant properties and that they show particularly strong free radical scavenging capacity (Bottone, et al., 2018). According to FAO 2021 data, Türkiye ranks 7th in world almond production (FAO, 2021).

Rhus coriaria L. (*R. coriaria*) belongs to the Anacardiaceae family and is commonly known as "sumac", derived from the Syriac word "sumaga" meaning "red" (Wetherilt and Pala, 1994). *R. coriaria* leaves and fruits are generally effective in treating bacterial and fungal infections (Gabr and Alghadir, 2015; Joseph et al., 2023; Özçelik, 1987). , *R. coriaria* leaves have traditionally been used in various treatments such as mouth sores (aphtha, thrush), antiseptic, sore throat, cracked hands-feet-lips, anti-inflammatory, antidiarrheal, stomach ache, heartburn, digestive system diseases, and wound healing (Aytar, et al., 2024; Fakir et al., 2009; Özhatay and Koçak, 2011; Yücel et al., 2011). It is stated that the fresh leaves of the plant, placed on the soles of shoes, heal cracks in the skin, and the fruit is used by chewing gum for mouth sores and stomach cramps (Tuzlacı, 2006; Tuzlacı, 2011). It has been reported that the sumac plant is used in dysentery, liver diseases, and loss of appetite, as well as in hair treatment and skin diseases such as burns and dermatitis (Ali-Shtayeh et al., 2013). *R. coriaria* components fatty acids, minerals, fiber, and phytochemicals give the plant many beneficial properties. Its nutritional value keeps this plant in a fluid state as a foodstuff or functional food (Grassia et al., 2021). In addition to its nutritional value, secondary metabolites such as gallic acid and vanillic acid, which are important for therapeutic use, are found in sumac (Schulze-Kaysers et al., 2015). It is effective in preserving foods due to its antimicrobial and antioxidant properties (Gulmez et al., 2006; Joseph et al., 2023; Shabbir, 2012; Zannou et al., 2025). In Türkiye, 17.25 thousand tons of, *R. coriaria* is produced annually between 2020-2024 (TAGEM, 2020).

Manufacturers often synthesize the antioxidants utilized in in vitro and in vivo research where antioxidant activity is examined. As a result, it is uncommon for research to employ naturally occurring antioxidants. Gallic acid and Trolox, two common antioxidants, were used in this investigation (Bardakçı 2017; Duysak et al., 2024a).

The aim of this study is to compare the antioxidant activities and total phenolic contents of the three plants mentioned above, which have been briefly discussed regarding their origins, areas of use, contents, and benefits. Additionally, another aim is to investigate the potential benefits that can be obtained from the leaves of *P. vera*, *P. dulcis*, and *R. coriaria* which are considered waste and are not utilized, in order to raise awareness regarding the evaluation of waste, one of the important topics of our time.

2. Materials and Methods

2.1. Plant Materials

P. vera (60 years), *P. dulcis* (15 years), and *R. coriaria* (10 years) plant leaves were collected from Gaziantep province in Turkey. These three trees grow spontaneously after being planted and do not require separate watering. The collected leaves were dried at room temperature. The dry leaves were ground into powder using a mortar and liquid nitrogen. Ethanol and methanol extracts were prepared from the plant powders. To prepare the alcohol extracts, 50 g of dry plant powder was weighed and extracted in 250 mL of ethanol and methanol in a horizontal shaking water bath at 50ºC for 72 hours. Every 24 hours, the mixture was filtered and fresh ethanol and methanol were added. The collected filtrates were gathered in a container, and the solvents were evaporated at 50°C using an evaporator. The dried extracts were stored in airtight bottles at 4ºC for experiments.

2.2. Determination of Total Phenolic Content

The total phenolic compound amounts in the leaves of *P. vera*, *P. dulcis*, and *R. coriaria* were determined using a modified version of the method developed by Slinkard and Singleton (1977). First, a 50 ml solution of 7.5% Na_2CO_3 was prepared. After preparing the stock solutions and performing the necessary dilution procedures, 40 µL of the sample and 200 µL of the Folin & Ciocalteu reagent were added to the plate wells and left to incubate for 5 minutes. Then, after adding 160 µL of Na_2CO_3, the samples were incubated for another 30 minutes, and the absorbance was measured at 765 nm using a spectrophotometer. Using the standard graph prepared with gallic acid, the results were calculated as mg Gallic Acid Equivalent (GAE)/g.

2.3. Determination of Antioxidant Capacity

2.3.1. DPPH method

The antioxidant capacity of ethanol and methanol extracts obtained from the leaves of *P. vera*, *P. dulcis*, and *R. coriaria* were determined according to the method developed by Brand Williams (1995). This method is based on measuring the scavenging effect of antioxidants against the stable and synthetic DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. In the reduction reaction that occurs in the presence of antioxidants, the DPPH solution loses its color, and the decrease in color intensity allows for easy measurement in a spectrophotometer. This method is an easy and quick way to evaluate the radical scavenging capabilities of antioxidants. DPPH, a dark purple radical, gives maximum absorbance at 517 nm. To prepare the DPPH solution, 39 mg of DPPH was dissolved in 100 mL of ethanol. Then, 210 µL of extracts and 70 µL of DPPH solution were added to each well of a 96-well

plate and shaken for about 1 minute. The samples were incubated for 30 minutes at room temperature and in the dark, and the absorbances were read at 515 nm. The standard antioxidant Trolox was used as the control sample. The results were calculated as % inhibition.

2.3.2. CUPRAC method

In the method used by Apak and colleagues, the Cu(II) Neocuproin complex is converted to Cu(I) Neocuproin through the action of antioxidant compounds in the environment, and this complex's absorbance at a wavelength of 450 nm is measured (Capanoglu, et al., 2018). To prepare the CUPRAC reagent, 0.4262 g of $CuCl_2 \bullet 2H_2O$ was weighed and dissolved in 250 mL of distilled water (10 mM). To prepare the acetate buffer, 19.27 g of NH_4Ac was dissolved in 250 mL of water. A 7.5 mM neocuproin solution was obtained by dissolving 0.039 g of neocuproin compound in a 25 mL volumetric flask with 96% pure ethanol. Then, solutions consisting of 60 µL $CuCl_2$, 60 µL acetate buffer, 60 µL neocuproin solution, and 66 µL extracts were mixed. After a 30-minute incubation, absorbances were measured at a wavelength of 450 nm. The standard antioxidant Trolox was used as the control sample. Calibration curves for the 1-100 µg/mL working range, where the absorbance graph is linear with respect to concentration, were plotted.

2.3.3. FRAP method

The antioxidant capacity measurement method based on electron transfer of ethanol and methanol extracts obtained from the leaves of *P. vera, P. dulcis,* and *R. coriaria* were determined using the method developed by Huang and colleagues (2005). First, a 300 mmol/L acetate buffer (pH=3.6) was prepared. 10 mM TPTZ was placed into a 100 mL volumetric flask, 40 mM HCl was added, and the final volume was adjusted to 100 mL. Finally, a 20 mmol/L $FeCl_3$ solution was prepared. From the prepared solutions, 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$, and 25 mL of acetate buffer were taken to obtain a total of 30 mL of FRAP solution. 10 µL of the extract sample and 200 µL of the FRAP solution were added to the plate wells, and after a 30-minute incubation, their absorbance was measured at 593 nm. The standard antioxidant Trolox was used as the control sample. Calibration curves for the 1-100 µg/mL working range, where the absorbance graph is linear with respect to concentration, were plotted.

2.4. Statistical Analysis

The graphs of the obtained data were plotted using GraphPad Prism Version 10.2.2 (397) Demo version. Additionally, the comparison between plants was conducted using the Tukey test in a one-way ANOVA. The P-value was expressed as P<0.0001 (****).

3. Results and Discussion

3.1. Total Phenolic Content

The total phenolic compound amounts of ethanol and methanol extracts prepared from the leaves of *P. vera, P. dulcis,* and *R. coriaria* were determined using the Folin-Ciocalteu Reagent (FCR). Gallic acid was used as a standard phenolic compound, and it was calculated as gallic acid equivalent from the equations obtained from the calibration curves of gallic acid (Table 1).

Table 1. The R^2 and linear range values of the equations formed with standard antioxidants prepared to measure the total phenolic content, DPPH, FRAP, and CUPRAC values of the leaves of *P. vera*, *P. dulcis,* and *R. coriaria*

		R^2	Linear range
DPPH (Trolox)	Ethanol	0,997	1-50
	Methanol	0,9946	1-40
CUPRAC (Trolox)	Ethanol	0,999	1-100
	Methanol	0,9981	1-70
FRAP (Trolox)	Ethanol	0,9989	1-90
	Methanol	0,9957	1-100
Total Phenolic Content (Gallic acid)	Ethanol	0,9976	1-100
	Methanol	0,9985	1-100

The total phenolic compound amount calculated in the samples according to the regression equations of the curves was determined as GAE/g for ethanol and methanol extracts (Figure 1). The total phenolic compounds of three plants were studied at three different concentrations (50, 125, and 250 µg/mL). According to the results obtained, it was determined that the highest total phenolic content was at a concentration of 250 µg/mL. Additionally, when comparing the high concentrations of ethanol and methanol extracts, it was found that the highest phenolic content was in the methanol extract of *P. vera* leaves (P<0.0001). Various in vitro and in vivo studies have investigated the antioxidant effects of pistachio flavonoids by comparing the activity of different parts of the peanut or different extracts (lipophilic or hydrophilic). It has been proven that polyphenols and flavonoids are commonly found in all parts of the pistachio (Gentile et al., 2007). Tomaino et al. showed that pistachio shells have a higher antioxidant activity and a higher content of antioxidant phenolic compounds than kernels (Tomaino et al., 2010). It has been shown that pistachio shells, which are considered as

agricultural waste, have high antioxidant properties by evaluating the oxidation of soybean oil after the heating process and then determining the peroxide value and thiobarbituric acid value (Goli et al., 2005). In this study, the high phenolic content of the leaf extracts of the three plants may play a supportive role in the prevention of oxidative stress-related diseases such as cardiovascular diseases, diabetes, cancer, and neurodegenerative diseases.

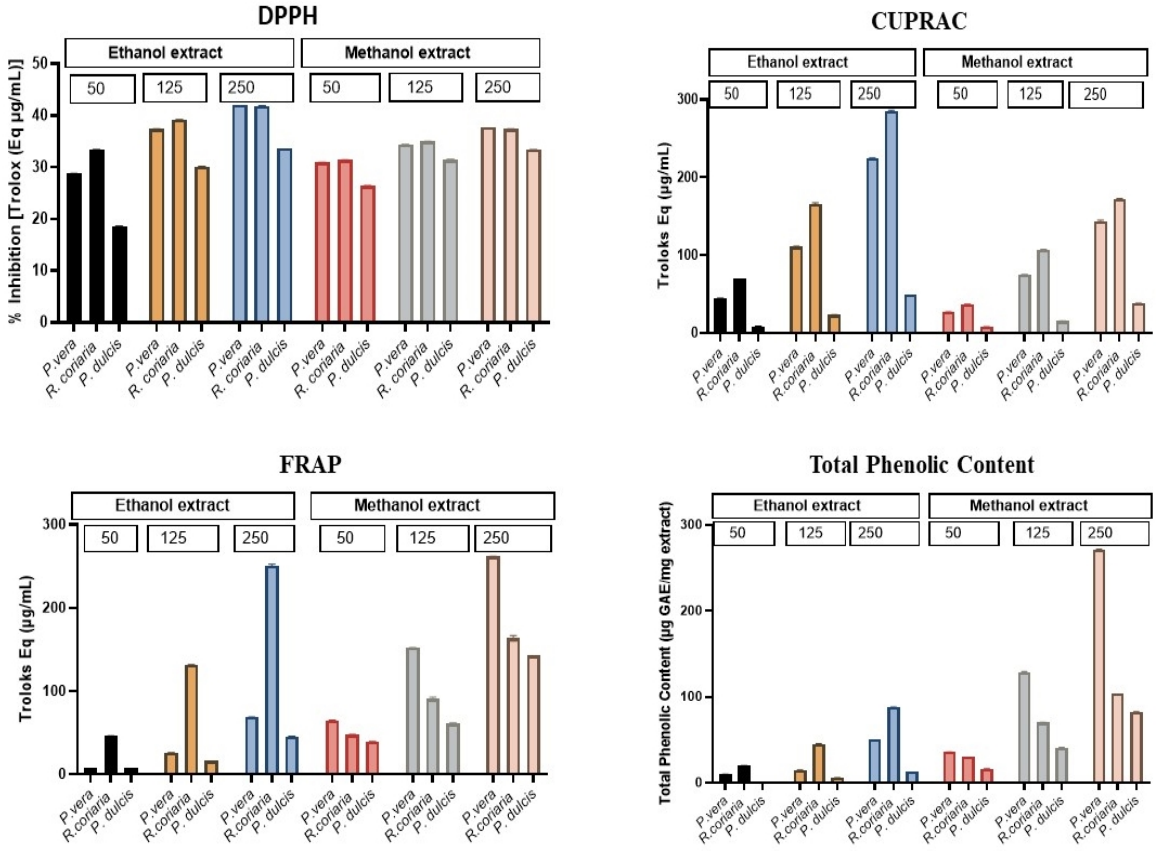

Figure 1. Total phenolic compound, DPPH, FRAP and CUPRAC values of ethanol and methanol extracts obtained from leaves of *P. vera*, *P. dulcis* and *R. coriaria* at concentrations of 50, 125 and 250 µg/mL.

3.2. Antioxidant Capacity

3.2.1. Results from DPPH radical scavenging studies

The concentration range of trolox, a standard antioxidant compound, was determined to be 1-100 µg/mL as a result of the studies conducted. A standard curve has been drawn and an equation obtained using trolox as a standard antioxidant compound (Table 1). The DPPH radical scavenging capacities of ethanol and methanol extracts prepared from the leaves of *P. vera, P. dulcis,* and *R. coriaria* at concentrations of 50, 125, and 250 µg/mL were calculated as % inhibition values. It has been determined that all three plants have the highest DPPH free radical scavenging capacity at a concentration of 250 µg/mL, and that the best effect is observed with a high dose of the ethanol extract (Figure 1). When comparing the concentration of ethanol extract at 250 µg/mL among three plants, it was found that there were differences among them, with the highest percentage of inhibition observed in the *P. vera* plant (Figure 2, $P<0.0001$). In support of our work, Hosseinzadeh et al. found in their study that the ethanol extract of *P. vera* leaves have a higher % inhibition value than the water extract (Hosseinzadeh et al., 2012). Boumaiza et al. found that all extracts tested in the DPPH assay of the *P. vera* plant exhibited good antioxidant properties. However, the antioxidant activity of the various extracts tested primarily increases based on their concentrations (Boumaiza et al., 2016).

3.2.2. Results of the copper ion reducing antioxidant capacity determination method (CUPRAC)

The conversion of ethanol and methanol extracts prepared from the leaves of *P. vera, P. dulcis,* and *R. coriaria*, as well as the Cu(II) neocuproin complex, into Cu(I) neocuproin through compounds with antioxidant effects in the medium, was carried out by measuring absorbance at 450 nm. The concentration range to be analyzed (1-100 µg/mL) was determined based on studies conducted on standard antioxidant compounds (Table 1) At the end of the study, it was observed that there is a correlation in antioxidant capacity proportional to the increasing amount of each extract, depending on the quantity of the extract. The reason for this is that as the

amount of extract increases, the quantity of active compounds in the extracts also increases. The reason for this correlation may be the many free radical scavenger groups found in plants, such as phenolic compounds with antioxidant effects (phenolic acids, flavonoids, coumarins, etc.), nitrogenous compounds (alkaloids, amines, etc.), vitamins, and terpenoids (Duysak et al., 2024b) (Figure 1).

When comparing the concentration of ethanol extract at 250 µg/mL among three plants, it was found that there were differences among them, with the best CUPRAC result observed in the *R. coriaria* plant (Figure 2) (P<0.0001). Studies have found that the leaves of *R. coriaria* exhibit high antioxidant capacity (Bursal and Köksal, 2011; Danış et al., 2014; Perna et al., 2018).

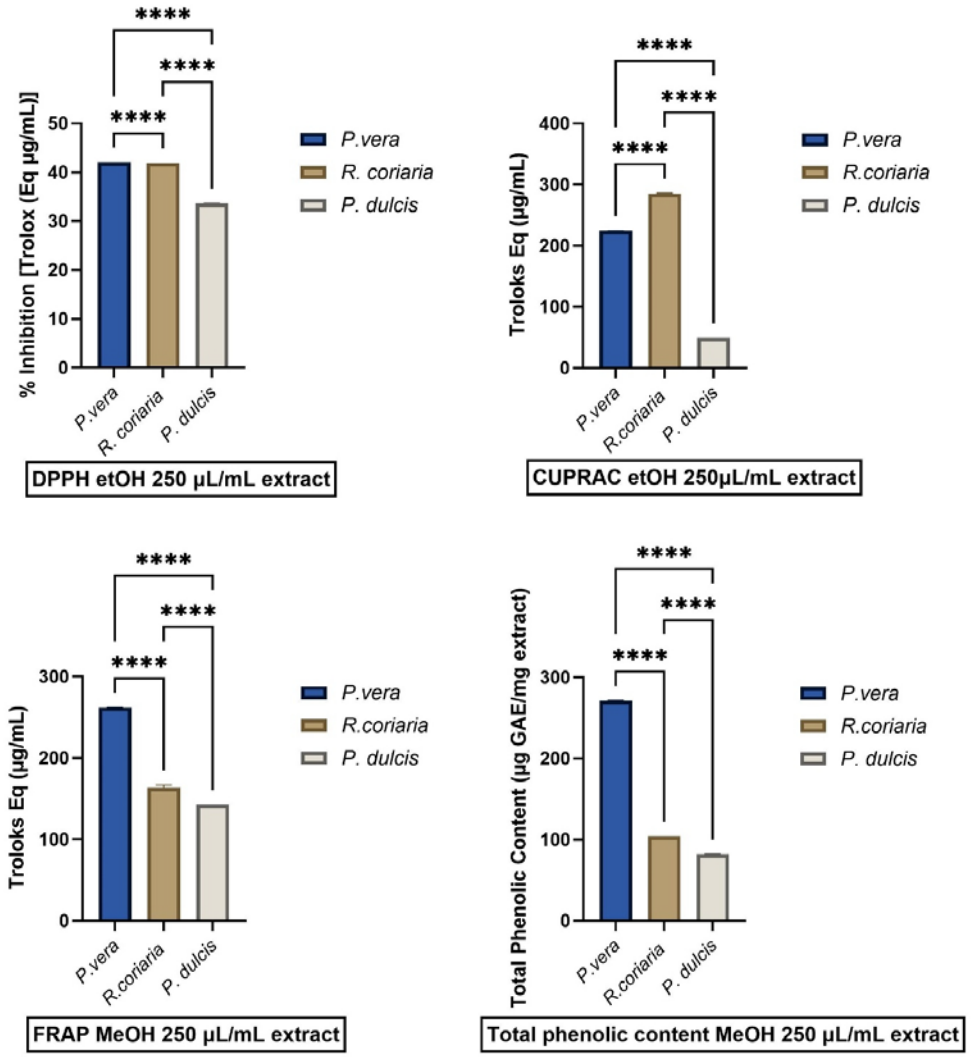

Figure 2. Comparison of the best acting solvent among plants (Statistics for antioxidant capacity and total phenolic compound measurements were made using GraphPad Prism Version 10.2.2 (397) version. Tukey test was used in one-way ANOVA for comparison among plants. p value was expressed as P<0.0001 (****).

3.2.3. Results of iron ion reducing antioxidant power (FRAP)

The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. As a standard antioxidant, trolox, iron (III) reducing/antioxidant potency activity reached the highest value at 100 µg/mL concentration. In line with these data, the concentration range of the extracts to be studied was determined as 1-100 µg/mL (Table 1). The iron (III) reducing/antioxidant powers of ethanol and methanol extracts prepared from *P. vera, P. dulcis,* and *R. coriaria* (50, 125 and 250 µg/mL) , standard antioxidant compounds were compared in terms of µg Trolox Equivalent Antioxidant Capacity (TEAC) (Figure 1). It was determined that the ethanol and methanol extracts prepared from the *P. vera, P. dulcis,* and *R. coriaria* had the highest iron ion reducing antioxidant power capacity, and it was determined that the methanol extract had a concentration of 250 µg/mL. When the 250 µg/mL concentration of methanol extract was compared in 3 plants, it was determined that there was a difference between them, and the best FRAP result was in the *P. vera* leaf. (Figure 2) (P<0.0001). A study showed that extracts from different parts of P. vera have antioxidant activity in various in vitro methods. It has been determined that the ethanolic extracts of leaves and fruits have higher antioxidant content (Hosseinzadeh et al., 2012). Boumaiza et al. demonstrated that the high phenolic content of *P.*

vera contributes to its antioxidant activity (Boumaiza et al., 2016). In another study, it has been shown that *P. vera* gum exhibits effective DPPH, FRAP testing for the Fe^{3+}-Fe^{2+} conversion, reducing capabilities for Cu^{2+} using the CUPRAC method (Sehitoglu et al., 2015).

4. Conclusion

The extractions of *P. vera, P. dulcis,* and *R. coriaria* were carried out separately with ethanol and methanol. The total phenolic content of the ethanol and methanol extracts of three plants, as well as their antioxidant capacities, were comparatively examined using the DPPH, FRAP, and CUPRAC methods. As a result, it has been determined that at concentrations of 50, 125, and 250 µg/mL, the antioxidant capacity increases with the concentration. The current study found that the total phenolic content and the Fe^{3+}-Fe^{2+} conversion in the FRAP test were highest in the methanol extract of the P. vera plant. In the DPPH test, it was found that the % inhibition value is present in the ethanol extract of the *P. vera* leaves. The CUPRAC method determined that the ethanol extract of R. coriaria L. leaves has the highest reducing property for Cu^{2+}. Based on this, the evaluation of the antioxidant properties of the leftover leaves of these plants, which are widely consumed for their fruits, indicates significant potential from both environmental and nutritional perspectives. In particular, the leaves of commonly consumed plants such as *P. vera* and *R. coriaria* can be used in the food industry or as natural food additives due to their high phenolic content. The evaluation of such waste will provide economic benefits and also enhance food security. In conclusion, the findings of this study can contribute to waste management strategies in the agricultural industry by investigating the antioxidant properties of plant leaves. In future studies, it is recommended that more research be conducted on the health effects and potential applications of these leaf extracts.

Author Contributions

The percentages of the author's contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	L.D.	B.Ö.
C	70	30
D	80	20
S	70	30
DCP	70	30
DAI	70	30
L	50	50
W	90	10
CR	80	20
SR	90	10
PM	70	30
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest Statement

The authors declare that they have no conflict of interest in this study.

Ethical Approval Statement

Since this study did not involve any studies on animals or humans, ethics committee approval was not obtained.

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