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Evaluation of bioactive components and antioxidant activities of *Rose canina* L., *Viburnum opulus* L., *Berberis vulgaris* L. and *Berberis integerrima* L. subjected to different extraction methods

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ABSTRACT

Rose hip (*Rosa canina* L.), gilaburu (*Viburnum opulus* L.), barberry (*Berberis vulgaris* L.) and black barberry (*Berberis integerrima* L.) are important endemic plants of Turkiye. This study determined the bioactive components, including total phenolic content, flavonoid compounds, and anthocyanin amounts. Antioxidant activity values of *Rosa canina* L., *Viburnum opulus* L., *Berberis vulgaris* L. and *Berberis integerrima* L. fruits grown in Türkiye were also evaluated after different extractions. According to the results, the highest total phenolic content was determined in the methanol extract of *Berberis integerrima* L.fruit as 3544.84 (mg GAE/100 g DM). The order of the total amount of phenolic substance for all three solvent extraction systems, from the highest to the lowest, was determined as: *Berberis integerrima* L.*>Viburnum opulus* L.*>Berberis vulgaris* L.*>Rose canina* L. In terms of total flavonoids, *Berberis integerrima* L. fruit extracted with methanol had the highest value of 2098.88 (mg CE/100 g). The order of total amount of flavonoid substance for all three solvent extraction systems, from the highest to the lowest, was determined as *Berberis integerrima* L. *> Viburnum opulus* L. *> Berberis vulgaris* L. *> Rose canina* was detected. The highest amount of Trolox equivalent (DPPH)(ABTS+) was determined as 2.95-3.30 (mM Trolox/100 g DM) in *Berberis integerrima* L. extracted with methanol. The highest total amount of anthocyanins found in the extracts was obtained for *Berberis integerrima* L. The highest amounts of vitamin C extracted with ethanol, methanol and water was determined for *Berberis integerrima* L.24.60; 33.22; 29.22 (mg/ 100 g), respectively. The highest value for CD, CI and T was obtained for *Berberis integerrima* L. extracted with methanol. The highest score for % R, %Y and % B was obtained for *Berberis vulgaris* L. exacted with water, for *Berberis integerrima* L. extracted with ethanol and *Berberis integerrima* L. extracted with water, respectively. The results demonstrated that the extraction solvent has a critical importance for the releasing of bioactive compounds for each used materials.

KEYWORDS
endemic plants of Turkiye; total phenolic content; total anthocyanin content; antioxidant activity

KEY CONTRIBUTION
This study highlights the critical importance of selecting the appropriate solvent for the extraction of bioactive components.
It was found that *Berberis integerrima L.*, a local plant from Turkey, is particularly rich in bioactive compounds and the highest values were obtained in samples extracted with methanol.
The plants used in the study were fruits rich in vitamin C and anthocyanin.
The highest score for % Redness, %Yellowness and % Blueness was obtained with BVW (*Berberis vulgaris* L. /water), BIE (*Berberis integerrima* L./ethanol) and BIW (*Berberis integerrima* L./water), respectively.

Introduction

The nutritional value of fruits is as important as the amount of food used in human nutrition. In recent years, the interest in the production of high-quality, nutritious food has been increasing rapidly. With the advancements in the aging process within nutrition and medical sciences, and the increase in living standards in many countries over the past 20 years, people have become more conscious of the quality of the foods they consume. As a result, they are increasingly aware of the impact these foods have on their health. The use of foods with high antioxidant capacity in human nutrition has been encouraged, and foods with high antioxidant capacity have been identified and announced to the consumer (Chobdar Rahim et al., 2021). The use of additives has increased rapidly (amounting to 200,000 tons) in recent years due to the benefits they provide, such as preservation, colouring, and antioxidant properties (Chobdar Rahim, 2017). Assuming that approximately 75% of processed food products are consumed in the kitchens of Western countries, it has been calculated that people receive approximately 5–6 kg of additives each year from these products. Parallel to this rapid increase in the consumption, reports indicate an increase in various side effects associated with these additives (Doğruyol, 2006).
Rosa canina L., *Viburnum opulus* L., *Berberis vulgaris* L. and *Berberis integerrima* L. are important endemic plants of Turkey. These plants were found in the whole Anatolian region. For this purpose, total phenolic, total flavonoid, total anthocyanin content and antioxidant activity of *Rosa canina* L., *Viburnum opulus* L. and two types of barberry fruits grown in Turkey were analysed and compared by using different extraction methods. Additionally, the colour values of the extracts obtained from these fruits were analysed with a spectrophotometer and evaluated. *Berberidaceae* is a family of 12 genera and 200 species of perennial herbaceous or shrub plants having a "berberine" component that gives the woody portions a yellow colour. The majority of these species are found in the Northern Hemisphere's temperate zones. In our country, *B. vulgaris*, *B. integerrima*, *B. crataegiana*, *B. cretica* species grow naturally in the genus Berberis (Talebi et al., 2019). Wild edible fruits are sources of bioactive compounds that possess different of nutritional, antioxidant, anti-inflammatory, anti-cancer, and anti-aging activities. Vitamins, phenolic, anthocyanins, flavonoids, tannins and other compounds in the fruits contribute to these properties (Belwal et al., 2016). Due to their nutritional and disease-prevention characteristics, many of these plants have become more popular (Faruque et al., 2011). Phenolic compounds are widely available compounds in these fruits and are particularly known for their preventive activity against reactive oxygen species and free radicals (Saini et al., 2014) by acting as

reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Miller and Rice-Evans, 1996). *Berberis vulgaris* L. is a red-coloured fruit that grows in Asia, North Africa and Europe (Gündoğdu, 2013). Since the presence of special phytochemical and bioactive components it has been used to prevent a variety of ailments and has a variety of health-promoting effects due to its antibacterial, anticarcinogenic, antibiotic, anti-inflammatory, antihypertensive and lipid-lowering characteristics (Zovko Končić et al., 2010; Hemmami et al., 2020). This fruit can be used fresh, dried, or as syrup, jam, or in jelly forms. Its powder form has also many uses as a spice or as an ingredient in food product formulations (Bakmohamadpor et al., 2021). In Turkiye, it usually grows in the Southeastern Anatolia region (Diyarbakır, Mardin and Şırnak) Sivas, Istanbul and Trakya. Since its different species grow in the Eastern Black Sea region, since ancient times, *Berberis vulgaris* L. has been utilized as an herbal cure. In previous studies, it has been found that the phytochemical content of *Berberis vulgaris* L., including specific phenolic compounds and anthocyanins, enhances its bioactivity and is influenced by different extraction methods (Abd El-Wahab et al., 2013; Annegowda et al., 2012). The dried fruit juice of berberis has been clinically tested and found to be effective against inflamed acne lesions, and it activates the immune system and helps in the prevention of scurvy (Belwal et al., 2017). Berberis species were determined to be highly active in many tests employed for evaluation of antioxidant activity (Hanachi and Sh, 2009). *Berberis vulgaris* L. fruits demonstrated a strong antioxidant activity and caused a reduction of cell viability in a human liver cancer cell line (HepG2).

The wild *Rosa canina* L., often known as the dog rose (*Rosa canina* L.), is a well-known and highly valued plant in many regions, with its natural habitat spanning North Africa and Asia Minor. It's a Rosaceae shrub with hooked thorns on floppy stems and single or double-serrated leaves divided into five or seven leaflets that grow up to 4 m tall. It blooms from May to June, with white or light pink flowers with five petals. Fruits ripen as orange-red, spherical to oval-shaped fruit-like structures from September to October. Wild rose is valued for its aesthetic qualities as well as its high concentration of bioactive compounds (Grys et al., 2009; Cendrowski et al., 2012). Polyphenolic compounds found in *Rosa canina* L. include flavonoids such as anthocyanins, procyanidins, catechins, quercetin, kaempferol, apigenin, and resveratrol, as well as phenolic acids like gallic and ellagic acids (Demir and Özcan, 2001; Cendrowskı et al., 2012; Fascella et al., 2019; Fent et al., 2020; Tabaszewska and Najgebauer-Lejko, 2020). Due to its diverse chemical content, *Rosa canina* L. is an important fruit for the food industry. *Rosa canina* L. fruits are processed and sold as a food source as well as a prophylactic and therapeutic treatment for inflammatory conditions. *Rosa canina* L. are a rich source of minerals (potassium, phosphorous), vitamins (vitamin C), carotenoids and flavouring components (Ahmad et al., 2016; Javanmard et al., 2018). The Viburnum is a genus of family Caprifoliaceae. *Viburnum opulus* L. is known by many names throughout Eastern Europe, North Asia, and Africa, including “guelder rose”, European “cranberrybush”, “snowball rose”, “cherry-wood” and “cramp bark” (Altan et al., 2005; Barak et al., 2019; Dursun et al., 2021; Polka et al., 2019; Yılmaz et al., 2020; Zarifikhosroshahi et al., 2020). With a dark-red colour, *Viburnum opulus* L. is rich in polyphenols, including flavonoids such as catechin, epicatechin, proanthocyanidin, quercetin, as well as phenolic acids like chlorogenic acid (Cemtekin et al., 2019; Koparal, 2019; Dursun et al., 2021). *Viburnum opulus* L. is consumed in various forms, including fruit juice, dried fruits, jam, and pickles. *Viburnum opulus* L. fruits are especially used to treat kidney problems. In addition, it was also reported that these fruits have antidiabetic and antispasmodic effects at a significant level (Altun et al., 2008; Özrenk et al., 2011; Moldovan et al., 2012; Kalyoncu et al., 2013; Ozola and Kampuse, 2018). The presence of bioactive components in V. opulus, such as phenolic compounds, vitamin C, carotenoids, triterpenes, iridoids, essential oils, saponins and dietary fiber contribute to the plant's beneficial properties (Polka et al., 2019; Česoniene et al., 2010; Perova et al.,

2014; Rop et al., 2010). *Viburnum opulus* L. fruits have also been utilized in Anatolian traditional medicine as a hypoglycemic and cough reliever (Barak et al., 2019; Fujita et al., 1995). A large number of pharmacological activity studies on *V. opulus* fruits have been done based on their folk medicine reputation. In vitro tests conducted to assess antibacterial and antioxidant activity yielded positive results (Charehsaz et al., 2015; Kraujalis et al., 2017).

The aim of this study was to evaluate the bioactive components (total phenolic compounds, total flavonoid content and total anthocyanin amounts), antioxidant activity values of *B. vulgaris*, *R. Canina* and *V. opulus* fruits extracted with different solvents and to interpret the relationship between the amounts of bioactive components, antioxidant activity and used extraction method.

Materials and methods

The fruits of *Berberis vulgaris* L., *Berberis integerrima*, *Viburnum opulus* L. and *Rose canina* were purchased from the market in Izmir, Turkey. The morphological properties were confirmed by botanical specialist of Ege University. Fruits were bought in dried form and immediately subjected to extraction. The fruits used in the study were extracted using three different solvent systems [80% methanol (Sigma-Aldrich), 80% ethanol (Sigma-Aldrich) and boiled distilled water]. Three different solvent systems (each system was 40 ml) were prepared according to the accepted method (Chobdar Rahim et al., 2021). For the ethanolic and methanolic extracts, 2 g of each plant sample was taken and ground (Sinbo Scm-2934, grounder) to the smallest particles. Then 40 ml of solvent was added and mixed by a mixer (Model-WiseShake-SHO-2D) at 100 rpm for 6 hours in dark conditions at room temperature. Afterwards, the extract was centrifuged at 4000 rpm for 20 minutes (Model-Mistral 1000) and the supernatant was separated to be used in the analyses. The water extract was prepared by taking 2 g of each plant sample and grinding it to the smallest particles, then adding 100 ml of distilled water and boiling it in the dark for 20 minutes. At the point of boiling, the amount of solvent was decreased to 50 mL. Afterwards, the extract was centrifuged at 4000 rpm for 20 minutes and the supernatant was separated to be used in the analyses (Eroğlu et al., 2020).

Physico-chemical analyses

Total phenolic contents analysis

The total phenolic content was measured by the Folin-Ciocalteu method, as suggested by Kumar et al. (2012) with slight modifications. Initially, the plant extract was diluted with a ratio of 1:10 (v:v) using distilled water (Kumar et al., 2012). The amount of 0.5 mL of the Folin–Ciocalteu reagent was added to 0.1 mL of diluted extract. After 2 min, allowing the reaction to be completed, the amount of 2 mL of sodium carbonate (7% *w/v*) was added. The final volume was adjusted to 7.5 mL by distilled water. Obtained mixture was kept in dark for 1 hour. The absorbances were measured at 760 nm using a spectrophotometer Shimadzu 1240 (Japan). Different concentrations (50-600 mg/L) of gallic acid (Merck, G7384) were prepared for drawing the calibration graph of gallic acid. Absorbance readings on the y-axis and concentration values on the x-axis at 760 nm were plotted on a standard processing chart. Calibration curve of the gallic acid standard solution was adopted to determine the total polyphenols concentration (mg GAE /100 g).

Total flavonoid contents analysis

The total flavonoid contents were determined using the aluminium chloride colorimetric method (Liu et al., 2009; Sánchez-Moreno, 2002; Singleton and Rossi 1965; Zhishen et al., 1999). Briefly, 250 µL sample

extract solution, was mixed with 1.50 ml distilled water and 75 µL of 5% sodium nitrite and the mixture was stored for 5 min. Then 150 µL of 10% $AlCl_3$ and 125 µL of 1 M NaOH were added. The absorbances were measured at 510 nm by using spectrophotometer after a reaction for 10 min. The standard curve was plotted by using +(−) catechin (50–600 mg/L) and total flavonoid contents were calculated as mg catechin equivalent (mg Catechin /100 g) extract.

Total anthocyanin contents analysis

The anthocyanin content of the samples was determined according to the pH differential method (Andrade et al., 2021). Anthocyanin amounts were determined in all samples in terms of cyanidin 3-glycoside (MW=449.2, molar absorbance, ε =26.900). Anthocyanin amounts of the samples were calculated according to the formula given below (Andrade et al., 2021).

$$\text{Anthocyanin mg / kg} = (\Delta A/\varepsilon \cdot L)\, 10^3 \times (MW) \times (DF) \quad (1)$$

ΔA: Absorbance difference (A_{520}nm − A_{700}nm) pH 1.0 − (A_{520}nm − A_{700}nm) pH 4.5
E: Molar extinction coefficient: 26,900 mol $L^{-1}cm^{-1}$
L: Optical path of cuvette (cm) = 1
MW: (Molecular weight) = 449.2 g mol^{-1}
DF: Dilution factor
10^3: Factor for conversion from g to mg

Vitamin C (HPLC-UV) content analysis

The vitamin C measurement was performed using the Agilent HPLC - UV system (Model Number, Agilent Technologies, Santa Clara, CA, USA) and a Eurosphere (C18, 5x4/6x250) column. The column was kept at 35 °C and the injection volume was 10 µL. 25 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid) was used as a mobile phase. The flow rate was set to 0.8 mL/min. The amount of vitamin C was calculated by comparing the surface area and retention time of the standard ascorbic acid diagram made with dilutions using the equations determined from the peaks. The samples' absorbance was measured at 230 nm (Klimczak and Gliszczynska-Wiglo, 2015).

Antioxidant activity by DPPH assays

The antioxidant capacity of the extracts obtained from the samples was measured according to the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical reducing activity method (Singleton and Rossi, 1965; Brand-Williams et al., 1995; Sánchez-Moreno, 2002;). The DPPH solution with a concentration of 6×10^{-5} M was prepared in methanol. The amount of 4.9 ml of methanolic DPPH solution was added to 0.1 ml of sample. The resulting mixture was kept in the dark for 30 minutes. The absorbance against the blank at 517 nm was recorded by using spectrophotometer (Shimadzu 1240). A calibration curve of (1 - 5 mM) Trolox was prepared for analysis. The results were expressed as (mM Trolox /100 g).

Antioxidant activity by $ABTS^+$ assays

The ABTS+ radical scavenging activity was performed according to Re et al., (1999). The ABTS+ radical cation was prepared by mixing 7 mM ABTS+ solution with 2.45 mM potassium persulfate solution and incubating the mixture in the dark at room temperature for 12-16 hours. For the measurements, the stock solution was diluted with distilled water until it provided an absorbance value of 0.700 at 734 nm. After the addition of 2 ml of ABTS+ solution to 0.1 ml of extract, it was kept in the dark for 6 minutes

and the absorbance was recorded at 734 nm (Shimadzu 1240). For the ABTS+ test, a Trolox calibration curve was prepared, and the results were expressed as (mM Trolox /100 g).

Colour parameters analysis

The colour intensity, hue / tint / tonality and pigments were analysed by the measurement of samples at absorbance of 420, 520, and 620 nm by using a Specord 400 spectrophotometer (Analytik Jena, Jena, Germany) (Glories, 1984). The colour intensity (CI), color tint / tonality / hue (T) and the proportion of yellow (% Ye), red (% Rd), and blue (% Bl) pigments were calculated as follows:

$$CI = Abs_{420} + Abs_{520} + Abs_{620} \quad (2)$$

$T = Abs_{420}/Abs_{520}$

$\%\ Ye = (Abs\ 420/CI) \times 100$

$\%\ Rd = (Abs_{520}/CI) \times 100$

$\%\ Bl = (Abs_{620}/CI) \times 100$

Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. The analysis of variance was performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Differences between means were considered at a significance level of $p < 0.05$, and Duncan's multiple-range tests were used for mean comparisons.

Results and discussion

The effect of used solvent on total phenolic substance and total flavonoid content

The amounts of total phenolic and flavonoid substances extracted by using methanol, ethanol and water regarding *Berberis vulgaris* L., black *Berberis integerrima* L., *Viburnum opulus* L. and *Rosa canina* L. samples are presented in Table 1. (p<0.05). As it is seen from the Table 1., the amounts of TPC and TFC demonstrated significant differences depending on the solvent used.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) values of samples extracted by different solvent system.

Total Phenolic Content (mg gallic acid /100 g DM)			
Sample	**Ethanol Extract**	**Methanol Extract**	**Water Extract**
Berberis vulgaris L.	1564.3 ± 4.88^{*b**}	2276.0 ± 3.65^{b}	2773.9 ± 5.41^{b}
Berberis integerrima L.	2736.4 ± 5.05^{d}	3544.8 ± 7.53^{d}	3506.3 ± 7.26^{d}
Viburnum opulus L.	2184.3 ± 5.57^{c}	3283.3 ± 4.71^{c}	2978.1 ± 7.49^{c}
Rosa canina L.	1463.5 ± 5.76^{a}	1896.9 ± 2.62^{a}	1990.6 ± 3.99^{a}
Total Flavonoid Content (mg Catechin/100 g DM)			
Sample	**Ethanol Extract**	**Methanol Extract**	**Water Extract**
Berberis vulgaris L.	342.22 ± 7.54^{*b**}	1597.50 ± 7.10^{b}	312.36 ± 8.94^{b}
Berberis integerrima L.	573.64 ± 12.29^{d}	2098.88 ± 10.40^{d}	641.53 ± 6.88^{d}
Viburnum opulus L.	515.00 ± 6.24^{c}	1851.23 ± 9.30^{c}	364.17 ± 12.58^{c}
Rosa canina L.	297.50 ± 11.46^{a}	583.33 ± 7.14^{a}	221.82 ±7.34^{a}

*Standard deviation.

**Different letters in each column; indicates that there is a statistical difference between the values (p <0.05).

The highest total phenolic content was found in the methanol extract of *Berberis integerrima* fruit with value of 3544.84 (mg GAE/100 g DM), while the lowest amount was determined in the ethanol extract of *Rosa canina* L. fruit with value of 1463.57 (mg GAE/100 g DM). The order of total amount of phenolic substance from the highest to the lowest value regarding all three solvent extraction systems, was determined as: *Berberis integerrima* > *Viburnum opulus* L. *> Berberis vulgaris* L. *> Rosa canina L.* In terms of total flavonoids, *Berberis integerrima* fruit extracted with methanol had the highest value of 2098.88 (mg CE/100 g), while *Rosa canina* L. fruit extracted with water had the lowest total flavonoid amount of 221.82 (mg CE/100 g). The order of the total amount of flavonoid substance in samples extracted by three solvent extraction systems, was: *Berberis integerrima > Viburnum opulus* L.*> Berberis vulgaris* L. *> Rosa canina L.*

In another study, the bioactive components of *Berberis crataegina* were examined and the total phenolic content was determined as 53.51 mg GAE /g and the total flavonoid amount was evaluated as 27.42 mg quercetin/g (Charehsaz et al., 2015). Fascella et al. (2019) investigated the total amount of phenolic substances in four species of Rose plants. The total amount of phenolic substances varied between 4057- 6784 mg GAE /100 g DW. In another study, the total amount of phenolic and flavonoid substances in five species of Rose plants was examined. The amount of total phenolic compounds was determined to be between 31.08 and 52.94 mg of gallic acid per gram of dry weight (DW), while the flavonoid content ranged from 9.31 to 10.81 mg of rutin per gram (Demir et al., 2014). In another study related to the topic, the total amounts of phenolic and flavonoid substances in water and methanol extracts of *Viburnum opulus* L. fruit were investigated and the phenolic content of the extracted materials were determined as 25.64 and 40.17 mg GAE/g DW, while the total flavonoid amounts were found to be 24.57 and 25.09 mg catechin/g DW, respectively (Barak et al., 2019).

The effect of used solvent on total amount of anthocyanins

The results were examined and statistically significant differences were determined in the total anthocyanin content of the fruits (P<0.05) (Table 2). The order of total amount of anthocyanins from the highest to the lowest values found in the extracts was obtained as: *Berberis integerrima > Berberis vulgaris* L. *> Rosa canina L. > Viburnum opulus* L.

In a study done by Ramezani, the total anthocyanin amount of *Berberis vulgaris* L. fruit was determined to be 39.56 mg cyanidin-3 glucoside/kg (Ramezani et al., 2021). In another study, the total amount of anthocyanins was evaluated 27.6 – 54.3 mg cyanidin-3-glucoside/100 g in their study on 11 genotypes of Gilaburun in Turkiye (Ozrenk et al., 2020). Cunja et al. (2015) investigated the bioactive components of *Rosa canina* L. grown in Slovenia during ripening and after frost damage and the total amount of anthocyanins was found to be in range of 9.2 – 125.7 µg cyanidin-3-glucoside/g DW. In a study conducted in different regions of Iran, the anthocyanin content of the Berberis *integerrima* was found to be in the range of 19–153 mg/100 g (Alemardan et al., 2013).

Table 2. Anthocyanin values of extracted samples.

Sample	Total Anthocyanin (mg Cyanidin-3-glucoside /100 g)
Berberis vulgaris L.	60.94 ± 5.30^{*b**}
Berberis integerrima L.	71.39 ± 6.60^{c}
Viburnum opulus L.	23.81 ± 2.52^{a}
Rosa canina L.	27.48 ± 2.18^{a}

*Standard deviation.

** Different letters in each column; indicates that there is a statistical difference between the values (p <0.05).

The effect of extraction methods on vitamin C in berry extracts

Vitamin C amounts of the samples extracted with different solvents varied significantly ($p<0.05$) (Table 3). The amounts of vitamin C from the highest to the lowest values in the ethanol extracts of different berry samples were determined as; *Berberis integerrima* > *Viburnum opulus* L. > *Rosa canina* L.> *Berberis vulgaris* L. In methanol extracts, the order amounts of vitamin C were found as: *Berberis integerrima* > *Viburnum opulus* L.> *Berberis vulgaris* L. > *Rosa canina L.* and water extracts *Berberis integerrima* > *Viburnum opulus* L. > *Berberis vulgaris* L. > *Rosa canina L.* The findings from other studies reviewed by Gündeşli et al. (2019) and our own results suggest that these berries contain significant amounts of vitamin C, which are influenced by factors such as their place of origin, harvesting time, and the extraction method used.

Table 3. Vitamin C amounts of different berry extracts subjected to extraction (mg/100 g).

	Vitamin C (mg/100 g)		
Sample	**Ethanol Extract**	**Methanol Extract**	**Water Extract**
Berberis vulgaris L.	18.5 2± 1.12*a**	28.26 ± 0.59[b]	22.17 ± 0.67[b]
Berberis integerrima L.	24.60 ± 2.43[b]	33.22 ± 0.59[d]	29.22 ± 0.56[d]
Viburnum opulus L.	20.21 ± 1.87[a]	30.65 ± 0.51[c]	25.79 ± 0.63[c]
Rosa canina L.	18.88 ± 0.90[a]	22.23 ± 1.24[a]	19.60 ± 0.57[a]

*Standard deviation.

** Different letters in each column indicate that there is a statistical difference between the values (p <0.05).

The effect of extraction methods on antioxidant activity

The antioxidant activity and free radical scavenging inhibition rates of samples prepared by methanol, ethanol and water extracts of *Viburnum opulus* L., black barberry, gilabur and *Rosa canina* L. samples are given in Table 4., Figure 1 and Figure 2. The order of antioxidant activity values for samples treated by all three solvent extraction systems from the highest to the lowest values was determined as: *Berberis integerrima* > *Viburnum opulus* L. > *Berberis vulgaris* L. > *Rosa canina L.*

In a different study, the antioxidant activities of red barberry, purple barberry and orange barberry species were determined by DPPH and CUPRAC methods. The antioxidant activity values determined by DPPH analysis were 96.63, 112.70 and 74.59 mg TE/g and 286.0, 290.10 and 182.92 for CUPRAC, respectively (Şensu et al., 2021). In another study, the antioxidant activities of *Berberis vulgaris* L. and *Berberis integerrima* fruits were evaluated from 11 different regions of western Azerbaijan, Iran and their amounts of antioxidants were determined by the FRAP method. The antioxidant activity values varied in the range 20.20–70.39 mmol Trolox / L. (Hassanpour and Alizadeh, 2016).

In another study, the antioxidant activities of water and methanol extracts of *Viburnum opulus* L. were investigated by DPPH, DMPD, FRAP, and CUPRAC methods. Water extract antioxidant activities (DPPH, DMPD, FRAP, and CUPRAC) were found to be 96.74 mg GHTE/g sample, 55.00 mg Trolox/g sample, 0.41 (mM $FeSO_4$/g sample, and 156.49 mg Ascorbic Acid/g sample, respectively. The antioxidant activities of the methanol extract were calculated as follows: 103.59 mg GHTE/g Sample, 52.55 mg Trolox/g Sample, 0.46 mM $FeSO_4$/g Sample, and 208.87 mg Ascorbic Acid/g Sample, respectively (Barak et al., 2019).

Tabaszewska and Najgebauer-Lejko, (2020) measured the antioxidant activity of *Rosa canina L. canina* fruit in samples with seeds, without seeds, fresh, frozen, hot air-dried and freeze-dried forms using the FRAP and ABTS+ methods. The results of the performed analyses with the FRAP method were as: (0.52, 0.95, 0.54, 0.94, 0.59, 0.89) measured µM Trolox/100 ml. The results of the analyses performed by the ABTS+ method, were obtained as: 127, 168, 160, 155, 119, 157 µM Fe^{2+}/100 ml (Ahmad et al., 2015).

In our study, the highest amount of Trolox equivalent and antioxidant inhibition percentage was determined in *Berberis integerrima* extracted with methanol, while the lowest amount was found in *Rosa canina L.* extracted with pure water.

Table 4. DPPH and ABTS antioxidant activity results of different extracted samples.

DPPH (mM Trolox / 100 g DM)			
Sample	**Ethanol Extract**	**Methanol Extract**	**Water Extract**
Berberis vulgaris L.	1.06 ± 0.17^{*b**}	2.34 ± 0.04^{b}	0.81 ± 0.05^{b}
Berberis integerrima L.	1.83 ± 0.12^{d}	2.95 ± 0.18^{d}	1.63 ± 0.11^{d}
Viburnum opulus L.	1.60 ± 0.12^{c}	2.75 ± 0.13^{c}	1.21 ± 0.05^{c}
Rosa canina L.	0.71 ± 0.49^{a}	0.86 ± 0.31^{a}	0.45 ± 0.02^{a}
ABTS^{+} (mM Trolox / 100 g DM)			
Sample	**Ethanol Extract**	**Methanol Extract**	**Water Extract**
Berberis vulgaris L.	1.69 ± 0,03^{*b**}	2.48 ± 0.05^{b}	2.33 ± 0.06^{b}
Berberis integerrima L.	2.95 ± 0,18^{d}	3.30 ± 0.10^{d}	2.63 ± 0.12^{d}
Viburnum opulus L.	2.61 ± 0,04^{c}	2.80 ± 0.04^{c}	2.43 ± 0.03^{c}
Rosa canina L.	1.31 ± 0,05^{a}	1.98 ± 0.04^{a}	1.28 ± 0.06^{a}

*Standard deviation.
** Different letters in each column indicate that there is a statistical difference between the values (p <0.05).

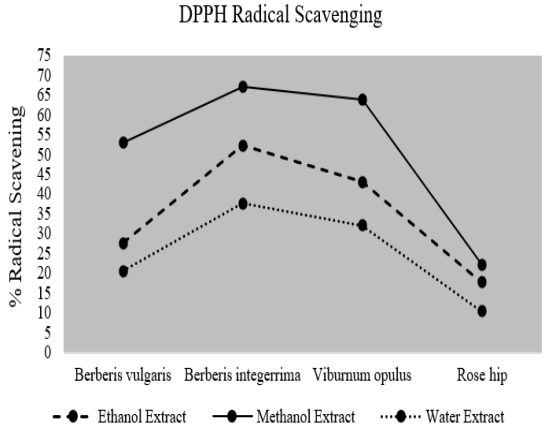

Figure 1. DPPH radical scavenging activity of various plant extracts.

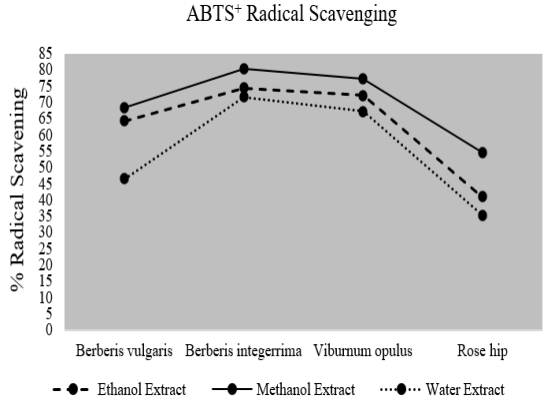

Figure 2. ABTS^{+} radical scavenging activity of various plant extracts.

The effect of used solvent on colour parameters of prepared extract samples

Colour parameters of plant extracts, such as the intensity of red, yellow and blue, are linked to the presence of compounds like flavonoids, anthocyanins and carotenoids. These compounds are not only responsible for the visible colour of the extracts but also significantly contribute to their antioxidant properties and biological activities. For instance, flavonoids are known for their strong antioxidant effects, which are crucial in preventing oxidative stress and related diseases. Similarly, carotenoids and anthocyanins, associated with specific colours, play role in anti-inflammatory and anticancer activities. (Mattioli et al., 2020; Mutha et al., 2021; Muzolf-Panek and Waśkiewicz, 2022).

The colour values of *Rosa canina* L., Viburnum opulus, *Berberis vulgaris* L. and *Berberis integerrima fruits* extracted with different solvents are presented in Table 5.

Table 5. Colour absorbance variables of extractions.

Sample	Abs 280nm	Abs 420nm	Abs 520nm	Abs 620nm	CD[1]	CI[2]	%T[3]	%Red[4]	%Yellow[5]	%Blue[6]
BIM*	0.5221	0.5067	0.1191	0.0251	0.6258	0.6509	4.2544	18.2977	77.8461	3.8562
RM	0.2684	0.1155	0.0353	0.0098	0.1508	0.1606	3.2720	21.9801	71.9178	6.1021
VM	0.4160	0.4121	0.1184	0.0242	0.5305	0.5547	3.4806	21.3449	74.2924	4.3627
BVM	0.5004	0.1170	0.0508	0.0092	0.1678	0.1770	2.3031	28.7006	66.1017	5.1977
BIE	1.0902	0.1811	0.0451	0.0039	0.2262	0.2301	4.0155	19.6002	78.7049	1.6949
RE	0.5561	0.0943	0.0247	0.0067	0.1190	0.1257	3.8178	19.6500	75.0199	5.3302
VE	1.0591	0.1496	0.0428	0.0030	0.1924	0.1954	3.4953	21.9038	76.5609	1.5353
BVE	0.7249	0.0549	0.0320	0.0017	0.0869	0.0886	1.7156	36.1174	61.9639	1.9187
BIW	0.6771	0.7641	0.2822	0.1194	1.0463	1.1657	2.7077	24.2086	65.5486	10.2428
RW	0.4872	0.2488	0.0945	0.0293	0.3433	0.3726	2.6328	25.3623	66.7740	7.8637
VW	0.6696	0.7538	0.2812	0.1030	1.0350	1.1380	2.6807	24.7100	66.2390	9.0510
BVW	0.4584	0.1702	0.1399	0.0233	0.3101	0.3334	1.2166	41.9616	51.0498	6.9886

*BIM; Berberis I/Methanol, RM; Rose H/Methanol, VM; Viburnum O/Methanol, BVM; Berberis V/Methanol, BIE; Berberis I/Ethanol, RE; Rose H/Ethanol, VE; Viburnum O/Ethanol, BVE; Berberis V/Ethanol, BIW; Berberis I/Water, RW; Rose H/Water, VW; Viburnum O/Water, BVW; Berberis V/Water extracts.

[1]Colour Density, [2]Colour Intensity, [3]Tint Value, [4]Proportion of Red Colour, [5]Proportion of Yellow Colour, [6]Proportion of Blue Colour

Considering the colour parameters of the samples extracted with different solvents, the order of colour density (CD) of the samples extracted with methanol from highest to the lowest was determined as: BIM > VM > BVM > RM, while the same values were found for the ethanol extract were as: BIE > VM > RE > BVE. The order of colour density obtained by was calculated as: BIW > VW > RW > BVW, respectively.

The colour intensity (CI) values of the sample extracts treated with methanol from highest to the lowest was as: BIM > VM > BVM > RM, while for samples treated with ethanol was as: BIE > VE > RE > BE. The order for samples subjected to extraction with water was as: BIW > VW > RW > BVW.

The order for the colour tone percent values (%T) of samples extracted with methanol and water was determined as: BIM > VM > RM > BVM, while for samples obtained with ethanol extraction the order was evaluated as: BIE > RE > VE > BE. The score values of colour intensity (CD), colour intensity (CI) and colour tone percent values (%T) are presented in Figure 3.

The highest value for % of red was determined in samples coded as BVW, followed by BVE and BVM. Regarding the % of yellow, the highest value was obtained for BIE, followed by BIM and VE. The order

for % of blue was different and was considered as: BIW > VW>RW. All score values of these parameters are presented in Figure 4.

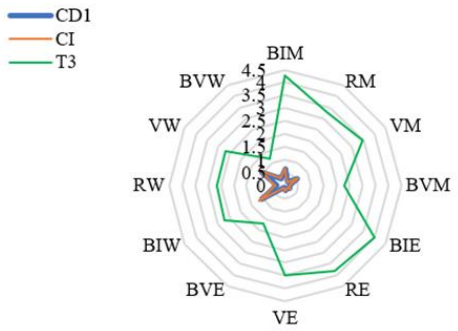

Figure 3. Colour correlations between extract samples of *Rosa canina* L., *Viburnum opulus*, *Berberis vulgaris* L. and *Berberis integerrima*.

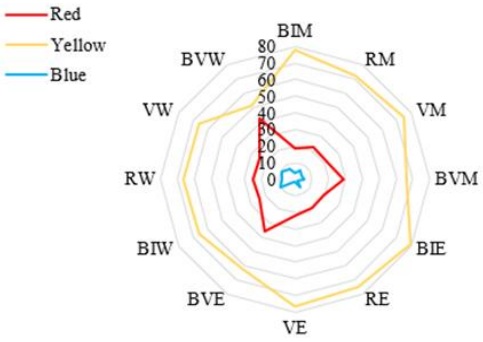

Figure 4. Colour change chart of extracts *Rosa canina* L., *Viburnum opulus*, *Berberis vulgaris* L. and *Berberis integerrima* L.

Considering of colour results, the highest value for CD and CI was obtained for BIM (*Berberis I*/Methanol), BIE (*Berberis I*/Ethanol) and BIW (*Berberis I*/Water) samples. The highest value for % T was obtained for BIM (*Berberis I*/Methanol), BIE (*Berberis I*/Ethanol). Regarding the highest score for % R, %Y and % B was obtained with BVW (*Berberis V*/water extracts), BIE (*Berberis I*/ethanol) and BIW (*Berberis I*/water), respectively.

Correlation between total phenolic compounds, flavonoid amount and antioxidant activity

In our study, a strong positive correlation was determined between total phenolic and flavonoid content amounts and antioxidant activity of fruit samples. While the correlation coefficient between antioxidant activity (DPPH) results and total phenolic content and flavonoid amounts was determined as r=0.65 and r=0.90, respectively, the correlation coefficient between antioxidant activity (ABTS+) and total phenolic, flavonoid substance amounts was determined as r=0.82 and r=0.62, respectively. All correlations among total phenolic compounds, flavonoid amount and antioxidant activity are presented in Figure 5 and 6.

The results of these correlations confirmed the high bioactivity of samples to be a consequence of the presence of high bioactive compounds. Polyphenols and flavonoids are key contributors to the antioxidant activity of plant extracts due to their ability to scavenge reactive oxygen and nitrogen species (ROS and RNS). These compounds neutralize free radicals through mechanisms like single electron transfer (SET) and hydrogen atom transfer (HAT), facilitated by their hydroxyl groups. The structure of

flavonoids, with their conjugated ring systems, allows for effective electron delocalization, which stabilizes free radicals and enhances their antioxidant properties.

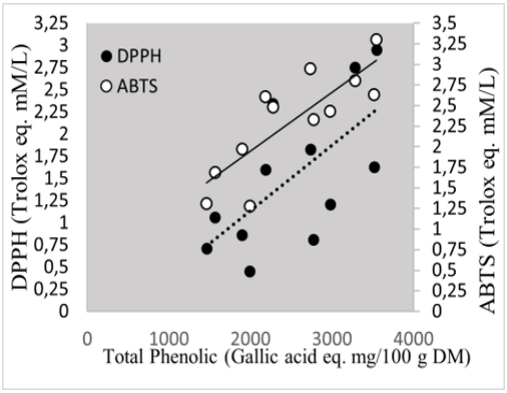

Figure 5. Correlation between DPPH, ABTS and total phenolic compound.

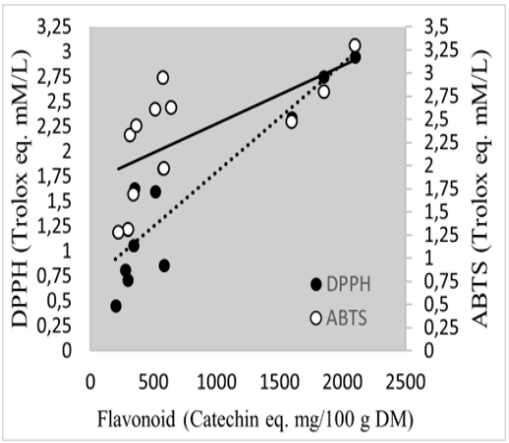

Figure 6. Correlation between DPPH, ABTS and flavonoid content.

Furthermore, as anthocyanins, a subclass of flavonoids, undergo pH-dependent structural changes, these modifications enhance their antioxidant properties. Consequently, the concentration of these bioactive compounds in the extract directly influences its ability to combat oxidative stress, with higher concentrations correlating to increased bioactivity and potential health benefits (Mattioli et al., 2020; Muzolf-Panek and Waśkiewicz, 2022).

Conclusions

Turkiye is a country with a very rich range of endemic plants. In the light of our results was demonstrated that some special fruits such as *Rosa canina* L., *Viburnum opulus* L., *Berberis vulgaris* L. and *Berberis integerrima* L. are rich in total bioactive components, anthocyanin, vitamin C and have high antioxidant activities. The extracts of *Berberis integerrima* L. and *Viburnum opulus* L. have high bioactivity and are richer in bioactive components than other fruits. The highest total phenolic content was determined in the methanol extract of *Berberis integerrima* fruit as 3544.84 (mg GAE/100g DM). The highest total flavonoids content was determined in *Berberis integerrima* fruit extracted with methanol had the highest value of 2098.88 (mg CE/100). The highest total amount of anthocyanins found in the extracts

was obtained as: *Berberis integerrima*. The highest amount of Trolox equivalent (DPPH)($ABTS^{+}$) was determined as 2.95-3.30 (mM Trolox/100 g DM) in *Berberis integerrima* extracted with methanol. The highest amounts of vitamin C extracted with ethanol, methanol and water was determined for *Berberis integerrima* 24.60; 33,22; 29.22 (mg / 100g) respectively. The highest value for CD, CI and T was obtained for *Berberis integerrima* extracted with methanol. The highest score for % R, %Y and % B was obtained with BVW (Berberis V/Water extracts), BIE (Berberis I/Ethanol) and BIW (Berberis I/Water), respectively. All of these results depend on the solvent which was used for extraction. The results of this study will promote the usage of these fruits in the food industry.

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