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ORIGINAL RESEARCH PAPER

INVESTIGATION ON THE ANTIOXIDANT COMPOUNDS AND ANTIOXIDANT CAPACITY OF ROMANIAN *PRUNUS* SPECIES

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Abstract

The current investigation delineates the application of the photochemiluminescence assay in two distinct extracts, namely hydrophilic and lipophilic, alongside the utilization of the DPPH method to quantify the antioxidative potential of five fruits belonging to the *Prunus* genus (sweet cherry, sour cherry, apricot, peach and plum). The findings of this study revealed that the analyzed fruit samples exhibited noticeable concentrations of phenolic compounds ranging from 31.14 ± 0.29 to 171.61 ± 0.98 mg of Gallic Acid Equivalent per 100 g of fresh weight. In reference to the flavonoid content, the observed concentrations ranged from 8.61±0.19 to 22.15 ± 0.31 mg Rutin Equivalent per 100 g fresh weight, whereas the anthocyanin content displayed concentrations ranging between 0.26 ± 0.01 and 5.26 ± 0.51 mg cyanidin-3-glucoside equivalent per 100 g fresh weight. Remarkably, the sour cherry exhibited the highest antioxidant capacity among the tested fruits in both hydrophilic and lipophilic systems, followed by sweet cherry and apricot.

Keywords: Prunus genus, phenolics, flavonoids, anthocyanins, antioxidant activity, photochemiluminescence

Introduction

Since ancient times, it has been well-established that antioxidants comprise a group of compounds that play a crucial role in neutralizing and reducing the concentration of free radicals, especially peroxyl radicals (ROO•), and reactive oxygen species (ROS) that are responsible for lipid peroxidation and autoxidation of organic substances (Abuajah *et al*., 2015). Food serves as a major source of exogenous antioxidants. A regular diet is thought to supply the organism more than 25,000 bioactive compounds. Vegetables, fruits, cereals, teas, legumes, nuts, and various other food products (FAO, 2001) represent abundant sources of antioxidants.

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The *Prunus* genus, belonging to the *Rosaceae* family, encompasses around 175 species distributed worldwide (Jang *et al*., 2018). *Prunus* is recognized as one of the most challenging genera in the *Rosaceae* family due to its complex taxonomy, primarily attributed to species polymorphism, wide ecological tolerance, and a vast number of cultivars (Dönmez and Yildirimli, 2000). The genus *Prunus* is further categorized into six subgenera: (1) *Amygdalus* (almonds and peaches); (2) *Prunus* (plums and apricots); (3) *Cerasus* (cherries); (4) *Lithocerasus* (represented by species like *Prunus pumila* - sand cherry); (5) *Padus* (represented by *Prunus padus* - bird cherry); and (6) *Laurocerasus* (represented by *Prunus laurocerasus* - cherry laurel) (Natic *et al.*, 2020).

In 2022, among stone fruits, peaches/nectarines had the highest world production, accounting for 26.3 million tons, followed by plums with 12.3 million tons. Apricot production ranked third with 3.8 million tons, while sweet cherries yielded 2.7 million tons, and sour cherries produced 1.6 million tons. China led in the peach/nectarine production, contributing with 16.8 million tons, followed by Italy and Turkey. The majority of plum production was concentrated in China along with Romania and Serbia. Turkey, Uzbekistan, and Iran led in annual apricot production, while sweet cherries were predominantly grown in Turkey, Chile, and Uzbekistan. Sour cherries were primarily produced in Russia, Poland, and Ukraine (Figure 1, Figure 2) (FAO, 2022).

Cherries (*Prunus* spp.) are the smallest members of the stone fruit family (Ferretti, 2010). Sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) are among the most popular cherry species traded and consumed as commodity fruits. Cherries are characterized by their high fiber and natural antioxidant content, offering relatively low-calorie consumption, and are typically consumed as fresh fruits. These small fruits are rich in phenolic compounds, particularly anthocyanins, which contribute to positive health benefits (Hu *et al*., 2021). In the case of apricots, phenolic compounds such as catechin, epicatechin, *p*-coumaric acid, caffeic acid, ferulic acid, and their esters have been identified (Sochor *et al.*, 2010). Studies investigating the antioxidant composition of peaches have revealed that phenolic compounds like chlorogenic and neochlorogenic acids, catechin, epicatechin, cyanidin, and quercetin derivatives play a significant role as potential antioxidants (Bento *et al*., 2020). Regarding plums, previous researches have demonstrated that these fruits are considered healthy food options due to their composition in carbohydrates, fibers, tannins, enzymes, minerals, and vitamins (Savic *et al*., 2021).

In Romania, the predominant fruit cultivars are represented by apples, pears, plums, apricots, sweet cherries, sour cherries, peaches, and nectarines (INSSE, 2022). Among these fruits, plum exhibits the highest production of 665,730 metric tons, followed by sweet cherry at 34,320 metric tons, and sour cherry at 28,970 metric tons (FAO, 2022). Apricot and peach production quantities stand at 23,500 metric tons and 12,370 metric tons, respectively. In terms of *Prunus* fruit consumption, plum ranks as the most consumed fruit with 7.7 kilograms per capita, followed by peach with 4 kilograms per capita, and cherries with 3.8 kilograms per capita (INSSE, 2022). It is known that *Prunus* species consumption provides health benefits

due to their nutritional components, acting also against the metabolic syndrome risk factors (Ullah *et al.*, 2020).

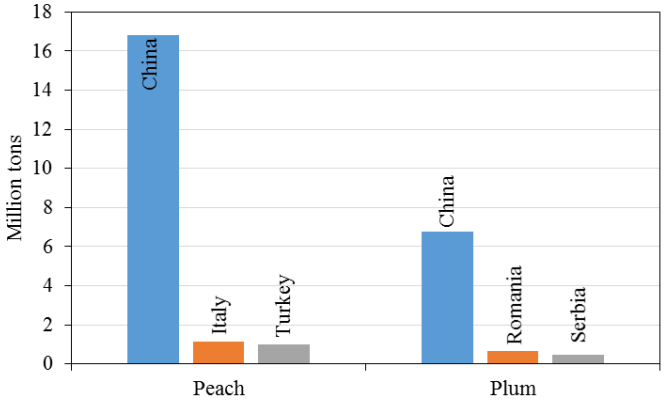

Figure 1. Top 3 producers of peach and plum in 2022

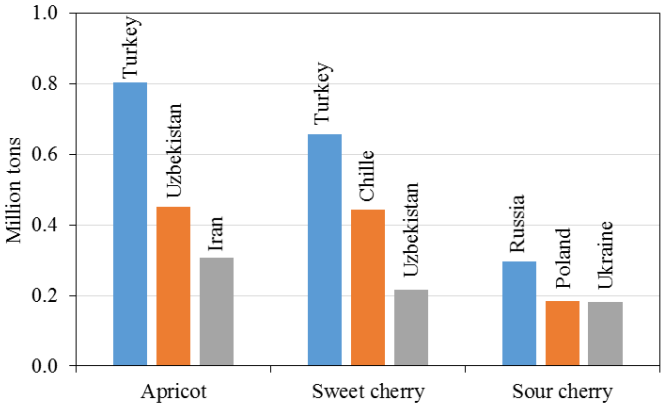

Figure 2. Top 3 producers of apricot, sweet cherry, and sour cherry in 2022

Considering the consumption patterns in Romania, this research aimed to evaluate the phenolic, flavonoid, and anthocyanin contents, but also the antioxidant capacities of five Romanian fruits belonging to the genus *Prunus*, known for their high nutritional value and globally widespread consumption.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (+)-rutin, gallic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (Switzerland). Folin–Ciocalteu's reagent was purchased from Merck (Germany). All chemicals used were of analytical grade. Standard solutions were prepared with distilled water.

Plant material

The selected Romanian samples (sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*), apricot (*Prunus armeniaca*), peach (*Prunus persica*), plum (*Prunus domestica*)) were purchased from a local market (Bucharest, Romania). The fruits used for the analysis were purchased one day before the experiment and were stored in the refrigerator at 4 °C until analysis. The samples were washed with tap water, the damaged parts were removed and the edible parts of the fruits (20-30 g of unpeeled fruits) were subjected to homogenization using a laboratory mixer.

Extraction procedure

For the analysis of total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and antioxidant activity on DPPH, 1.0 gram of fresh fruits was weighed and mixed with 30 mL of 50% aqueous methanol. The mixtures were vortexed for one hour at 2,000 rpm using a vortex mixer (Heidolph Instruments Multi Reax) in order to extract the compounds of interest. Following the vortexing step, the extracts were subjected to centrifugation at 10,000 rpm for 10 minutes, at 4 °C (Multescu and Susman, 2022). After centrifugation, the supernatant was freezed at -20 °C until further analysis.

Determination of Total Phenolic Content (TPC)

The determination of TPC was carried out using the Folin-Ciocalteu method with minor adjustments, as described by Multescu et al. (2022). In brief, a volume of 500 µL of the extract was mixed with 5 mL Folin-Ciocalteu reagent and 500 µL of 20% sodium carbonate. The resulting mixtures were incubated in darkness for 20 minutes to facilitate the formation of the molybdenum-tungsten complex development. The absorbance was measured at 765 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). The standard curve was established using various concentrations (ranging from 10 to 50 µg/mL) of Gallic acid under the same experimental conditions as the samples (R^2 = 0.9999). The total phenolic content was expressed as mg of Gallic acid equivalent per 100 g of fresh weight (mg GAE/100 g FW).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was evaluated using the $AlCl_3$ method (Multescu and Susman, 2022). Briefly, 0.1 mL of extract was mixed with 0.1 mL 10% sodium acetate and 0.12 mL 2.5% $AlCl_3$, the final volume being adjusted to 1 mL with ethanol 70%. The samples were vortexed and incubated in the dark for 45 min. The absorbance was measured at 510 nm. A standard curve was plotted by using different concentrations (10–60 µg/mL) of rutin (R^2 = 0.9996). Total flavonoid content was expressed as mg rutin equivalent/100 g of fresh weight (mg RE/100 g FW).

Determination of Total Anthocyanin Content (TAC)

The determination of TAC was conducted utilizing the pH differential method, capitalizing on the variations in the spectral absorbance of the samples containing anthocyanin compounds under varying pH conditions, according to AOAC (2005) method with slight modification. In brief, 3 mL of extract were mixed with 30 mL solution of pH 1.0 buffer (potassium chloride, 0.025M). Another 3 mL were mixed

with 30 mL of pH 4.5 buffer solution (sodium acetate, 0.4M). Subsequently, the optical properties of these solutions were quantified using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). Measurements were taken at two specific wavelengths: 520 nm and 700 nm. TAC was calculated applying the formula described in the AOAC (2005) method and presented bellow and the results were expressed in units of mg C3G/100 g FW:

$$\frac{A\ x\ MW\ xDF\ x\ 10^3}{\varepsilon\ x1}$$

Where A=(A_{520nm}-A_{700nm})pH 1.0 - (A_{520nm}-A_{700nm})pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (C3G); DF = dilution factor; 1 = pathlength in cm; ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1}, for C3G; and 10^3 =conversion factor (g to mg).

Determination of the antioxidant activity

DPPH radical scavenging activity was determined according to Horszwald and Andlauer (2011) with slight modifications. The reaction mixture consisted of 1 mL of methanolic extract and 6 mL of DPPH radical solution, which was incubated for 30 min in the dark. Then, the absorbance was measured at 517 nm. Antioxidant activity was calculated using a calibration curve (0.0156–0.0625 µg/mL) obtained with Trolox (R^2 = 0.9998). The results were expressed in µmol TE/100 g FW.

Photochemiluminescence Assay

For the photochemiluminescence assay, two different solvents, namely the ACW (aqueous) and ACL (methanolic) were employed. For the ACW, 1.0 gram of fruits was mixed with 30 mL of water, while for the ACL, 1.0 gram of sample was mixed with 30 mL of 100% methanol (Popov and Lewin, 1996). The mixtures were vortexed for one hour at 2,000 rpm using a vortex mixer (Heidolph Instruments Multi Reax) in order to extract the compounds of interest. Following the vortexing step, the extracts were subjected to centrifugation at 10,000 rpm for 10 minutes, at 4 °C (Multescu and Susman, 2022). After centrifugation, the supernatant was frozen at -20 °C until further analysis.

The assay described by Popov and Lewin (1996) was commercially distributed as a comprehensive system known as Photochem® by Analytik Jena AG (Jena, Germany). In the Photochemiluminescence (PCL) assay, the process involves the photochemical generation of (O_2 •−) free radicals combined with sensitive detection through chemiluminescence. To initiate the assay, the photosensitizer (S) is optically excited, leading to the formation of the superoxide radical anion. The process can be represented as follows:

$S + h\upsilon + O_2 \rightarrow [S^*O_2] \rightarrow S^{\bullet +} + O_2^{\bullet -}$

There are two distinct protocols in this assay: ACW (water-soluble antioxidant capacity) and ACL (lipid-soluble antioxidant capacity). These protocols allow separate measurement of both hydrophilic and lipophilic antioxidants. The conditions used in the assay are standardized, ensuring that the results obtained can be compared to other assays. The antioxidant potential is determined by measuring

the lag phase (ACW) and by calculating the area under the curve (ACL) at various concentrations.

Hydrophilic system (PCL-ACW)

The extracts were dissolved in water. The reactions were carried out using kits designed for determining the antioxidant capacity of water-soluble substances (Analytik Jena, Jena, Germany). The reaction mixture comprised 1000 µL of diluent (reagent 1), 1000 µL of buffer solution (reagent 2), 25 µL of luminol (reagent 3), and 50 µL of the fruit extract. The measurements were performed using a Photochem device with PCL soft software (Analytik Jena). A calibration curve was prepared using ascorbic acid. The results are expressed as µmol of ascorbic acid equivalent per 100g of fresh weight (µmol AA/100g FW).

Lipophilic system (PCL-ACL)

The extracts were dissolved in methanol. For the reactions, kits designed for determining the antioxidant capacity of lipid-soluble substances were utilized (Analytik Jena, Jena, Germany). The reaction mixture consisted of 2300 µL of methanol (reagent 1), 200 µL of buffer solution (reagent 2), 25 µL of luminol (reagent 3), and 50 µL of the fruit extract. The measurement was carried out using a Photochem device with PCL soft software (Analytik Jena). A calibration curve was prepared using Trolox. The results are expressed as µmol of Trolox equivalent (TE) per 100g fresh weight (µmol TE/100g FW).

Statistical analysis

All methods were applied for samples characterisation in at least three repetitions. Results are presented as means ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey test to assess differences between means (Minitab software, Minitab Inc., Coventry, UK). Differences were considered to be significant at $p < 0.05$. The principal component analysis (PCA) was also carried out using Minitab considering as variables TPC, TFC, TAC and DPPH; the type of matrix was correlation.

Results and discussion

Bioactive compounds content

In Table 1. the TPC, TFC and TAC of the analyzed Romanian cultivars from the *Prunus* genus are presented. The TPC ranged from 31.14±0.29 to 171.61±0.98 mg GAE/100 g FW. Among the five fruit types studied, sour cherry exhibited the highest TPC value of 171.61±0.98 mg GAE/100 g FW, whereas peach and plum showed lower TPCs, measuring 31.73±0.81 mg GAE/100g FW and 31.14±0.29 mg GAE/100 g FW, respectively. The concentration of polyphenols was significantly different ($p<0.05$) among the fruit samples except for peach and plum. In a prior investigation, Hu *et al.* (2021) determined phenolic compounds in four different Australia-grown sweet cherry cultivars, revealing TPC values ranging from 87±0.09 to 173±0.90 mg GAE/100 g FW. Furthermore, Chaovanalikit and Wrolstad (2004) reported a total phenolic content of 185.0 mg GAE/100 g FW in cherries, which exceeded the values obtained in our study. Prvulovic *et al.* (2012) analyzed two

Serbia-grown sweet cherry cultivars, and they found a TPC values of 76.05±4.85 mg GAE/100 g FW and 110.96±13.33 mg GAE/100 g FW, respectively, which were consistent with our findings.

Table 1. Total phenolic (TPC), flavonoid (TFC) and anthocyanins (TAC) content in the analyzed fruits

Sample	TPC (mg GAE/100 g FW)	TFC (mg RE/100 g FW)	TAC (mg C3G/100 g FW)
Sweet cherry	105.52±1.26^{b}	22.15±0.31^{a}	3.89±0.16^{b}
Sour cherry	171.61±0.98^{a}	17.25±0.28^{b}	5.26±0.51^{a}
Apricot	74.92±0.59^{c}	15.32±0.32^{c}	0.26±0.01^{d}
Peach	31.73±0.81^{d}	8.61±0.19^{e}	0.37±0.02^{d}
Plum	31.14±0.29^{d}	10.33±0.26^{d}	2.42±0.15^{c}

The values are expressed as means ± standard deviations (n =3). Values followed by different letters in the same column are significantly different ($p < 0.05$)

Sokół-Łętowska *et al.* (2020) determined the phenolic content in 21 sour cherry cultivars, reporting values ranging from 96.56±2.14 to 268.98±0.32 mg GAE/100 g FW. Vuletić *et al.* (2017) investigated how different locations (Osijek and Zadar), years (2010-2012), and cultivars influenced polyphenol content in fruits of various sour cherry cultivars. The average total polyphenol content over three years in the Osijek location ranged from 487±0.74 to 1276±0.53 mg GAE/100 g FW, while in the Zadar location, it was slightly higher, ranging from 561±0.56 to 1373±0.48 mg GAE/100 g FW. These values were higher than those obtained in our study. Khoo *et al.* (2011) investigated 34 sour cherry cultivars for their total phenolic content, finding a TPC between 74±2.50 to 754±13.40 mg GAE/100 g FW. Previous studies also reported variable total phenolic content values of sour cherries, ranging from 78 to 500 mg GAE/100 g FW (Bonerz *et al*., 2007; Kim *et al*., 2005; Dragovic´-Uzelac *et al*., 2007). Najafzadeh *et al.* (2014) evaluated Iranian sour cherries for TPC, and the total phenolic content across the genotypes ranged from 184.10 to 625.38 mg GAE/100 g FW.

Kalyoncu *et al*. (2009) determined the phenolic content in apricots, and the results indicated TPC values ranging between 58.4±6.20 to 309.5±17.80 mg GAE/100 g FW. Gecer *et al*. (2020) investigated the phenolic content in 15 wild and 1 cultivated apricots from Turkey, reporting TPC values ranging from 34.2 to 52.8 mg GAE/100g for wild apricots, and the Aprikoz cultivar had a TPC value of 37.8 mg GAE/100 g FW. Total phenolic content in 10 wild apricot genotypes ranged between 68.3 to 81.4 mg GAE/100g FW (Karatas, 2022). Hegedus *et al.* (2010) found a TPC between 82-2891.60 mg GAE/L in apricot germplasm. Karaat and Serce (2019) analyzed 12 Turkish apricots grown in Malatya and they found a phenolic content between 351.83 and 1208.33 µg GAE/g FW. Our results are in concordance with those obtained in previous studies.

Zhao *et al.* (2015) conducted an assessment of 17 Chinese peach cultivars to determine their phenolic content. The total phenolic content in the peach peel ranged

from 458 to 1268 mg GAE/100 g dry weight (DW), while in the pulp, it varied from 82 to 652 mg GAE/100 g DW. Manzoor *et al.* (2012) investigated the TPC in both peel and pulp parts of various peach varieties from Pakistan. The peel and pulp extracts exhibited a considerable amount of total phenolics, with values ranging from 1209.30±22.70 to 1354.50±18.60 mg GAE/100 g DW and 711.70±14.90 to 881.30±12.30 mg GAE/100 g DW, respectively.

Nineteen European plum cultivars and one Japanese plum genotype were evaluated for their total phenolic content (Rupasinghe *et al*., 2006). The results showed that TPC ranged from 86±8.00 mg GAE/100 g FW to 413±11.00 mg GAE/100 g FW. Kim *et al.* (2003) observed a range of TPC of 174±1.50-375±3.80 mg GAE/100 g FW in six commercial cultivars of plums. In another study, Chun and Kim (2004) reported that TPC in 13 plum cultivars from New York varied between 138.1±2.90-833.6±4.80 mg GAE/100 g FW. The total phenolic content in plum genotypes was within the range of 72.42±1.10 to 211.07±2.63 mg GAE/100 g FW (Miletic *et al*., 2012).

The differences in phenolic values observed in the current study compared to previous investigations may be attributed to variations in the origin of cultivars, environmental conditions, and fruit maturity levels (Hegedus *et al*., 2010). Similarly, previous findings have indicated that factors such as sunlight exposure, soil composition, seasonal variations, agronomic practices (Joshi *et al*., 1991), and the choice of analytical method can contribute to divergent results and variations in the levels of phenolic compounds (Hegedus *et al*., 2010; Leccese *et al.*, 2011).

The flavonoid content in *Prunus* fruits ranged from 8.61±0.19 mg RE/100 g FW to 22.15±0.31 mg RE/100 g FW. Data in table 1. showed that sweet cherry had the highest content of TFC (22.15±0.31 mg RE/100 g FW), followed by sour cherry (17.25±0.28 mg RE/100 g FW). On the other hand, peach presented the lowest value of flavonoid content (8.61±0.19 mg RE/100 g FW). The concentration of flavonoids was significantly different ($p<0.05$) among the studied fruits. These results emphasize the significance of species selection in relation to flavonoid content in the studied fruits.

The flavonoid content in four Australian-grown sweet cherry cultivars ranged between 31±0.05-51±0.02 mg quercetin equivalent (QE)/100 g FW (Hu *et al*., 2021). In contrast, Gao *et al.* (2017) reported a higher concentration of 550 mg RE/100 g FW for a sweet cherry cultivar from China. Similarly, De Souza *et al*. (2014) found a TFC value of 59.92±3.76 mg CE/100 g FW for sweet cherries in Brazil. Gu *et al*. (2019) determined the TFC value of sweet cherry to be 34±0.01 mg QE/100 g FW. These variations in flavonoid content highlight the influence of geographical location and cultivar selection on the flavonoid profiles of sweet cherries.

In previous studies, Karatas (2022) found a concentration between 9.2-15.1 mg equivalent catechin (CE)/100 g FW in 10 wild apricot genotypes from Anatolia. Saeed *et al*. (2021) reported a total flavonoid content between 0.044±0.01–7.623±1.97 µg QE/ g FW in apricots. According to Alajil *et al*. (2021) the TFC in apricot ranged between 5±0.83–15.46±0.63 mg CE/100 g FW. Carbone *et al*. (2018) reported a TFC in Italia-grown apricot cultivars between 1.9±0.1 and 12.0±0.2 mg CE/ g FW.

Being a potential source of bioactive compounds, Manzoor *et al*. (2012) investigated the TFC in peel and pulp parts of various peach varieties. The peel extract exhibited a TFC range of 599.70±11.70-785.50±15.30 mg CE/100 g DW, whereas the TFC in the pulp extract decreased to levels of 301.30±7.70-499.70±9.40 mg CE/100 g DW. These results underscore the distinction in flavonoid content between the peel and pulp portions of peach fruits. The flavonoid content was between 1.8-30.9 mg CE/100 g FW in 218 genotypes from 15 peach and nectarine breeding progenies (Cantin *et al*, 2009). Mrázová *et al.* (2021) analyzed 34 peach cultivars of diverse origin including 20 cultivars from USA, 6 from Yalta, 5 from Italy, 1 from Czech Republic, 1 from France, and 1 from Slovakia. The flavonoid concentration varied from 1.12±0.02 to 95.1±0.8 mg (+)-catechin equivalents (CAE)/100 g FW.

Cosmulescu *et al*. (2014) determined the TFC in 12 Romanian plum cultivars which was between 5.40±0.31-20.43±1.02 mg QE/100 g FW. These are in concordace with our results. In the research made by Kim *et al*. (2003b), the TFC ranged from 118±2.60 to 237±6.30 mg CE/100 g FW in various cultivars of plum. The results showed that the levels of total flavonoid compounds changed depending on cultivars and fruit parts.

The results demonstrated significant variability in the total flavonoid content, which was observed to be influenced by the specific cultivars as well as by the distinct anatomical parts of the fruits.

The TACs in the analyzed *Prunus* species from Romania were between 0.26±0.01-5.26±0.51 mg C3G/100 g FW. High levels of anthocyanins were measured in sour cherry (5.26±0.51 mg C3G/100 g FW), sweet cherry (3.89±0.16 mg C3G/100 g FW) and plum (2.42±0.15 mg C3G/100 g FW). On the other hand, low values were recorded in peach and apricot, 0.37±0.02 mg C3G/100 g FW and 0.26±0.01 mg C3G/100 g FW, respectively. The anthocyanins content was significantly different ($p<0.05$) except for apricot and peach. Mrázová *et al*. (2021) analysed 17 peach cultivars and they reported a TAC between 0.05±0.03-3.7±0.20 mg C3G/100 g FW. The results are in accordance with those found in this article. Variations in sour cherry fruit total anthocyanin content have been reported by Kim *et al.* (2005) (49.1±0.8–109.2±6.2 mg C3G/100 g FW), Simunic *et al.* (2005) (2.7±0.0–28.0±0.1 mg C3G/100 g FW), and Papp *et al*. (2010) (11.31-93.48 mg C3G/100 g FW). The anthocyanin content in four plum cultivars from Slovenia exhibited a range of concentrations, specifically measured in terms of cyanidin-3-glucoside, ranging from 0.01±0.00 mg to 5.57±2.616 mg/100 g FW (Usenik *et al.*, 2009). The results align with those discovered in this article. Bureau *et al*. (2009) conducted an analysis of two apricot cultivars, examining both the skin and flesh parts in terms of their anthocyanin content. In the skin part, the TAC was found to be 4.7±0.6 mg C3G/kg FW and 31.1±9.7 mg C3G/kg FW for the two respective cultivars. Regarding the flesh part, the anthocyanin content was detected at 0 mg and 3.6 mg C3G/kg FW, for each of the two cultivars, respectively.

Antioxidant Activity of Prunus species

Prunus fruits contain a high amount of antioxidants (Zehiroglu *et al.*, 2019). Previous studies reported on the health benefits of *Prunus* species consumption regarding

cardiovascular, diabetes, inflammatory pathologies, bone, gastric, liver and brain health (Ullah *et al.*, 2020).

As presented in Figure 3. the antioxidant activity of selected fruits ranged between 177.21±4.01-263.40±3.12 µmol TE/100 g FW. The fruits extracts of sour cherry, sweet cherry and apricot showed the highest activity with values of 263.40±3.12, 235.16±5.32, and 232.64±2.96 µmol TE/100 g, while peach and plum extract presented the lowest DPPH scavenging activity. The DPPH values are significantly different ($p<0.05$).

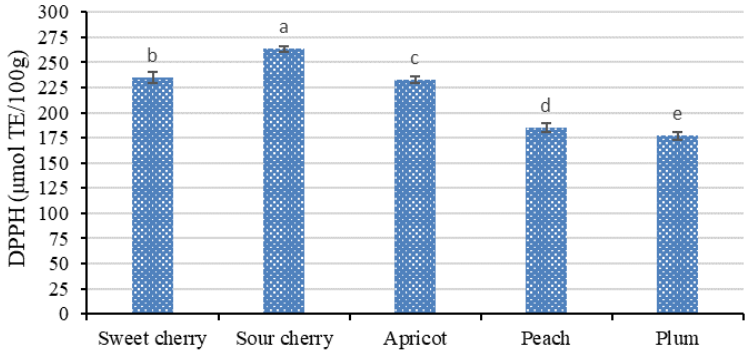

Figure 3. Antioxidant activity of 50% methanolic extracts from analyzed *Prunus* species

In prior investigations, the DPPH activity of distinct *Prunus* cultivars has been extensively studied using various calculation methodologies. Regarding sweet cherry cultivars, Li *et al*. (2020) reported a DPPH value of 4.61 mmol TE/kg for the Bing cultivar and 3.49 mmol TE/kg for the Rainier cultivar, while Zhao *et al*. (2019) observed a DPPH value of approximately 3.8 mmol TE/kg for the Lapins cultivar. Khoo *et al.* (2011) examined the antioxidant activity of 34 sour cherry cultivars. The findings revealed a range of antioxidant activity levels from 9±3.1 to 63±7.5µmol TE/g. Antioxidant activity measured using DPPH• test in the genotypes of apricot varied within the interval from 8.3±0.7 to 555.6±48.6 mg Trolox/100 g FW (Sochor *et al*., 2010). Zhao *et al.* (2015) analyzed antioxidant activity in peel and pulp extract of 17 peach cultivars. The DPPH values varied from 6.35±0.63 to 19.84±0.48 mg TE/g DW in the peel and from 1.05±0.07 to 15.01±2.36 mg TE/g DW in the pulp. Cosumlescu *et al.* (2014) investigated the antioxidant activity of various components of plum fruit, namely skin, pulp and fruit itself. The antioxidant capacities exhibited variations among different plum genotypes and in different parts of fruits. Specifically, the antioxidant activity ranged from 2.54±0.24 to 6.0±0.32 mmol Trolox/100 g FW in the skin, from 0.23±0.01 to 0.46±0.03 mmol Trolox/100 g FW in the pulp, and from 0.48±0.01 to 1.02±0.05 mmol Trolox/100 g FW in the fruit. Slimestad *et al.* (2009) studied six Norwegian plum cultivars to assess their antioxidant activity, and they observed values ranging from 290 µmol/100g FW to 814 µmol/100g FW. Cantin *et al.* (2009) evaluated the antioxidant activity in 218 genotypes from 15 peach and nectarine breeding progenies. They measured the relative antioxidant capacity (RAC) and found that it varied significantly among the

different genotypes, with values ranging from 227.3 to 629.9 µg of Trolox/100 g FW, and an average of 405 µg of Trolox/100 g FW. Mihalache Arion *et al.* (2014) highlighted differences in antioxidant capacity and antioxidant content between cultivars by testing 6 summer and 6 autumn cultivars obtained from the Fruit Research Station, Miroslava, Iasi, Romania. The DPPH values for summer cultivars ranged between 203-730 µmol TE/100 g FW, and from 132 to 554 µmol TE/100g FW for autumn cultivars respectively.

A strong correlation was observed between TPC and TFC with DPPH method. The correlation coefficients (r) for these relationships were 0.9471 and 0.7952, respectively. A positive relationship (r = 0.5952) was presented between total anthocyanins and DPPH test (Table 2).

Table 2. The correlation coefficients between total phenolic (TPC), flavonoid (TFC) and anthocyanins (TAC) content with antioxidanta acitvity (DPPH)

	TPC	**TFC**	**TAC**
DPPH	0.9471	0.7952	0.5952

Antioxidant Capacity of Water Soluble Compounds (ACW) and Lipid Soluble Compounds (ACL)

The results of ACW and ACL antioxidant capacity, determined by the PCL method, are presented in Table 3. The ACW values ranged from 28.79±0.39 to 430.78±0.21 µmol AA per 100 g FW. As shown in Table 3, the sour cherry sample exhibited the highest ACW value, reaching 430.78±0.21 µmol AA/100 g FW. Following that, the sweet cherry (360.31±0.62 µmol AA/100 g FW) and apricot (293.32±0.79 µmol AA/100 g FW) samples showed ACW values approximately around 300 µmol AA/100 g FW. Obtained data for the peach and plum extract were the lowest (under 100 µmol AA/100 g FW).

Table 3. Values of antioxidant capacity of the water soluble (ACW) and lipid soluble compounds (ACL)

Sample	**PCL-ACW (µmol AA/100 g FW)**	**PCL-ACL (µmol Trolox/100 g FW)**
Sweet cherry	360.31±0.62^{b}	1276.02±1.01^{b}
Sour cherry	430.78±0.21^{a}	1647.75±0.96^{a}
Apricot	293.32±0.79^{c}	1222.96±1.10^{c}
Peach	28.79±0.39^{e}	866.04±0.62^{d}
Plum	90.72±0.64^{d}	727.00±0.59^{e}

The values are expressed as means ± standard deviations (n =3). Values followed by different letters in the same column are significantly different ($p < 0.05$)

Usenik *et al.* (2008) assessed the antioxidant capacity of 13 cherry cultivars by quantifying it in terms of ascorbic acid equivalent. The recorded values for the antioxidant capacity ranged from approximately 7.99±0.38 to 17.2±0.30 mg/100 g FW. The findings from their study indicate lower values compared to the results we have reported. Homoki *et al*. (2016) analysed the antioxidant capacity ACW and ACL of different Hungarian sour cherry varieties. The results were between

4.92±0.33-15.36±0.27 µg AA/g FW for ACW system and between 3.40±0.11-10.61±0.29.

Lipid soluble antioxidant capacity data of the 5 *Prunus* fruits were between 727.00±0.59 and 1647.75±0.96 µmol TE/100 g FW (Table 2). Among these fruits, sweet cherry showed the highest value of 1647.75±0.96 µmol TE/100 g FW followed by apricot (1276.02±1.01 µmol TE/100 g FW) and sour cherry (1222.96±1.10 µmol TE/100 g FW). The lowest ACL value was observed in plum extract, which exhibited an ACL value of 727.00±0.59 µmol TE/100 g FW.

Hegedus *et al.* (2010) investigated the antioxidant capacities using ACW and ACL system in 15 apricot cultivars. The ACW values ranged from 1601.7±250.5 to 33587.5±980.8 nmol AA/L, while the ACL values varied from 0.55±0.11 to 78.65±2.50 nmol TE/L.

Principal Component Analysis

The aim of the principal component analysis (PCA) is to reduce a big number of variables to a few variables, referred to as principal components (PCs) (Granato et al., 2018). PCA was employed to explore similarities among the fruit samples in relation with the analyzed parameters (TPC, TFC, TAC and DPPH); these parameters are called variables within the statistical software. PCA graph projected onto the first principal component (PC1)/second principal component (PC2) plane is presented in Figure 3.

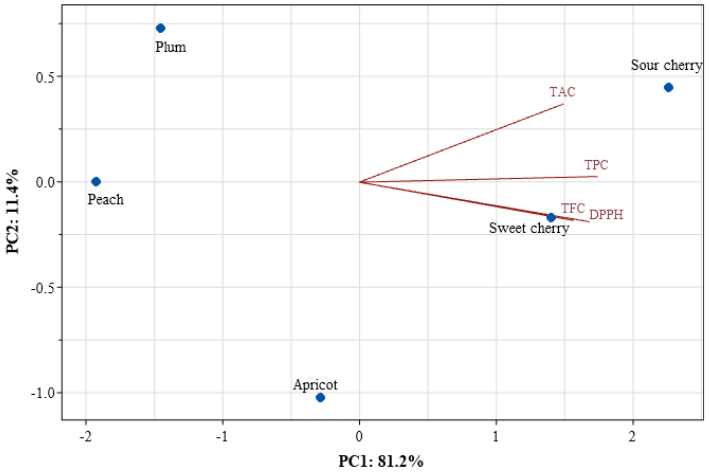

Figure 4. Principal component analysis to project fruit samples based on TPC (total phenolic content), TFC (total flavonoid content), TAC (anthocyanins content) and DPPH (antioxidant activity through DPPH)

The PC1 and PC2 described 81.2% and 11.4% of variance, respectively, and the total contribution rate of PC1 and PC2 was 92.6%. The plot indicates similarity between sour cherry and sweet cherry (the samples located in the right side of the graph) and between plum, peach and apricot (the samples are on the left side of the PCA plot). The analyzed parameters (TPC, TFC, TAC and DPPH) are positively correlated, being in close proximity and on the same side of the graph. TPC, TFC and

antioxidant activity were also pooled together in a previous study dealing with edible plants analysis (Rana *et al.*, 2019). Also, in a study investigating the properties of common Indian fruits and vegetables, PCA analysis showed positive relations amongst TPC, TFC, DPPH and ABTS antioxidant activities, stating that phenolic compounds were mainly responsible for the antioxidant activities of fruits and vegetables (Singh *et al.*, 2016). In different *Citrus* species, the relationship between flavonoid content and antioxidant capacity performed by PCA, revealed that the characteristic flavonoids for each species contributed largely to the antioxidant capacity (Chen *et al.*, 2020).

Conclusions

In this research, we examined the phenolic content, flavonoid content, anthocyanin content, antioxidant activity, and the antioxidant capacity in both water and lipid-soluble system for five distinct *Prunus* fruits. The outcomes of our analysis revealed the remarkable richness of these fruits in a diverse array of biologically functional compounds, with a pronounced emphasis on their substantial total phenolic content.

In the assessment of antioxidant activity, the DPPH method was utilized, while the evaluation of water soluble compounds and lipid soluble compounds was conducted using the PCL method. The findings revealed distinct variations in antioxidant capacities across different fruit species. Notably, among all the fruits investigated, sweet cherry and sour cherry exhibited the highest levels of antioxidant capacity.

Furthermore, the divergence in polyphenol content and antioxidant capacity among the fruits was found to be influenced by their specific varieties.

In summary, this study's results underline the *Prunus* genus's role as a rich source of phenolic compounds possessing potential antioxidant properties. These bioactive constituents represent valuable functional ingredients with favorable impacts on human health. The incorporation of *Prunus* fruits or their derived extracts into diets and functional foods has the potential to contribute to the enhancement of overall health and well-being, offering protection against oxidative stress-related ailments.

Subsequently, an evaluation of total antioxidant capacity of fruits with local consumption in Romania and the establishment of a database with the obtained results are desired.

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