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Original research paper

POLYPHENOLIC COMPOSITION AND ANTIOXIDANT CAPACITY IN THE BRANCHES AND LEAVES OF SELECTED *PRUNUS* AND *PYRUS* SPECIES

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Abstract: Plants of the genus *Prunus* and *Pyrus* have been valued and used for centuries due to their rich nutritional and therapeutic properties. The fruit itself is primarily consumed, while other parts of the plants are often overlooked despite their biological activity. In this study, branches and leaves of 10 plant species were investigated, including nine from the *Prunus* genus (wild *P. avium*, cultivated *P. avium*, *P. mahaleb*, *P. fruticosa*, *P. cerasus*, *P. domestica*, *P. persica*, *P. cerasifera*, *P. cerasifera pissardii*) and one from the *Pyrus* genus (*P. communis*). This study aimed to determine their polyphenolic composition and antioxidant capacity. The powders from branches and leaves of *Prunus* and *Pyrus* species were extracted in 70% ethanol using ultrasound. The quantitative analysis of polyphenols involved spectrophotometric determination of total phenolic content, total flavonoid content, total hydroxycinnamic acid content, and total anthocyanins content. The study evaluated the antioxidant capacity of branches and leaves using FRAP and DPPH assays. *Pyrus communis* demonstrated the highest total phenolic content in both branches (591.55 mg gallic acid equivalents/g dry weight) and leaves (685.62 mg GAE/g d. w.). *P. communis* also showed strong antioxidant potential in both assays. Among *Prunus* species, *P. cerasifera pissardii* demonstrates an exceptional antioxidant capacity, and a high amount of total anthocyanins, phenolic acids, and total phenols. This study provides a preliminary insight into the phytochemical profile of underutilized by-products, such as branches and leaves. These parts of plants can also generally be regarded as valuable resources of biologically significant compounds. Based on the results, we can conclude that by-products from *Pyrus communis* and *Prunus cerasifera pissardii* have the potential for wider chemical and biological investigations.

Keywords: *Prunus, Pyrus, by-products, antioxidant activity, polyphenols.*

INTRODUCTION

The Rosaceae family, with approximately 105 genera and around 3000 species, is highly diverse and widespread (Hummer & Janick, 2009). Within this family, the genera *Prunus* and *Pyrus* are of particular significance. The genus *Prunus* includes around 430 species of deciduous and evergreen shrubs and trees and is prevalent in temperate regions of the Northern Hemisphere (Agrawal et al., 2024). These species are cultivated for their edible fruits and seeds and are highly regarded as ornamental plants because of their flowers. The plum (*Pru-*

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nus domestica) and pear (*Pyrus communis*) are of significant importance in Serbia's fruit production sector. Serbia ranks as the third-largest producer of plums globally, with the majority of the harvested fruits being utilized in the production of brandy (Tomić, Štampar, Glišić, & Jakopič, 2019).

It has been shown that the daily intake of fruits rich in polyphenols may have beneficial effects on human health and positively influence biochemical processes in the body (Telichowska et al., 2020). *Prunus* and *Pyrus* genera are recognized as a rich source of various polyphenolic compounds, including phenolic acids, flavonols, and anthocyanins (Kolniak-Ostek, 2016; Popović et al., 2021), so they are recognized for their multiple health benefits, such as antidiabetic, anticancer, anti-neurodegenerative, anti-inflammatory, and cardioprotective properties (Hong, Lansky, Kang, & Yang, 2021; Ullah, De Filippis, Khan, Xiao, & Daglia, 2020).

During harvesting, large amounts of by-products, like branches and leaves are produced. However, these resources remain underexplored in terms of their phytochemical properties as well as biopotential. The reuse of by-products represents significant potential for the food industry, as well as the pharmaceutical and cosmetic industries (Munekata et al., 2023).

These by-products are particularly rich in compounds such as antioxidants, sweeteners, antimicrobial agents, and natural colourants, which can serve as a sustainable alternative to synthetic additives in the industry (Ueda et al., 2022). The leaves, twigs, and bark of various species from the *Prunus and Pyrus* genera exhibit a range of medicinal properties that have been utilized in traditional medicine for centuries. For instance, the leaves of *Prunus spinosa* are known for their lithontriptic and diuretic effects and are often recommended in the diet of individuals with peptic ulcers. The stems of *P. avium* and *P. cerasus* are valuable in treating certain heart diseases, while an infusion of the leaves and bark of *P. cerasoides* is utilized in traditional medicine in the treatment of cough, asthma, diarrhoea, and dyspepsia (Poonam et al., 2011). In addition, leaves from *P. domestica* have demonstrated pharmaceutical potential and have traditionally been used as antihelminthic, laxative, and sedative agents (Maatallah et al., 2020). In traditional Chinese medicine, the branches and leaves of some *Pyrus* species *(P. pashia)* have been used to treat gastrointestinal ailments (Singh, Verma, & Sharma, 2024).

Starting from the fact that by-products of *Prunus* and *Pyrus* species are still under-explored in terms of their chemical composition and biological properties, this research aimed to provide a preliminary insight into the chemical composition and *in vitro* antioxidant capacity of leaves and branches from selected species.

Ultrasound-assisted ethanol extraction of bioactive compounds was applied to extract bioactive compounds from branches and leaves. The prepared extracts were spectrophotometrically analyzed to determine the content of total phenols, flavonoids, phenolic acids, and anthocyanins. Furthermore, the extracts were evaluated for their antioxidant capacity *in vitro* using the FRAP and DPPH assays.

MATERIALS AND METHODS

Chemicals

Gallic acid (98 %) and caffeic acid (98%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Quercetin (97%) was obtained from Xi'an Pincredit Bio-tech Co (Xi'an, Shaanxi Province, China). Folin-Ciocalteu reagent was supplied by Biochem Chemopharma (ZA Cosne sur Loire, France). 2,4,6-tris(2-pyridyl)-s-triazine (98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fluka (Buchs, Switzerland Germany) provided 2,2-diphenyl-1-picrylhydrazyl (DPPH, 85%).

Plant material

This study analyzed branches and leaves of various species from the *Prunus* and *Pyrus* genera. Species belonging to the *Prunus* genus were: wild cherry (*P. avium, w*), cultivated cherry (*P. avium, c*), mahaleb cherry (*P. mahaleb*), European dwarf cherry (*P. fruticosa*), sour cherry (*P. cerasus*), European plum (*P. domestica*), peach (*P. persica*), cherry plum (*P. cerasifera*), and purple-leaved cherry plum (*P. cerasifera pissardii*). In addition, the pear (*Pyrus communis*) from *Pyrus* genus was also analyzed. All samples were harvested at the same time in Novi Sad area in 2023. After a week of drying in a dark, ambient-temperature environment, the branches and leaves were ground into a powder using a laboratory blender. The resulting powder was then stored in paper bags for future use.

Extraction procedure

The powdered branches and leaves from ten species of the *Prunus* and *Pyrus* plants (1 g) were immersed in 10 mL of 70% ethanol, following the modified method described by (Ghafoor, Choi, Jeon, & Jo, 2009). The mixture was then subjected to ultrasonic extraction in a water bath (Elmasonic S 100H 37 kHz) at 50 °C for 25 minutes. Following this, the samples were centrifuged at RCF 3920 × g for 15 minutes. The supernatants were collected into Eppendorf tubes and stored at +4 °C for further analysis.

Total phenolic content

The total phenolic content in extracts was determined using the Folin-Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventós, 1998). A millilitre of 0.1 M Folin-Ciocalteu reagent was combined with 0.2 milliliters of the diluted extract. After 10 minutes, saturated Na2CO3 solution (0.8 mL) was added to the mixture. The mixture was incubated for an hour at room temperature in a dark place, and the absorbance of the obtained blue mixture was measured at λ_{max} = 765 nm. Gallic acid was used to prepare the standard calibration curve (concentration range 0–1 mg/mL), with a total of 13 concentration levels measured. Total phenolic content was expressed as mg of gallic acid equivalents per gram of dry branches and leaves (mg GAE/100 g d.w.).

All spectrophotometric measurements were carried out using the UV-visible spectrophotometer Thermo Scientific Evolution 220.

Total flavonoid content

The aluminium chloride colourimetric method was used to determine the total flavonoid content in branches and leaf extracts, modified from the procedure reported by Chang, Yang, Wen and Chern (2020). Quercetin was used to make the calibration curve (0 – 200 μg/mL). The diluted extracts (0.5 mL) were mixed with 800 μL of 96% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate, and 1.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at λmax = 415 nm using a spectro-photometer. In the blank sample, 10% aluminium chloride was substituted by the same amount of distilled water. The total flavonoid content in the extracts was determined using the calibration curve for quercetin and expressed as milligrams of quercetin equivalent per gram of dry plant material (mg QUE/100 g d.w.).

Total hydroxycinnamic acids

The spectrophotometric method, based on the Polish Pharmacopoeia VI (2002) was applied, with modifications, to determine the total hydroxycinnamic acid content. To prepare the solution, 0.2 mL of the extract was mixed with 1 mL of distilled water, 0.2 mL of 0.5 M HCl, and 0.2 mL of Arnow's reagent, a mixture of equal volumes of 10% NaNO2 and 10% Na-molybdate. Next, 0.2 mL of NaOH and 0.2 mL of distilled water were added. The absorbance of the resulting red solution was then measured at λmax = 490 nm. The content of hydroxylcinnamic acids was reported as milligrams of caffeic acid equivalents per gram of dry plant material (mg CAE/100 g d.w.).

Total anthocyanin content

The total anthocyanin content was quantified using the pH differential method, according to Cheng and Breen, (1991), with slight modifycations. Six test tubes contained 0.2 mL of the samples; 1.8 mL of pH 1.0 buffer was added to three of them. In another three, the same volume of pH 4.5 buffer was added. Samples were incubated for approximately 30 minutes. Blank samples were prepared using pH 1.0 and pH 4.5 buffers, with ethanol used in place of extracts. The samples were diluted in ethanol, and absorbance readings at λ_{max} = 520 nm and λ_{max} = 700 nm were recorded.

Ferric reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power (FRAP) assay, introduced by Benzie and Strain (1996), was employed to assess the ability of the extracts to convert Fe^{3+} ions to Fe^{2+}. To prepare the FRAP reagent, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, 10 mM in 40 mM HCl), a 20 mM $FeCl_3$ solution, and acetate buffer (pH 3.6) were combined in a volumetric proportion of 1:1:10. Three milliliters of the reagent were mixed with 50 μL of the diluted extract. After incubating for six minutes at room temperature, the absorbance of the blue solution was measured at λmax = 593 nm, using water as the blank. A calibration curve of ascorbic acid (AA) was used to calculate the results, which are expressed as ascorbic acid equivalents (AAE) per gram of dry weight of plant material (mg AAE/100 g d. w.).

DPPH scavenging capacity

To evaluate the ability of the extracts to scavenge radicals, the simple and popular DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Hussain et al. (2012), was used with mi-nor modifications. Serial dilutions of the extracts in water (0.2 mL) were combined with 2 mL of DPPH radical solution in methanol (90 µM).

The mixtures were incubated in a dark environment at room temperature for one hour. The absorbance of the resulting coloration was then measured at λ_{max} = 517 nm. For control samples, ethanol was used instead of the DPPH radical solution under identical experimental conditions. The results of this method are expressed as IC50 values in µg/mL, which represent the concentration of the sample required to achieve the neutralization of 50% of the DPPH radicals.

Statistical analysis

Data are expressed as means ± standard deviation (SD). The data were evaluated for normality before the statistical testing and subsequently analyzed using one-way ANOVA, followed by Tukey's post hoc test (Statistica®, version 14.0.1.25, TIBCO Software, USA). Results were regarded as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In order to obtain a preliminary insight into the polyphenolic profile of the investigated species, spectrophotometric measurements of total phenolic content, total flavonoids, total hydroxycinnamic acids, and total anthocyanins were performed.

Total phenolic content

The results obtained for the total phenolic content (TPC) of *Prunus* and *Pyrus* species are presented in Fig. 1A and B. Among the branch extracts, the highest total phenolic content was measured in *P. communis* (591.55 mg gallic acid equivalents/100 g d. w.) followed by *P. cerasifera pissardii* (550.23 mg GAE/100 g d. w.), *P. fruticosa* (384.70 mg GAE/100 g d. w.),and *P. cerasifera* (353.20 mg GAE/100 g d. w.). *P. cerasus*, wild and cultivated *P. avium*, *P. domestica*, and *P. persica* shared a similar value of total phenolic compounds with a TPC value ran-ging from 234.02 mg GAE/100 g d. w. to 309.82 mg GAE/100 g d. w. The branch extract of *P. mahaleb* contained the lowest level among other branch extracts (195.21 mg GAE/100 g d. w.).

A similar manner in TPC values was observed among the leaf extracts. As in the case of branch extract, the highest TPC was in *P. communis* (685.62 mg gallic acid equivalents/100 g d. w.) and *P. cerasifera pissardii* (591.55 mg GAE/100 g d. w.) leaf extract, while *P. mahaleb* contained the lowest content of total phenolic compounds (155.14 mg GAE/100 g d. w.). Comparable values for TPC were observed among *P. fruticosa*, *P. cerasus*, and *P. domestica* ($p > 0.05$).

Literature data on total phenolic content in ethanol extract of by-products from *Prunus* and *Pyrus* species are scarce. Our results harmonise with those from the study performed by Zahid (2019), which showed TPC values in ethanol leaf extract from *P. domestica*, *Pyrus communis*, and *P. persica* similar to our findings.

In addition, Nowak et al. (2016) reported that the total phenolic content in *P. cerasus* leaves was 3.17 mg GAE/g, which is in line with our findings. Elsayed et al. (2020) showed that ethanol extract of *P. domestica* leaves contained 66.50-143.7 mg GAE/g dried leaves, while Nunes et al. (2021) showed a higher amount of total phenols in stems than in leaf *P. avium* extracts (301.38 and 100.71 mg GAE/g d. w, respectively).

The discrepancies in the results may be explained by several factors. Total phenolic content may be influenced by climate, seasonal, and environmental conditions, and also cultivar genotype. Moreover, sample preparation methods, the extraction process, solvent, and other preparation and storage factors may contribute to the different results for the same vegetal parts (Jesus, Gonçalves, Alves, & Silva, 2022).

Total flavonoids

Total flavonoids (TF) in extracts were measured using a method based on the complexation of Al(III) with flavonoids. The results are shown in Fig. 2A and B. Among the tested branch extracts, the ethanol extract of wild *P. avium* contained the highest amount of TF (9.35 mg QUE/100 g d. w.). No flavonoids were measured in the branch extracts of *P. cerasus*, *P. domestica*, *P. communis*, and *P. cerasifera*. The remaining samples contained a similar concentration of TF ($p > 0.05$).

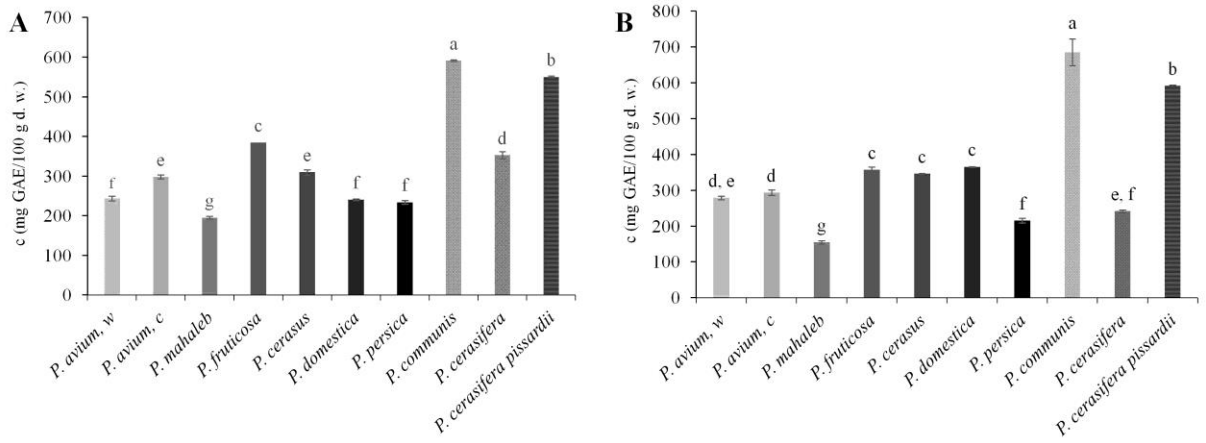

Figure 1. Total phenolic content in branches (A) and leaf extracts (B) of *Prunus* and *Pyrus* species; expressed as mg gallic acid equivalents/100 g of dry weight. The data are expressed as the mean ± standard deviation. Different letters represent significant differences, as evaluated using Tukey's test ($p < 0.05$)

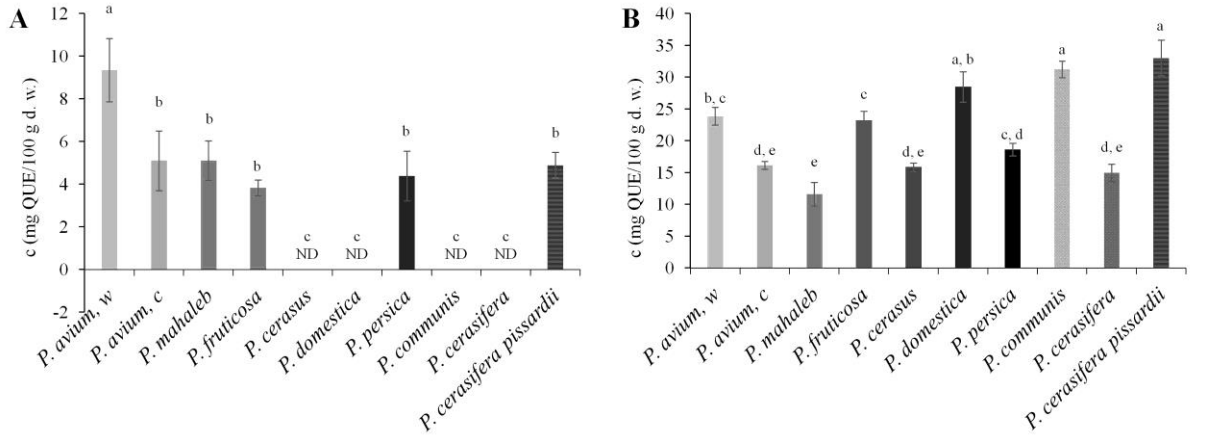

Figure 2. Flavonoid content in branches (A) and leaf extracts (B) of *Prunus* and *Pyrus* species, measured as mg quercetin equivalents per 100 g of dry weight. ND – not observed. Values are expressed as the mean ± standard deviation. Different letters represent significant differences, as evaluated using Tukey's test ($p < 0.05$)

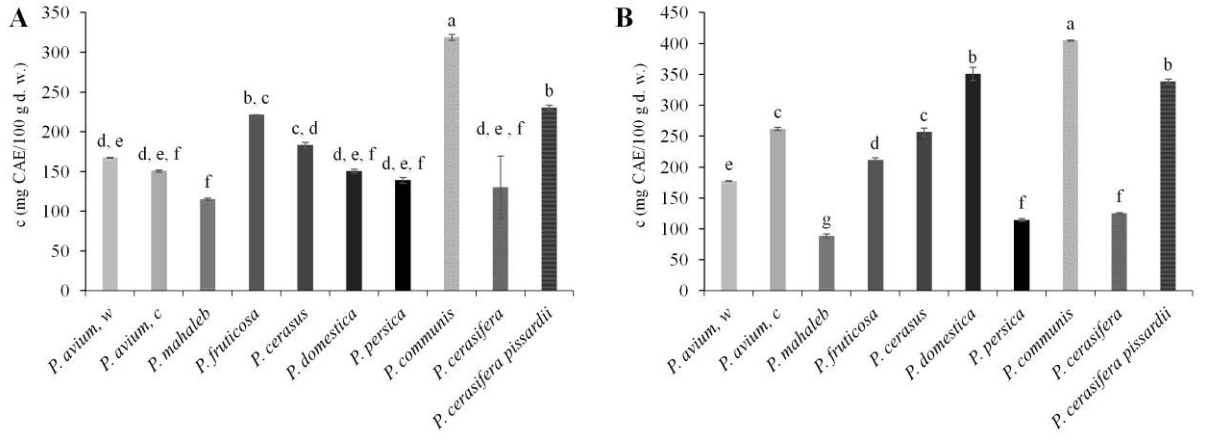

Figure 3. Phenolic acid content in branch (A) and leaf extracts (B) of *Prunus* and *Pyrus* species, expressed in mg of caffeic acid equivalents/100 g of dry weight. Results are presented as the mean ± standard deviation. Significant differences between samples are marked with different letters, based on Tukey's test ($p < 0.05$)

On the contrary, *P. cerasiferra pissardii*, *P. communis*, and *P. domestica*, were the richest in TF among the leaf extracts (33.00, 31.28, and 28.47 mg QUE/100 g d. w, respectively), while

TF in *P. mahaleb* was almost three-fold lower (11.62 mg QUE/100 g d. w.).

Yüksekkaya et al. (2021) reported that stalk and leaf extracts of *P. avium* contained almost 8 times higher amounts of TF compared to pulp and seeds. In a study by Zymonė, Liaudanskas, Lanauskas, Nagelytė and Janulis (2024), the leaf extracts from nine *P. cerasus* genotypes were analyzed for their phytochemical profile. The results ranged from 7.27 to 11.75 mg rutin equivalents/g d. w, while chromatographic analysis confirmed the presence of rutin, quercitrin, avicularin, and hyperoside in *P. cerasus* leaves, pointing out the variations in dominant compounds among different genotypes. Additionally, UPLC-MS/MS analysis conducted by Oszmiański, Wojdyło, Lamer-Zarawska and Swiader (2007) showed the presence of flavan-3-ols (catechin and its derivatives), flavonols (quercetin, kaempferol, and isorhamnetin derivatives), and luteolin derivative in sour cherry (*P. cerasus*) leaves. Studying the phytochemical profile of by-products of several cultivars of *P. persica*, Maatallah et al. (2020) demonstrated the presence of quercetin 3-galactoside and quercetin 3-rutinoside in the peach leaves extract, suggesting that peach leaves had important content of polyphenolic compounds.

Total hydroxycinnamic acids

Phenolic acids are a group of phytochemicals found in plants, characterized by a phenolic ring structure with a carboxylic acid group. Two main types of phenolic acids are hydroxybenzoic and hydroxycinnamic acids. In our study, a method based on the colouring reaction between nitrite and hydroxyl group on the aromatic ring of hydroxycinnamic acids in an alkaline medium was applied.

The total hydroxycinnamic acids content (THCA) of various *Prunus* and *Pyrus* species is presented in Fig. 3A and B. For branch extracts, THCA concentrations ranged from 115.44 mg caffeic acid equivalents/100 g d. w. (*P. mahaleb*) to 319.11 mg CAE/100 g d. w. (*P. communis*). Similar THCA values were observed between *P. cerasifera pissardii* and *P. fruticosa* (230.69 and 221.55 mg CAE/100 g d. w; $p > 0.05$). Regarding leaf extracts, a similar pattern was observed as for branch extracts: the highest THCA was measured in *P. communis* (405.08 mg CAE/100 g d. w.), while almost 5-fold lower content was recorded in *P. mahaleb* (88.82 mg CAE/100 g d. w). No significant differences were noted between *P. cerasifera pissardii* and *P. domestica* ($p > 0.05$) as well as between cultivated *P. avium* and *P. cerasus* ($p > 0.05$).

Studying different anatomical parts of the pear (*P. communis*), Kolniak-Ostek (2016) reported that the phenolic acids were presented in significantly higher yield in leaves (995.3 mg/100 g d. w.) than in pulp, peel, or seeds. Jiao et al. (2024) found chlorogenic acid as the most abundant phenolic acid in *P. communis* leaves.

Zymonė et al. (2024) showed that THCA in *P. cerasus* leaves was in the range of 9.03 - 12.82 mg/g, while Nowak, Czyzowska, Efenberger and Krala (2016) detected the three hydroxycinnamic acids in the leaves of *P. cerasus*: chlorogenic, neochlorogenic, and *p*-coumaric acids. The presence of chlorogenic and caffeic acids and their derivatives in high abundance in other *Prunus* leaf extracts was also confirmed by other authors (Elsayed, Hammad & Abd El-Salam, 2020; Liu, Nisar, & Wan, 2020; Nunes et al., 2021; Oszmiański & Wojdyło, 2014). Additionally, Willig et al. (2022) identified chlorogenic acid (3.81 mg/g) and neochlorogenic acid (0.42 mg/g) as the predominant phenolic acids in *P. avium* branches. Caffeic acid was also detected in the branch extract of blackthorn (*P. spinosa*).

The results indicate that chlorogenic acid and caffeic acid are the predominant phenolic acids across various *Prunus* species, suggesting that these by-products may serve as a potential source for the targeted ex-traction of these compounds.

Total anthocyanins

Anthocyanins are a class of water-soluble pigments located in all parts of the higher plants: fruits, flowers, and other vegetative organs, which are often used as natural food colourants. Chemically, they are glycosides of anthocyanidins, flavonoid derivatives produced via the phenylpropanoid pathway (Mattioli, Francioso, Mosca, & Silva, 2020).

The pH differential method was used to measure the total anthocyanin content (TAC) in tested samples. Among the branches extracts, *P. cerasifera pissardii* contained the highest TAC (41.86 mg cyanidin 3-glucoside equivalents/100 g d. w.),

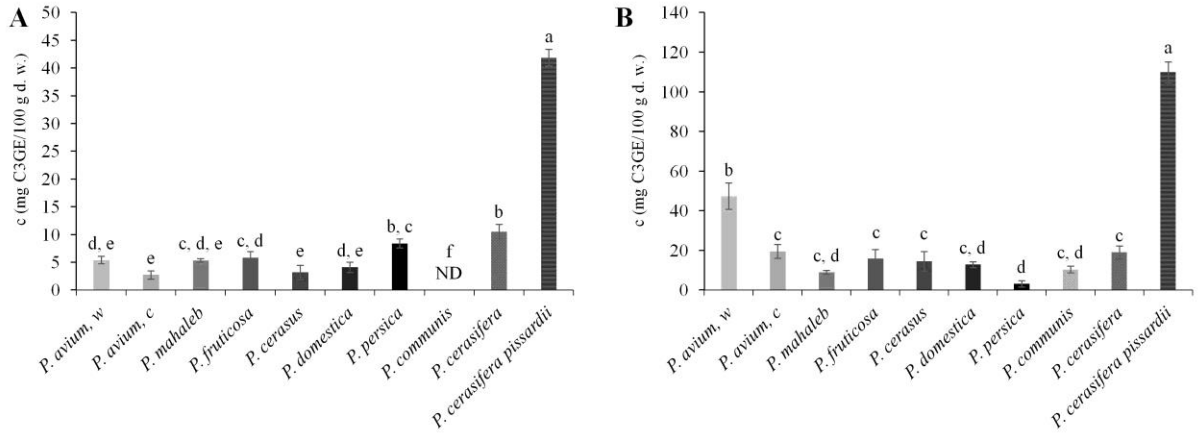

Figure 4. Anthocyanin levels in branches (A) and leaf extracts (B) of *Prunus* and *Pyrus* species, presented as mg of cyanidin-3-glucoside equivalents per 100 grams of dry weight. ND – not observed. Results are provided as the mean ± standard deviation. Significant differences across samples are indicated by different letters, determined through Tukey's test ($p < 0.05$)

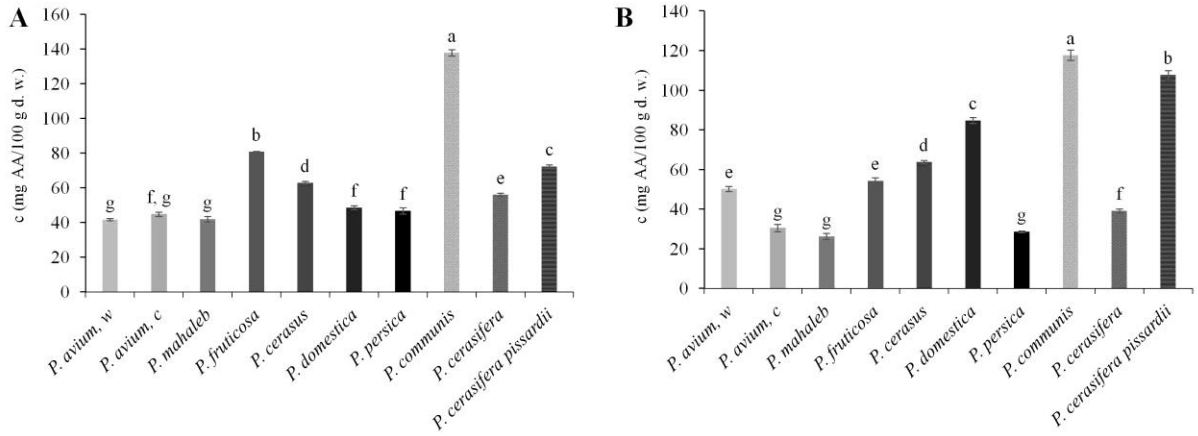

Figure **5.** Antioxidant capacity by FRAP assay in branches (A) and leaf extracts (B) of ten *Prunus* and *Pyrus* species; expressed as mg ascorbic acid equivalents per 100 gram of dry weight. Results are presented as the mean ± standard deviation. Significant differences across samples are indicated by different letters, as determined by Tukey's test ($p < 0.05$)

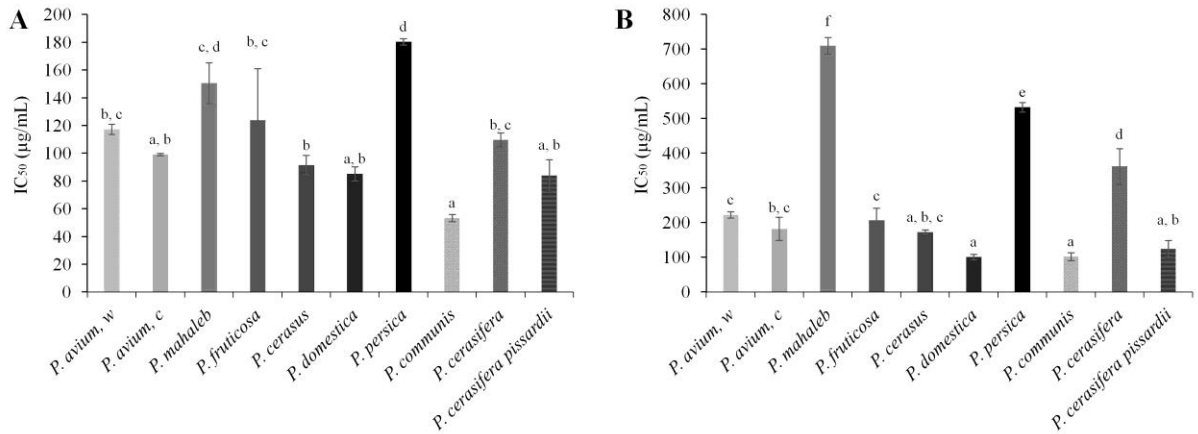

Figure 6. Neutralization of DPPH radical by branches (A) and leaf extracts (B) of ten *Prunus* and *Pyrus* species; presented as the minimum inhibitory concentration (IC_{50}; µg/mL). Results are expressed as mean ± standard deviation. Different letters represent significant differences, as evaluated using Tukey's test ($p < 0.05$)

which is almost 4-fold higher than the next samples - *P. cerasifera* (10.52 mg C3GE/100 g d. w.) and *P. persica* (8.41 mg C3GE/100 g d. w.). The lowest TAC among branch extracts was measured in cultivated *P. avium*. However, no anthocyanins were measured in *P. communis* branch extracts.

P. cerasifera pissardii leaves extract also contained the highest TAC among the leaf extracts (109.88 mg C3GE/100 g d. w.), followed by wild *P. avium* (47.31 mg C3GE/100 g d. w.). Our results showed the lowest TAC in *P. persica* (3.17 mg C3GE/100 g d. w.). The remaining leaf extracts did not differ significantly in terms of TAC ($p > 0.05$).

Compared to the anthocyanin profile of fruits, literature data on the anthocyanin content of *Prunus* and *Pyrus* by-products are insufficient. Similar to our results, Wojdyło, Nowicka, Turkiewicz, Tkacz & Hernandez (2021) showed that leaves of *P. communis* contained less than 2.4 mg of anthocyanins per 100 g. However, anthocyanins were not presented in the leaves and petioles extracts *of P. avium* (Dziadek et al., 2018). Chen, Sang, Zhang and Sang (2018) showed that cyanidin derivatives were the dominant anthocyanins in *P. cerasifera* leaves.

Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay is used to assess the antioxidant activity of extracts, and the results are shown in Fig. 5A and B. The FRAP assay was employed to evaluate the antioxidant activity of extracts by assessing their potential to act as electron donors. This characteristic enables the compounds to neutralize oxidants by donating electrons and stabilizing them in the process. The highest FRAP activity among the branches extracts was observed in *P. communis* (137.83 mg ascorbic acid equivalents per 100 g d. w.), while the lowest values were obtained for *P. mahaleb* and wild *P. avium* (41.96 and 41.76 mg AAE/100 g d. w.). The range of FRAP activity for the remaining branch extracts was 44.89 - 80.75 mg AAE/100 g d. w.

In the leaf extracts, FRAP ranged from 26.30 mg AAE/100 g d. w. (*P. mahaleb*) to 117.78 mg AAE/100 g d. w. in *P. communis*. No significant differences were observed among *P. avium*, *P. mahaleb*, and *P. persica* ($p > 0.05$), suggesting that these samples shared the lowest ability to donate electrons among the tested leaf extracts.

Comparing different anatomical parts of pear, Kolniak-Ostek (2016) revealed that the antioxidant activity of leaves was quite higher than for pulp, peel, or seeds, with a result of 3539.26 μmol Trolox equivalents per 100 g, suggesting the importance of considering by-products in the evaluation of biological activity. Additionally, Ušjak et al. (2021) recorded a value of 5.90 mmol Fe^{2+}/g for the ethanolic extract of pear branches. In a study by Wojdyło, Nowicka, Turkiewicz & Tkacz (2021), significant differences between FRAP results among fruit and leaves extracts of *P. avium* and *P. cerasus* were reported, suggesting that fruit exhibits a higher ability to donate electrons, which was correlated with anthocyanins content. Zymonė et al. (2024) reported that the FRAP activity of ethanolic extract of *P. cerasus* was in the range from 1111.1 to 30658 μmol Trolox equivalents per 100 g of fresh weight. However, comparisons of the results from various studies are often limited and challenging, because of the variations in experimental protocols across studies and specific interpretations of the obtained data.

DPPH scavenging activity

The radical scavenging capacity of samples was evaluated using the DPPH method and the obtained results are presented in Fig. 6. The data are interpreted as the minimum inhibitory concentration required to neutralize 50% radicals, so a lower IC50 value signifies greater antioxidant capacity.

For branch extracts, IC50 values were in the range from 53.34 μg/mL (*P. communis*) to 180.44 μg/mL (*P. persica*). The IC50 values for branch extracts of *P. cerasifera pissardii*, *P. domestica*, wild *P. avium*, and *P. cerasus* were quite similar to the results of the most potent branch extract (*P. communis*) ($p > 0.05$), while *P. mahaleb* exhibits a similar capacity to neutralize DPPH radicals as *P. persica*.

The highest antioxidant capacity in leaf extracts was recorded in *P. domestica* and *P. communis* (100.85 μg/mL and 102.16 μg/mL, respectively), which display the highest antioxidant capacities, while *P. mahaleb* demonstrated the lowest antioxidant capacity among the leaf extracts, with IC50=710.24 μg/mL. Similar antioxidant capacities to those of the most potent species are detected in *P. cerasifera pissardii* and *P. cerasus*. *Prunus* species exhibiting comparable IC50 values include wild *P. avium*,

cultivated *P. avium*, *P. fruticosa*, and *P. cerasus*.

A similar order of the results for the leaf extracts was observed by Pompeu et al. (2021), where DPPH scavenging activity was decreased in the following manner (*P. domestica* > *P. communis* > *P. avim*; 49.51, 44.66, and 37.41 μM Trolox equivalents/ g d. w., respectively). Nunes et al. (2021) reported an antioxidant capacity of 35.17 mg quercetin equivalents/g d.w for the hydroethanolic leaf extract of *P. avium*. Similarly, Ušjak et al. (2021) recorded an IC50 value of 6.60 μg/mL for the bark extracts of *Pyrus communis*.

CONCLUSIONS

This study broadens the understanding of the phenolic composition and antioxidant capacity of branches and leaves from the *Prunus* and *Pyrus* species, highlighting their potential as valuable sources of bioactive compounds. The obtained results emphasize the potential of these underexplored plant parts for innovative uses in the food and health industries. *Pyrus communis* was identified as the most abundant source of polyphenols and antioxidants, underlining its industrial relevance. Similarly, *Prunus cerasifera pissardii* demonstrated promising antioxidant properties, and also high anthocyanin and phenolic contents.

These findings pave the way for future studies to optimize extraction methods, expand bioactive applications, and explore commercial opportunities, promoting innovation and sustainable use of the underutilized plant resources.

AUTHOR CONTRIBUTIONS

Investigation, formal analysis, data curation, writing - original draft, T.J.; Data curation, writing - review & editing, R.Ž.P.; Data curation, writing - review & editing, M.P.; Jelena Radević: Statistical analysis, writing – review & editing, J.R.; Statistical analysis, writing – review & editing, S.H.; Conceptualization, methodology, resources, validation, supervision, funding acquisition, B.M.P.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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POLIFENOLNI SASTAV I ANTIOKSIDATNI KAPACITET EKSTRAKATA GRANČICA I LISTOVA ODABRANIH VRSTA RODA *PRUNUS* I *PYRUS*

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Sažetak: Biljke iz roda *Prunus* i *Pyrus* vekovima su cenjene i korišćene zbog svojih bogatih nutritivnih i terapeutskih svojstava. Plod biljaka se primenjuje u najvećoj meri, dok su ostali delovi biljaka često zanemareni i nedovoljno istraženi. U ovoj studiji ispitivane su grančice i listovi 10 vrsta voća, uključujući devet iz roda *Prunus* (*P. avium* (divlja i gajena), *P. mahaleb*, *P. fruticosa*, *P. cerasus*, *P. domestica*, *P. persica*, *P. cerasifera*, *P. cerasifera pissardii*) i jedna iz roda *Pyrus* (*P. communis*). Cilj ovog rada jeste da se ispita polifenolni sastav i antioksidatni potencijal ekstrakata grančica i listova pomenutih vrsta voća. Usitnjene grančice i listovi su ekstrahovani ultrazvučnom ekstrakcijom uz korišćenje 70% etanola kao rastvarača. Kvantitativna analiza polifenola obuhvatala je spektrofotometrijsko određivanje ukupnog sastava fenola, flavonoida, fenolnih kiselina i antocijana. Antioksidantna aktivnost ekstrakata grančica i listova određena je pomoću FRAP i DPPH testova. Na osnovu rezultata, *Pyrus communis* je pokazala najviši ukupni sadržaj fenolnih jedinjenja u ekstraktima grančica (591,55 mg ekvivalenata galne kiseline/100 g suve materije) i listova (685,62 mg EGK/100 g s. m.). *Pyrus communis* je takođe pokazala i snažan antioksidativni potencijal u oba testa. Među vrstama *Prunus*, *P. cerasifera pissardii* je pokazala izuzetnu antoksidantnu aktivnost i visoku količinu ukupnih antocijana, fenolnih kiselina i ukupnih fenola. Ova studija pruža preliminarni uvid u fitohemijski profil nedovoljno iskorišćenih nus-proizvoda, kao što su grančice i listovi biljaka koje su široko zastupljene u voćarskoj proizvodnji. Ovi delovi biljaka takođe se generalno mogu smatrati vrednim resursima biološki značajnih jedinjenja. Na osnovu dobijenih rezultata možemo zaključiti da sporedni proizvodi iz *Pyrus communis* i *Prunus cerasifera pissardii* imaju potencijal za šira hemijska i biološka istraživanja.

Ključne reči: *Prunus, Pyrus, grančice, listovi, antioksidantna aktivnost, polifenoli*

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