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TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *CORNUS MAS* L. FRUIT EXTRACTS

SUMMARY

In this study, the contents of phenolic compounds (phenols, hydrolysable tannins, flavonoids, and monomeric anthocyanins) and the antioxidant capacity of extracts obtained from *Cornus mas* L. fruits were determined. The plant material was collected in September 2023 at Duklja, near Podgorica, Montenegro. Destoned fruits were used to obtain the juice, the extraction of which was performed by the Kutscher-Steudel liquid-liquid extraction method. Soxhlet solid-liquid extractions were performed on the remaining by-products: pomace (consisting of exocarp and mesocarp) and stones/pyrenes (endocarp and seed). Different solvents were applied depending on the fruit part and the extraction method used. The highest phenolic and flavonoid contents were detected in the pyrene extracts: 85.85-105.86 mg/g gallic acid equivalents (GAE) and 60.37-71.77 mg/g quercetin equivalents (QE), respectively. The highest tannin content was found in the extracts of pomace (6.61-25.19 mg/g tannic acid equivalents, TAE) and juice (17.59-19.75 mg TAE/g). The strongest antioxidant activity, assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, was observed in the acetone extract from pyrenes and the methanol extract from pomace (IC_{50} of 58.27 and 63.66 µg/mL, respectively). The ferric reducing antioxidant power (FRAP) assay showed the highest activity in the acetone extract from pyrene (40.01 mmol Fe^{2+}/g). For comparison, all analyses were also performed using freshly squeezed juice; the contents of phenols, tannins, flavonoids, and anthocyanins were 1383.56 mg GAE/L, 1529.68 mg TAE/L, <5 mg QE/L and 79.49 mg/L cyanidin-3-glucoside equivalents, respectively. The antioxidant capacities of the juice were IC_{50} 20.97 µg/mL (DPPH) and 39.07 mmol Fe^{2+}/L (FRAP).

Keywords: cornelian cherry; extraction; fruit extract; bioactive compounds; DPPH; FRAP

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INTRODUCTION

Cornus mas L. (cornelian cherry, cornel, dogwood; CM) is a shrub species belonging to the family Cornaceae and is native to Southern and Central Europe and parts of Asia (Kazimierski *et al.*, 2019). It can reach up to 9 m in height and grows well in various soil types, including clay and sandy soils with low pH values, as well as in areas with low temperatures. However, it does not tolerate coastal regions with high air and soil salinity (Dokoupil and Řeznicek 2012). The plant is entomophilous, heliophilous, and thermoxerophytic. Its fruit is a drupe, approximately 12 mm long and 5 mm wide, with an elliptic, smooth endocarp containing a seed (Šilić, 1990). The fruit ripens in late summer, and its color ranges from yellow to red depending on the stage of maturity (Da Ronch *et al*., 2016). Fruit weight varies from 2 to 9 g and is influenced by genotype, cultivation practices, and environmental conditions (Szczepaniak *et al*., 2019).

CM is valued for its nutritional and therapeutic properties and is classified as a medicinal plant (Kaya and Koca, 2021). Traditionally, it has been used to treat inflammation, kidney and intestinal disorders, maintain blood glucose levels, prevent liver fat accumulation, and potentially support cardiovascular health and cancer prevention (Hosseinpour-Jaghdani *et al*., 2017). Due to the durability and hardness of its wood, it has historically been used in weapon making and construction (Da Ronch *et al.*, 2016). In Balkan folklore, CM is considered a magical plant and was often planted near homes to promote health and longevity (Radaković, 2012). The fruit can be consumed dried or used to prepare compotes and teas (D' Antuono *et al.*, 2014). Among the most important bioactive compounds in CM are phenolic compounds, which are stable secondary metabolites with protective roles in plants. They contribute to defense against herbivores and pathogens and may influence plant growth and interactions with the environment (Pratyusha, 2022). In human nutrition, phenolic compounds contribute to reducing oxidative stress and may offer benefits in the prevention or management of cardiovascular diseases, diabetes, and cancer (Lin *et al.*, 2016). Although phenolic composition and antioxidant activity of CM fruits have been previously studied, limited attention has been given to the chemical composition and bioactive potential of pyrenes. This represents a significant research gap, considering that pyrenes are often treated as by-products despite their possible functional value. Moreover, in this study, juice samples were extracted using a Kutscher-Steudel apparatus, a liquid-liquid extraction technique that has been applied in only a few previous studies. This approach provides a more efficient separation of bioactive compounds and represents a methodological innovation in the analysis of CM fruit constituents.

The aim of the study was to investigate the phenolic composition and antioxidant activity of different extracts of CM fruit, with particular emphasis on pyrenes and pomace, in order to evaluate their potential as valuable bioactive sources.

MATERIAL AND METHODS

Sampling of Cornelian Cherry

Plant material was collected in September 2023 at the Duklja site (42.467638, 19.263933) near Podgorica. The harvested fruits were mostly dark red, indicating full ripeness. On the same day, the fruits were processed by removing the endocarp with seed (pyrene), and juice was manually squeezed from the remaining pulp using a pestle and mortar. The juice was strained through cheesecloth (a simple traditional method for juice preparation) and stored in glass bottles at low temperature. The remaining dry residue (pomace) and pyrenes were dried for 20 days in a well-ventilated, dark area. Additionally, freshly squeezed juice and concentrated juice (obtained by water evaporation under heating) were included as samples.

Liquid-liquid Extraction

Juice samples were extracted using a Kutscher-Steudel apparatus according to a previously reported methodology (Božović *et al.*, 2025). This liquid-liquid extraction apparatus is suitable for the analysis of volatile solvents, as it requires temperatures typically near the boiling point of the selected solvent (Rausch, 2008). The device is intended for the extraction of aqueous samples using solvents that are less dense than water; in this study, ethyl acetate and diethyl ether were used. An 80 mL aliquot of the prepared juice sample was subjected to extraction for 24 hours. The obtained extracts were evaporated and stored in dark glass vials at 4°C until further analysis.

Solid-liquid Extraction

Soxhlet extraction was performed according to a previously published method (Božović *et al.*, 2026). This technique is based on the cyclic percolation of the plant material, which is continuously wetted by a solvent that extracts the bioactive compounds and collects them in a round-bottom flask. The apparatus is used for solid-liquid extraction, in which the selected plant material (in this study, the endocarp with seeds and dry residue of pulp) is first pulverized to increase the contact surface, thereby enhancing extraction efficiency. A total of 20 g of material was weighed, pulverized in an electric blender, and packed in filter paper. For the pyrenes, diethyl ether, hexane, and acetone were used as solvents, while for the pomace, methanol, hexane, and ethyl acetate were employed. The volume of solvent used for each extraction was 300 mL. The extraction cycles lasted on average 6-7 hours, with a total of 20 cycles, each lasting approximately 20 minutes.

Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). From each 1 mg/mL stock solution, 1 mL was taken, mixed with 0.5 mL of Folin-Ciocalteu reagent and 2.5 ml of 7.5% sodium carbonate. The mixture was kept in the dark for 120 minutes before absorbance

was measured at 740 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g of dry extract, or per L in the case of juice. All samples were tested in triplicate, and values are expressed as mean ± standard deviation (SD).

Total Flavonoid Content (TFC)

The aluminum chloride ($AlCl_3$) colorimetric method was used to determine total flavonoids (Ardekani *et al*., 2011). Each reaction mixture consisted of 1.2 mL of extract and 1.2 mL of 2% $AlCl_3$. After 60 minutes at room temperature, absorbance was measured at 420 nm. Results were calculated using a quercetin calibration curve and expressed as mg of quercetin equivalents (QE) per g of dry extract, or per L in the case of juice. All samples were analyzed in triplicate, and values are expressed as mean ± standard deviation (SD).

Total Anthocyanin Content (TAC)

TAC was measured using the pH differential method (Lee *et al*., 2005), following the procedure described by Giusti and Wrolstad (2003), which is based on the structural transformation of anthocyanins as a function of pH in two buffer solutions: 0.025 M potassium chloride at pH 1.0 and 0.4 M sodium acetate at pH 4.5. Each test included 2 mL of extract (stock solution) and 8 mL of buffer. Absorbance was measured at 520 and 700 nm after 50 minutes. Values were expressed as mg of cyanidin-3-glucoside equivalents (C3GE) per g of extract, or per L in the case of juice. Assays were performed in triplicate, and values are expressed as mean ± standard deviation (SD).

Total Tannin Content (TTC)

Tannins were determined using the modified Prussian blue method described by Price and Butler (1977). The stock solutions were diluted 10-fold, and 500 µL from each diluted solution was mixed with 8 mL of distilled water, 0.5 mL of 0.1 M $FeCl_3$, and 0.5 mL of 0.008 M $K_3Fe(CN)_6$. After 10 minutes at room temperature, absorbance was measured at 720 nm. Tannic acid was used as the standard and the results were expressed as mg tannic acid equivalents (TAE) per g of dry extract, or per L in the case of juice. Assays were performed in triplicate, and values are expressed as mean ± standard deviation (SD).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The method is based on the reduction of the purple-colored DPPH radical by antioxidants (Gülçin and Alwasel, 2023; Baliyan *et al*., 2022). From 0.1 g of extract dissolved in 10 ml methanol, 2 ml was taken and diluted to prepare 11 concentrations ranging from 1 to 0.0009375 mg/mL. Each concentration was tested in triplicate. A volume of 3 mL of each diluted extract was mixed with 1 mL of 0.1 mM DPPH solution and kept in the dark for 30 minutes. Absorbance was measured at 517 nm. Vitamin C was used as the standard. The experiment was performed in triplicate. The inhibition percentage of antioxidant activity (%AA) was calculated by the equation:

$$\%AA = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract or the standard solution. The IC_{50} (the concentration required to inhibit 50% of the DPPH free radical; µg/mL) value of the extracts or ascorbic acid was calculated using log dose inhibition curve; the lower IC_{50} value means a higher antioxidant activity.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the ability of the extract to reduce Fe^{3+} to Fe^{2+}, forming a blue Fe^{2+}–TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) complex (Benzie and Devaki, 2017). Each test used 3 ml of TPTZ reagent, 1 mL of distilled water, and 100 µL of extract. After incubation at 37°C for 15 minutes and cooling for 5 minutes, absorbance was measured at 595 nm. Results were expressed as µmol Fe^{2+} equivalents per mL of sample. The standard was prepared using $FeSO_4 \cdot 7H_2O$.

Statistical Analysis

All statistical analyses were performed using Statistica 10.0 software package. Pearson correlation coefficients ($p < 0.05$) were used to examine relationships between antioxidant activity (DPPH and FRAP values) and total content of phenols, flavonoids and tannins. Correlation analyses were initially conducted for all extract types. However, as no statistically significant correlations were observed for most of the extracts, only the results for pomace are presented. A total of 18 correlations were calculated for pomace, including three phytochemical parameters (TPC, TFC, TTC) and two antioxidant assays (DPPH and FRAP) across all solvent extracts.

RESULTS AND DISCUSSION

Table 1. Yields of extracts (E1–E10) obtained from different fruit parts of CM and corresponding solvents

Extract	Fruit part	Solvent	Yield%
E1	juice	Ethyl acetate	3.03
E2	juice	Diethyl ether	0.75
E3	pomace	Ethyl acetate	6.05
E4	pomace	Methanol	45.3
E5	pomace	Hexane	1.4
E6	pyrene	Diethyl ether	8.45
E7	pyrene	Acetone	11.2
E8	pyrene	Hexane	5.13
E9[a]	concentrated juice	–	15.2
E10[a]	freshly squeezed juice	–	n.a.[b]

a E9 and E10 represent concentrated and freshly squeezed juice respectively. They are labeled as "E" for consistency with the extract samples (E1–E8), although they are not obtained by extraction. b n.a. = not applicable (the yield was not determined). "–" indicates that no solvent was used.

The yields (%) of all extracts obtained and analyzed in this study are summarized in Table 1, with the corresponding fruit part and solvent indicated for each extract.

The results of the analyses of TPC, TFC, TAC, and TTC are presented in Table 2. Analyses of freshly squeezed juice and juice concentrated by evaporation were performed directly, without any additional extraction steps. Results for extracts are expressed per gram of dry extract, whereas juice samples are expressed per liter due to differences in sample matrices and preparation methods. This approach ensures that concentrations are presented appropriately for each sample type and allows for meaningful comparison of antioxidant activity and phytochemical content within each matrix.

Table 2. Analytical results of phenolic compounds of CM fruit extracts

Extract [a]	TPC (mg GAE/g)	TFC (mg QuE/g)	TTC (mg TAE/g)	TAC (mg C3GE/g)
E1	48.39 ± 3.38	< 5 [b]	17.59 ± 0.73	n.d. [c]
E2	51.18 ± 2.67	< 5	19.75 ± 0.81	n.d.
E3	52.38 ± 2.83	< 5	24.76 ± 0.85	n.d.
E4	52.54 ± 3.26	8.31 ± 0.33	25.19 ± 0.98	n.d.
E5	34.38 ± 1.48	< 5	6.61 ± 0.38	n.d.
E6	85.85 ± 4.46	63.53 ± 2.30	10.86 ± 0.61	n.d.
E7	105.86 ± 4.34	60.37 ± 2.17	19.81 ± 0.70	n.d.
E8	86.35 ± 3.62	71.77 ± 2.25	10.03 ± 0.53	n.d.
E9	36.47 ± 1.58	< 5	9.96 ± 0.60	n.d.
E10 [d]	1383.56 ± 95.63	< 5	1529.68 ± 101.26	79.49 ± 1.73

a The extract names (E1–E10) are consistent with those used in Table 1. E9 and E10 correspond to concentrated and freshly squeezed juice, respectively, and are labeled as "E" for uniformity, although they were not obtained by extraction. b Below the detection limit of 5 mg/g). c n.d. = not detected; the compound was below the detection limit of the applied method and standard concentration range. d The contents of phenols, flavonoids, tannins, and anthocyanins for E10 are expressed per liter (mg/L)

TPC ranged from 34.38 to 105.86 mg GAE/g extract (Table 2); the lowest value was observed in the hexane extract of the dry pomace (E5), whereas the highest phenolic content was recorded in the acetone extract of the pyrenes (E7). Among the solvents used, methanol was the most effective for extracting phenolics from the pomace, whereas acetone was the most effective for the pyrenes, whose extracts exhibited the highest values among all samples. The TPC of freshly squeezed juice was 1383.56 mg GAE/L.

For comparison, the present results are evaluated alongside previously reported values. In the study by Cosmulescu *et al*. (2018), phenolic values ranged from 163.69 to 359.28 mg GAE/100 g FW, depending on genotype and processing method, while Gąstoł *et al*. (2013) reported values of approximately 45.6 mg GAE/g, which are similar to those obtained in the present study. In a

study conducted in Serbia (Stanković *et al*., 2014), the mean phenolic contents were 31.36 mg GAE/g for methanol extracts, 179.05 mg GAE/g for ethyl acetate extracts, and 55.38 mg GAE/g for acetone extracts. Among these solvents, methanol showed values most similar to those obtained in the present study, whereas ethyl acetate and acetone differed considerably; the value for ethyl acetate was approximately three times higher, while that for acetone was about half of the value reported in this paper. In a recent study conducted in Ukraine (Lidiková *et al.*, 2024), TPC ranged from 0.583 to 1.877 mg GAE/g FW. These differences can be attributed to analyses performed on fresh fruits, as opposed to extracts analyzed in the present study.

Flavonoid contents ranged from 8.31 to 71.77 mg QE/g extract, the lowest being observed in the methanol extract of the pomace (E4) and the highest recorded in the hexane extract of the pyrenes (E8). Moreover, extracts of the pyrenes obtained with all three solvents exhibited high flavonoid contents. For the pomace, flavonoids in the ethyl acetate and hexane extracts were not detected (below the detection limit of 5 mg/g), and the same was observed for the juice extracts, and both concentrated and freshly squeezed juice. Therefore, the results indicate that flavonoids are predominantly concentrated in the pyrenes.

In the study by Aurori *et al*. (2023), flavonoid contents averaged 0.139 mg/g, with ethanol used as the extraction solvent. Considering that most extracts in the present study were below measurable levels, these low values support the conclusion that flavonoids are weakly present in this plant's fruit. Gelo (2022) reported TFC values in fruits from Croatia without the endocarp, using methanol as the solvent, ranging from 0.18 to 1.07 mg QE/g. The authors also noted that the aluminum chloride method may be inadequate for determining flavonoid content in this plant, due to the presence of glycosylated hydroxyl carbon groups, which interfere with chelation by the reagent (Gelo, 2022; Mammen and Daniel, 2012).

Anthocyanins were not detected in the extracts of juice, pomace, or pyrenes, nor in concentrated juice, but were detected in freshly squeezed juice at 79.49 mg C3GE/L (E10). This suggests that anthocyanins are likely rapidly degraded during storage and upon exposure to high or low temperatures. Moldovan and David (2020) reported a TAC value of 93.43 mg C3GE/L in juice, measured using the pH differential method, which is comparable to the value obtained in the present study.

Tannin contents ranged from 6.61 to 25.19 mg TAE/g extract; the lowest value was found in the hexane extract of the pomace (E5), whereas the highest was recorded in the methanol extract of the pomace (E4). When comparing the same solvents across different parts of the fruit, ethyl acetate yielded higher tannin contents in the pomace than in the juice extract, hexane was more effective for the pyrenes than for the pomace, and diethyl ether resulted in higher values in the juice extract compared to the one obtained from pyrenes. Freshly squeezed juice contained 1529.68 mg TAE/L. In the study by Blagojević *et al.* (2023), using the percentage method prescribed by the European Pharmacopoeia (Ph.

Eur. 9.0), tannin content was higher in the pomace (0.81%) than in juice (0.65%), a result that aligns with the observations of the present study.

Given that only ripe fruits were used in the present study, this may explain the relatively low tannin content, as tannin levels are known to decrease during fruit ripening (Gunduz *et al.*, 2013). Bayram and Ozturkcan (2020) noted that only few studies have investigated tannin content in CM, highlighting the limited available data. Tannins in pomegranate (Fernandes *et al.*, 2015), which was selected for comparison with the present results, showed varying values depending on the part of the fruit analyzed. Notably, tannins were not detected in pomegranate juice, whereas in CM juice, they were present at significant level. Tannin contents in pomegranate seeds ranged from 2.4 to 12 mg/g, which is markedly lower than that observed in the pyrene extracts analyzed in the present study.

The results of antioxidant properties are presented in Table 3. Antioxidant activity measured by the DPPH assay showed IC_{50} values ranging from 58.27 to 4560.89 μg/mL; the lowest value, indicating the highest antioxidant activity, was observed in the acetone extract of the pyrenes (E7), whereas the highest value was recorded for the hexane extract of the pyrenes (E8).

Table 3. DPPH (IC50) and FRAP values of extracts (E1–E10) from CM fruits

Extract [a]	DPPH (IC_{50} μg/mL)	FRAP (mmol Fe^{2+}/g)
E1	134.54 ± 4.57	13.69 ± 0.45
E2	362.09 ± 9.25	15.66 ± 0.55
E3	108.96 ± 4.36	15.96 ± 0.55
E4	63.66 ± 2.34	13.69 ± 0.56
E5	1273.07 ± 60.38	6.75 ± 0.35
E6	534.85 ± 25.10	7.51 ± 0.40
E7	58.27 ± 2.60	40.01 ± 1.52
E8	4560.89 ± 260.72	7.21 ± 0.40
E9	447.19 ± 17.87	7.09 ± 0.41
E10	20.97 ± 1.15	39.07 ± 1.37 [b]

a The extract names (E1–E10) are consistent with those used in Table 1. E9 and E10 correspond to concentrated and freshly squeezed juice, respectively, and are labeled as "E" for uniformity, although they were not obtained by extraction. b The FRAP value for juice is expressed per liter (mmol Fe^{2+}/L)

Large differences in IC_{50} values were observed in the pomace extracts, highlighting the significant influence of solvent choice in this assay; methanol was the most effective solvent (E4), while hexane was the least effective (E5). The juice extract obtained with ethyl acetate (E1) showed higher activity than that obtained using diethyl ether (E2). A comparison of the same solvents across sample types revealed that ethyl acetate was slightly more effective in the pomace

extracts than in the juice extracts. The hexane extracts displayed generally low antioxidant activity, although those obtained from pomace performed better than those from pyrenes. Diethyl ether, in contrast, was more effective in the juice extracts than in those obtained from pyrenes. These results indicate that acetone and methanol are the most suitable solvents for maximizing antioxidant activity, while hexane and diethyl ether are less effective. The low antioxidant activity observed in concentrated juice is likely attributable to thermal degradation occurring during the juice concentration process. Freshly squeezed juice exhibited an IC_{50} value of 20.97 μg/mL.

IC_{50} values reported by Stanković *et al.* (2014) for fruit extracts prepared with methanol, ethyl acetate, and acetone, were 251.87, 11.06, and 107.99 μg/mL, respectively. In their study, ethyl acetate was the most effective solvent, whereas in the present research, acetone showed the highest activity and ethyl acetate was the least effective. These differences are likely due to the fact that Stanković *et al.* (2014) used whole CM fruits. In the study by Milenković-Anđelković *et al*. (2015), DPPH activity expressed as IC_{50} was evaluated in fruits and leaves. Fruit extracts had IC_{50} values of 1090 μg/mL in the first year and 940 μg/mL in the following year. These values indicate considerably weaker antioxidant activity compared to the present results, which may be attributed to differences in extract preparation and solvent choice.

FRAP activity measured in juice by Gąstoł *et al.* (2013) was 23.5 mmol Fe^{2+}/L, which is comparable to the values obtained for freshly squeezed juice in the present study. In Turkey, Tural and Koca (2008) reported FRAP values ranging from 16.21 to 94.43 mmol Fe^{2+}/g, which partially correspond to the samples tested here; however, among 24 native plants covered in their work, some showed substantially higher FRAP values than those measured here. FRAP activity measured by Blagojević *et al.* (2023) in juice and pomace of CM from Montenegro was exceptionally low, ranging from 0.000094 to 0.000112 mmol Fe^{2+}/g and 0.000107 to 0.000120 mmol Fe^{2+}/g, respectively. In comparison, the present study showed considerably higher values for juice than for pomace across all solvents, and in general, higher FRAP values were detected. These differences may reflect variation in sample composition, extraction methods, and experimental conditions, rather than solely the analytical procedure.

Correlation analysis of phenolic content and antioxidant activity

In this study, the correlation between phenol, flavonoid and tannin contents and antioxidant activity was calculated using the Pearson correlation coefficient, with significance set at $p<0.05$ (Table 4).

The content of phenolic compounds in ethyl acetate, methanol, and hexane extracts showed a negative correlation with antioxidant activity as determined by the DPPH assay. In contrast, the FRAP assay revealed significant positive correlations between phenolic compound content and antioxidant activity. A strong positive correlation was observed for phenols extracted with ethyl acetate ($r=0.85$, $p<0.05$), while a moderate positive correlation was found for methanol

extracts (r=0.53, p<0.05). Regarding flavonoid content, a high significant positive correlation was found between ethyl acetate extracts and FRAP activity (r=0.94, p<0.05). For tannins, a very strong significant correlation was observed between ethyl acetate extracts and FRAP activity (r=0.99, p<0.05).

These results indicate that ethyl acetate is an effective solvent for extracting phenols, flavonoids, and especially tannins, which contribute significantly to antioxidant activity measured by the FRAP assay. Methanol also showed a positive correlation between flavonoid content and FRAP activity, suggesting it can influence the antioxidant potential of flavonoids.

Table 4. Correlation of phenol, flavonoid, anthocyanin, and tannin contents with antioxidant activity in extracts of CM

	TPC EA	**TPC M**	**TPC H**	**TFC EA**	**TFC M**	**TFC H**	**TTC EA**	**TTC M**	**TTC H**
DPPH EA	-0,99			-0,99			-0,92		
DPPH M		-0,69			-0,83			-0,85	
DPPH H			-0,88			-0,58			-0,98
FRAP EA	**0,85***			**0,94***			**0,99***		
FRAP M		**0,53**			-0,73			0,29	
FRAP H			-0,46			-0,02			-0,69

**Bold values represent strong correlations (p <0.05); TPC=Total Phenolic Content; TFC=Total Flavonoid Content; TTC=Total Tannin Content; EA=Ethyl Acetate; M=Methanol; H=Hexane; DPPH=antioxidant activity measured by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (IC50, µg/mL); FRAP=antioxidant activity measured by the Ferric Reducing Antioxidant Power assay (mmol Fe^{2+}/g)*

These findings are generally in agreement with previous studies. For instance, Guzel (2021) reported strong positive correlations between antioxidant activity and the content of phenolic compounds, including total phenols (r=0.965), flavonoids (r=0.890), and hydrolysable tannins (r=0.882). Similarly, Blagojević *et al.* (2023) demonstrated a high correlation between flavonoid content and antioxidant capacity measured by the FRAP assay (r=0.96), whereas the association with DPPH activity was minimal, which is consistent with the trends observed in the present study. In contrast to Blagojević *et al.* (2023), who reported very low correlations for tannin content with both DPPH and FRAP assays (r=–0.04 and r=0.14, respectively), the results in the study presented herein indicate a strong positive correlation between tannins and FRAP activity, underscoring their substantial contribution to the overall antioxidant potential. These discrepancies may reflect differences in extraction solvents, plant material, or methodological approaches, emphasizing the influence of experimental conditions on the observed antioxidant activities.

CONCLUSIONS

It can be concluded that acetone and methanol were the most effective solvents for extracting antioxidant compounds, while hexane was less successful. Nevertheless, the highest flavonoid content in the pyrenes extracts was obtained using hexane, and in pomace samples, methanol successfully extracted flavonoids

despite lower overall values. Both tests showed similar trends, indicating that the highest antioxidant activity was found in the acetone extract of pyrenes, whereas the lowest activities were recorded in hexane extracts. Among fruit parts, the extracts obtained from pyrenes exhibited the strongest antioxidant activity.

These findings provide valuable insights into the composition and bioactivity of CM, particularly the pyrenes, which has been the least investigated fruit part to date. Future research could focus on the mineral composition of fresh juice, the potential uses of the pyrenes, and other under-examined organs such as flowers, bark, and leaves. Environmental factors influencing antioxidant activity, including altitude and proximity to the sea (salinity being a known limiting factor), should also be considered. Additionally, exploring different extraction methods could further enhance understanding of the plant's chemical composition and medicinal properties, supporting its potential applications in pharmaceutical, cosmetic, and food industries.

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