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NATURAL DRUGS

ANTIOXIDANT POTENTIAL OF CORNELIAN CHERRY (*CORNUS MAS* L.) FROM MONTENEGRO

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Abstract: In this work, total phenols, flavonoids, tannins, anthocyanins, and the antioxidant capacity (by the DPPH and FRAP methods) were determined in the juice and pomace of Cornelian cherry from the Montenegro area. Also, the content of microelements and macroelements in the juice, pomace and fruit of Cornelian cherry were investigated. In the studied Cornelian cherry samples, the content of phenols ranged from 159.57 to 244.93 mg GAE/100 g, flavonoids from 71.74 to 165.5 mg QE/100 g, tannins from 0.33 to 0.81%, and total anthocyanins 0.057-0.310%. HPLC analysis identified the following anthocyanins: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, pelargonin-3-*O*-glucoside and its derivative. The most abundant essential microelement in the studied Cornelian cherry samples was Fe (23.44-37.69 mg/kg), followed by Zn (3.14-6.25 mg/kg), Cu (1.50-1.80 mg/kg) and Mn (0.58-0.78 mg/kg). The most abundant essential macroelement in the examined samples was K (2427.8-3276.2 mg/kg), followed by Ca (361.5-475.7), Mg (95.41-146.5 mg/kg), and Na (8.347-16.59 mg/kg). Pb, Cd and Ni were not detected in the tested samples. A high degree of correlation was obtained between the content of total flavonoids and the antioxidant value measured by the FRAP method (R = 0.96), as well as between the content of total anthocyanins and antioxidant activity measured by the DPPH method (R = 0.89). Of the individual anthocyanins, high correlation values were shown by cyanidin-3-*O*-galactoside (R = 0.96), cyanidin-3-*O*-glucoside (R = 0.95) and pelargonin-3-*O*-glucoside (R = 0.94) with antioxidant activity measured by the DPPH method.

Keywords: *Cornus mas* L., antioxidant activity, polyphenolic compounds, metals.

Phenolic compounds—as parts of secondary metabolites—are present with enormous structural diversity in plant species. They can exist as glycosides or aglycones, matrix or freely bound compounds, which mainly consist of polymerized or monomeric structures. Major classes of phenolic compounds in plants include phenolic acids, flavonoids, tannins, and stilbenes (1). Phenols are perhaps the most researched natural compounds for their potential health benefits, as shown in numerous studies (2). They are associated with antioxidant, anti-inflammatory, anti-allergic, anti-cancer, anti-hypertensive, cardio-protective, anti-arthritic, and antimicrobial effects. Recently, a considerable efforts have been made to provide sensitive and selective analytical methods for the determination and characterization of phenols (3).

The Cornelian cherry fruit (*Cornus mas* L., Cornaceae) is considered to be one of those medicinal plants with important nutritional and therapeutic properties (4). It can be found naturally in the central and southeastern regions of Europe (5). Its fruits are characterized by an oval or oval-oblong shape, with colors ranging from light yellow to dark cherry. Cornelian cherry is known to have antimicrobial, antioxidant, anticancer, and anti-inflammatory effects due to its composition of phenolic compounds (4).

The main health promoting properties of the Cornelian cherry are related to the presence of anthocyanins (5). It is well known that this fruit is

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used in different countries for different purposes—for the treatment of intestinal and kidney diseases, strengthening immunity, and preventing some types of cancers and others (4). It is also used in traditional medicine for treating fever, cholera, kidney stones, malaria, urinary tract infections, heat stroke, and bleeding. Many studies have reported the positive effect of Cornelian cherry in regulating blood sugar and preventing fat accumulation in the liver (4).

Numerous studies emphasize the high phenolic compound content (flavonoids, anthocyanins) and antioxidant potential of Cornelian cherry, noting that it is among the highest among many other fruits (6, 7). They indicated that the main *C. mas* anthocyanins were cyanidin and pelargonin-3-*O*-glucosides and among the flavonoids were quercetin, kaempferol and aromadendrin 3-*O*-glucosides (7). However, it is known that mineral content as well as antioxidant potential varies between different populations as a result of differences in genotype and environmental variables, as well as geographical origin, time of sampling, and microclimatic conditions of the locality where the Cornelian cherry fruits were collected (8). Based on the available literature, phenolic compounds and mineral content in Cornelian cherry fruits from Montenegro were not the subject of special studies. Therefore, this is the first study that provides comprehensive data on the antioxidant potential of Cornelian cherry from two different regions of Montenegro.

The aims of this study were to determine total phenolic (TP), total tannin (TT), total flavonoid (TF), and total anthocyanin (TA) content in the Cornelian cherry juice and pomace and the mineral composition in the Cornelian cherry juice, pomace and fruit. To identify and quantify major anthocyanin compounds present in the investigated samples, the HPLC method was employed. To assess the relationship between antioxidant activity and phenolic compound content as well as the metal content of the investigated samples, two common antioxidant activity assays—DPPH and FRAP—were applied. To estimate the nutritional value of Cornelian cherry fruits for dietary intake, the concentrations of eleven elements including macroelements (Ca, Na, K and Mg) and microelements (Cd, Cu, Mn, Ni, Pb, Fe and Zn) were determined. In addition, the obtained results were marked in regard to the geographical origin of the investigated Cornelian cherry samples.

MATERIALS AND METHODS

Sample Collection

Samples of Cornelian cherry fruits were collected from two environmentally different towns in Montenegro (Figure 1). Namely, Šavnik (42.953889°N 19.091389°E) is located in the northern part of Montenegro and it is under the influence of mountain climate, at an altitude of 995 m. The climate of the Šavnik is characterized by long, cold

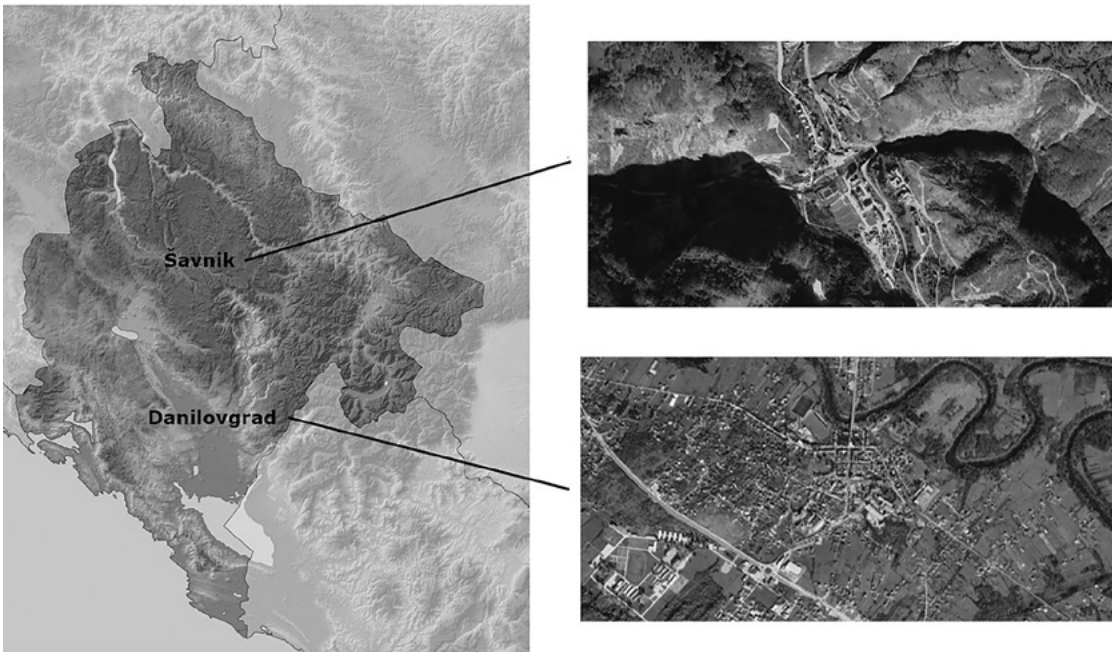

Figure 1. Map of Montenegro with sampling sites.

and snowy winters and short summers. Danilovgrad (42.541666°N 19.103166°E) is located in the central region of Montenegro, at an altitude of 48 m. This town is in the submediterranean zone of the Mediterranean climate region. Due to the different climatic conditions, the Cornelian cherry in Šavnik ripens in October, while the Cornelian cherry in Danilovgrad ripens earlier, in September.

After squeezing, the samples were divided and labelled as Cornelian cherry fruits (F_1), Cornelian cherry juice (S_1), and Cornelian cherry pomace (T_1) from Šavnik; and Cornelian cherry fruits (F_2), Cornelian cherry juice (S_2), and Cornelian cherry pomace (T_2) from Danilovgrad. The samples were frozen and stored before analysis.

Determination of Total Phenol, Tannin, Flavonoid and Anthocyanin Content in Cornelian Cherry Juice and Pomace

Total phenolic (TP), total tannin (TT), total flavonoid (TF), and total anthocyanin (TA) content were determined by spectrophotometric method using a BUCK Scientific 105 UV-VIS spectrophotometer.

The total phenolic (TP) content was measured according to a slightly modified Folin-Ciocalteu method (9, 10). One hundred microliters of water solution of the investigated samples—S_1, T_1, S_2, T_2 (25.41 mg/mL, 25.17 mg/mL, 25.25 mg/mL, 25.11 mg/mL, respectively)—was mixed with 0.75 mL of 10% Folin-Ciocalteu reagent (VWR chemicals) and allowed to stand at 22° C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22° C, absorbance was measured at 740 nm. Gallic acid (5 mg/mL) was used to prepare a standard curve. The calibration curve showed the linear regression at $r^2 > 0.99$, and the results are expressed as milligrams of gallic acid equivalents per 100 gram of plant matter (mg GAE/100 g). The content of total phenolics is presented as the mean of three determinations.

Total flavonoid (TF) content was determined according to a slightly modified procedure described by Chang et al., (2002); 1 mL of the investigated samples (S_1, T_1, S_2, T_2) were mixed with 3 mL of deionized water and 0.3 mL of $NaNO_2$. After 5 minutes at room temperature, 3 mL of 1% $AlCl_3$ and 2 mL of 1M NaOH were added to the mixture. The samples were quantitatively transferred into a volumetric flask (10 mL) and diluted with deionized water. Absorbance was measured at $\lambda = 415$ nm. Quercetin (1 mg/L) was used to prepare a standard curve. The results are expressed as milligrams of quercetin equivalents per 100 g of plant material (mg QE/100 g) and represent the mean value of three independent measurements (11).

Using the method described in the European Pharmacopoeia 9.0 (12), the total tannin (TT) content was calculated as a percentage. The investigated samples (S_1, T_1, S_2, T_2) were treated with phosphomolybdotungstic reagent in alkaline medium after and without treatment with hide powder. Absorbance was measured at 760 nm. The percentage of tannins (the mean of three determinations), expressed as pyrogallol (%), was calculated from the difference in absorbance of total polyphenols (A_1) and polyphenols not adsorbed by hide powder (A_2), using the following expression:

$$62.5(A_1 - A_2) \times m_2/(A_3 \times m_1)$$

where m_1 represented the mass of the sample to be examined, in grams; and m_2 the mass of pyrogallol, in grams.

Total anthocyanin (TA) content was investigated according to the procedure described in European Pharmacopoeia 9.0 (12). Shortly, the investigated samples (S_1, T_1, S_2, T_2) were hydrolyzed under reflux with a MeOH/HCl mixture. The absorbance of the solution was measure at 528 nm. The percentage content of anthocyanins (mean of three determinations), expressed as cyanidin-3-glucoside chloride, was calculated using the following expression:

$$A \times 5000/(718 \times m)$$

where A was absorbance at 528 nm, and m was the mass of the extracts to be examined in grams.

HPLC Analysis

Identification and quantification of anthocyanins in the examined samples was achieved using Agilent Technologies 1200 HPLC system equipped with a Lichrospher 100RP 18e column, applying gradient elution of two mobile phases, i.e., "A/B" ("A"—0.2M solution of phosphoric acid, and "B"—a pure acetonitrile), at flow–rates of 1 mL/min, with photodiode-array (PDA) detection (UV at 260, 325 nm), always within 70 min. The best combinations were 89–75% A (0–35 min); 75–60% A (35–55 min); 60–35% A (55–60 min); and 35–0% A (60–70 min). The concentrations of the investigated samples were 99.36 mg/mL, 100.58 mg/mL, 298.65 mg/mL, 314.20 mg/mL for S_1, S_2, T_1, T_2, respectively. Prior to injection, the samples were filtered through a PTFE membrane filter. For the standard used in the investigation, the concentrations were: 0.46 mg/mL for delphinidin-3-glucoside,

0.49 mg/mL cyanidin-3-galactoside, 0.50 mg/mL cyanidin-3-glucoside, and 0.48 mg/mL pelargonin-3-glucoside. The volume of the standard solutions being injected, as well as for the tested sample extracts, was 4 μL. For the purpose of anthocyanin identification and determination in the investigated samples, the mixtures of the standards were prepared, with the already mentioned concentrations. Identification was based on retention times and spectral matching. Once spectra matching succeeded, the results were confirmed by spiking with the respective standard to achieve complete identification by means of the so-called peak purity test. Quantification was performed by external calibration with the standards. The content of anthocyanins are presented as the mean of three determinations.

Methods for Antioxidant Activity Determination

Radical Scavenging Activity (DPPH Test)

The diluted samples (300 μL of the stock solution at concentrations of 46.47 mg/mL,50.52 mg/mL, 42.20 mg/mL, 49.56 mg/mL for S_1, S_2, T_1, T_2, respectively) and 2.7 mL of 0.1 mM ethanol DPPH solution were mixed. After 30 min of incubation at room temperature in the dark, absorbance was recorded at 517 nm against ethanol as a blank. Free radical scavenging activity was calculated with respect to the control solution containing ethanol instead of the test solution using the formula:

$$\text{DPPH radical scavenging capacity (\%)} = 100-[(A_S - A_B) \times 100 / A_C]$$

where A_S was the absorption of the ethanol solution of the samples treated with DPPH radical solution; A_B was the absorption of the ethanol solution of the samples which was not treated with DPPH radical solution; and A_C was the absorption of the ethanol solution of the DPPH.

The inhibition percentage was plotted against the sample concentration, and IC_{50} values were determined by linear regression analysis. The synthetic antioxidant *tert*-butyl hydroxytoluene (BHT) was used as a positive control.

Ferric-Reducing Antioxidant Power (FRAP Test)

The diluted extract (100 μL of the stock solution at concentrations of 46.10 mg/mL, 46.39 mg/mL, 44.64 mg/mL, 43.88 mg/mL for S_1, S_2, T_1, T_2, respectively) and 3.0 mL of freshly prepared FRAP reagent (25 mL of 300 mM acetate buffer pH 3.6 plus 2.5 mL of 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3x6H_2O$) were mixed. Absorbance was recorded at 593 nm against a blank, containing 100 μL of reagent-resembling solvent, after 30 min incubation at 37° C. The FRAP-value was calculated from the calibration curve of $FeSO_4x7H_2O$ standard solutions (100-1000 mmol/L) and expressed as mmol Fe^{2+}/g of plant material.

Preparation of Cornelian Cherry Fruits, Juice and Pomace for Metal Determination

A total of 1 g of Cornelian cherry juice and 0.5 g of Cornelian cherry pomace and Cornelian cherry fruits transferred into PTFE cuvettes, and 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 were added. After microwave digestion, the samples were quantitatively transferred into a volumetric flask (25 mL), diluted with distilled water, and saved for ICP-OES analysis.

Inductively Coupled Atomic Emission Spectrometer, ICP-OES (Thermo Scientific, United Kingdom), model 6500 Duo, equipped with a CID86 chip detector was used for the determination of elements. A multielement standard (1000 mg/L) was used for the preparation of standard solutions in 2% HNO_3 as well as in matrix-matched solutions for the digest solutions. The stock solution was diluted to obtain the following concentrations (mg/L): 0.05, 0.20, 1.0, 2.0, 5.0, 10, and 20. Blank solutions were prepared in the same media. Calibration ranges were modified according to the expected concentration ranges of the elements of interest.

Dietary Elemental Intake Determination

To calculate elemental intakes per day, 300 g of fresh weight portion of Cornelian cherry fruits per meal were used. Such a portion contained about 48 g of dry matter. Daily mineral intake (DMI,%) and recommended daily allowance (RDA) values were calculated according to the European Economic Community (EEC) (13). DMI was calculated using:

$$DMI = C \times 100/RDA$$

where C was the elemental content (mg) in 300 g of fruits.

Daily intake (DI,%) for Ni, Cd and Pb was calculated for 300 g of Cornelian cherry fruits using:

$$DI = C \times 100/MDI$$

where MDI corresponded to the maximum tolerable daily intake established by the EFSA (14-16).

Statistical Analysis

Linear regression analyses and Pearson's correlation coefficient (R) to determine the relationships between two variables were calculated using MS-Windows software (Excel, 2007).

RESULTS AND DISCUSSION

Polyphenolic Compound Content in Cornelian Cherry Juice and Pomace

In this study, the TP content (Figure 2a) found in Cornelian cherry juice was 229.94 mg GAE/100 g in S_1 and 244.93 mg GAE/100 g in S_2, while in the Cornelian cherry pomace was 159.57 mg GAE/100 g in T_1 and 184.7 mg GAE/100 g in T_2. The obtained results were in accordance with the available literature data. De Biaggi found that the average total phenol content in Cornelian cherry fruits from northwestern Italy was 196.68 mg GAE/100 g (17), while Cosmulescu in Romanian fruits noticed that TP content had a range from 163.69 mg GAE/100 g to 359.28 mg GAE/100 g (18). Perova found a slightly higher total phenolic content (from 150 mg GAE/100 g to 400 mg/100 g) (19) in samples of fresh-frozen Cornelian cherry fruits from Russia, while Popović obtained a range from 494 mg GAE/100 g to 704 mg GAE/100 g in fruits from Serbia (20). In addition, the Cornelian cherry samples (S_2 and T_2) from Danilovgrad were richer in TP compounds compared to the samples (S_1 and T_1) from Šavnik. The obtained results can be explained by the influence of different abiotic environmental factors (temperature, water deficiency, irrigation, and nutrient stress) on the quantitative and qualitative content of phenolic compounds in the plant (21, 22). The Šavnik location is such that it is affected by the mountain climate, while Danilovgrad belongs to the Mediterranean climate region. Also, a higher total phenolic content was found in the Cornelian cherry juice (S_1 and S_2) than in the pomace (T_1 and T_2) regardless of the geographical location of the collection sites (Figure 2a).

Furthermore, the obtained TF content (Figure 2b) in Cornelian cherry juice was 71.71 mg QE/100 g in S_1 and 134.45 mg QE/100 g in S_2, while in the pomace was 165.58 mg QE/100 g in T_1 and 94.62 mg QE/100 g in T_2. Our results were in accordance with the previously reported data for Cornelian cherry fruits. Cosmulescu found a TF content from 12.14 mg QE /100 g to 64.48 mg QE /100 g in Romanian Cornelian cherry fruits (18),

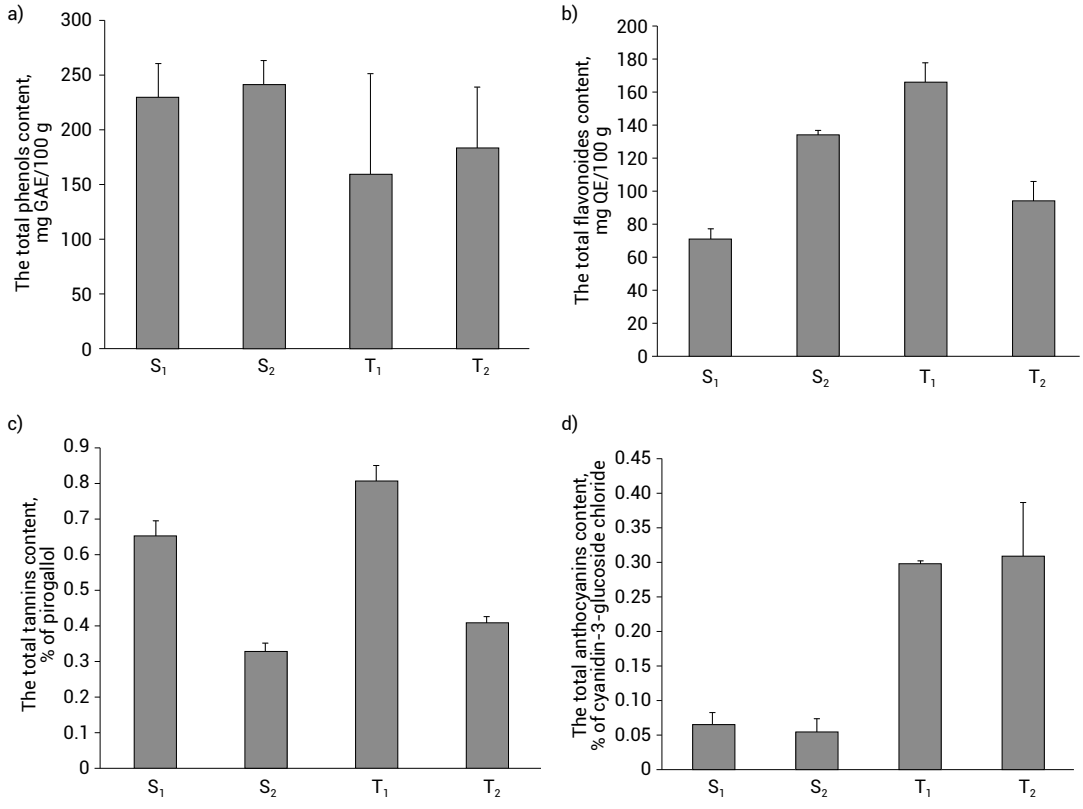

Figure 2. Content of (a) total phenolic compounds (TP); (b) total flavonoids (TF); (c) total tannins (TT); (d) total anthocyanins (TA) content in the investigated samples.

while Karaaslan noticed a slightly higher TF content in Turkish fruits (255.75 mg QE /100 g) (23). Our results (Figure 2b) revealed that Cornelian cherry juice from fruits collected at a higher altitude (S_1) had a lower TF content than the juice from a lower altitude (S_2). On the other hand, the TF content was higher in the Cornelian cherry pomace from Šavnik (T_1) than pomace from Danilovgrad (T_2). According to previous findings, it was suggested that environmental temperature plays a significant role in flavonoid content, being more pronounced in cold weather (24). During stress, more phytochemicals are produced in plants to withstand the adverse conditions. Studies conducted on plants in stress conditions showed higher production of flavonoids and anthocyanins (24).

The TT content (Figure 2c) found in Cornelian cherry juice was 0.65% in S_1 and 0.33% in S_2, while in the Cornelian cherry pomace was 0.81% in T_1 and 0.41% in T_2. The obtained results were in accordance with the available literature data. Bijelić noticed a TT content in Serbian Cornelian cherry fruits from 0.73% to 1.21% (25); while the percentage of tannin content in the Turkish Cornelian cherry fruits varied from 0.19% to 0.45%, depending on the degree of fruit maturity (26). Also, we found that the TT content (Figure 2c) was higher in Cornelian cherry pomace (T_1 and T_2) than in the juice (S_1 and S_2), regardless of the geographical location of the collection sites. Furthermore, a higher content of these compounds was determined in the Cornelian cherry samples (S_1 and T_1) from Šavnik than in the same corresponding samples of this plant from Danilovgrad (S_2 and T_2). This difference in TT content might be due to the fact that their amount decreased with the ripening of the plant fruits (26). In our study, samples from the north of Montenegro were harvested before full maturity, while Cornelian cherry fruits from the central part were harvested in the maturation phase. Therefore, we cannot exclude that the difference in TT content in the samples from the two locations is the result of different maturation stages.

The percentage of total anthocyanin content (Figure 2d) found in Cornelian cherry juice was 0.067% in S_1 and 0.057% in S_2, while in the pomace was 0.299% in T_1 and 0.310% in T_2. The obtained results were in accordance with the available literature data. Novruzov noted that the percentage of anthocyanins in Cornelian cherry fruits from Azerbaijani ranged from 0.053% to 0.434% (27), while in Cornelian cherry fruits from Italy De Biaggi found that the average content was 0.134% (17). Bijelić found the percentage of anthocyanins varied from 0.036% to 0.116% in the fruits from Serbia (25). Also, the total anthocyanin content (Figure 2d) was higher in Cornelian cherry pomace (T_1 and T_2) than in the juice (S_1 and S_2) regardless of the geographical location of the collection sites (Danilovgrad and Šavnik). The percentage of anthocyanins in Cornelian cherry juice from Šavnik (S_1) was higher than in juice from Danilovgrad (S_2), while the content of these phenolic compounds was higher in Cornelian cherry pomace from the central part (T_2) compared to the northern area (T_1). Our data revealed that the concentration of anthocyanins in plants depended mostly on the different plants' biological habitats and growing conditions, as well as on the nature of the associations that phenolics might form with other plant components such as carbohydrates and proteins (28). According to previous findings, it was suggested that total anthocyanin content increased during maturation due to the degradation of chlorophylls (29).

HPLC Analysis

Anthocyanins belong to a large group of secondary plant metabolites collectively known as flavonoids (30). They are water-soluble glycosides of anthocyanidins, which are largely responsible for the attractive pale yellow, orange, red, magenta, violet and blue color of a wide range of plant tissues, principally flowers, leaves and fruits, besides storage organs, roots, tubers, stems and grains (31).

Recent studies revealed the presence of different anthocyanins in Cornelian cherries (32-34). Seeram et al. (2002) mentioned delphinidin 3-*O*-galactoside, cyanidin 3-O-galactoside and pelargonidin 3-*O*-galactoside as most abundant. Tural and Koca (2008) mentioned cyanidin 3-O-glucoside, cyanidin 3-*O*- rutinoside and pelargonidin 3-*O*-glucoside. Pawlowska et al. (2010) mentioned cyanidin 3-*O*-galactoside, pelargonidin 3-*O*-glucoside and pelargonidin 3-*O*-rutinoside (7, 35, 36,).

On the other hand, our study revealed the presence of four individual anthocyanins and one non-identified derivative of pelargonin-3-*O*-glucoside (Figure 3, Table 1). The identification of the above-mentioned anthocyanins was important considering that numerous studies confirmed their potential preventative and/or therapeutic effects on health, including obesity prevention, cardiovascular diseases, antibacterial, anti-inflammatory, and anti-cancer effects (31).

The results obtained by HPLC analysis showed that in the Cornelian cherry samples, from both of collection sites, the content of identified

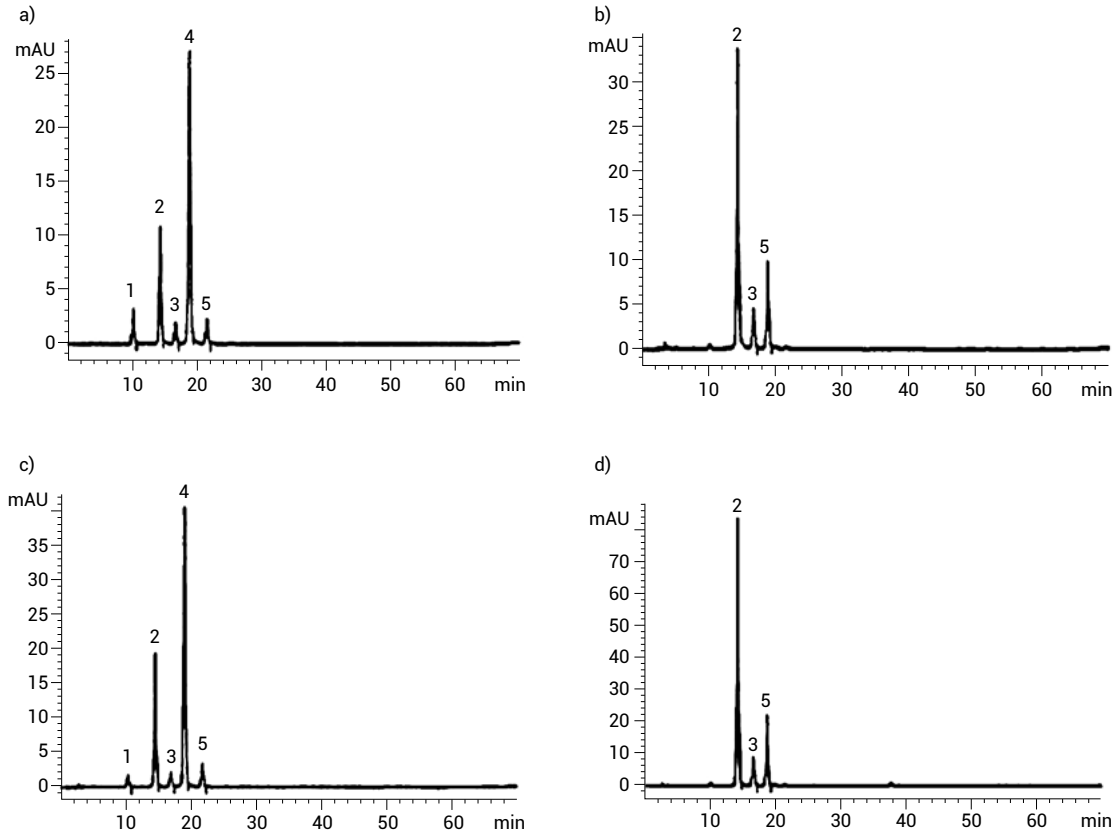

Figure 3. HPLC chromatograms of the investigated samples and anthocyanin (1. delphinidin 3-*O*- glucoside, 2. cyanidin 3-*O*-galactoside 3. cyanidin-3-*O*-glucoside, 4. pelargonin-3-*O*-glucoside derivative, 5. pelargonin-3-*O*-glucoside) identified at 520 nm in: (a) S_1; (b) S_2; (c) T_1; (d) T_2

anthocyanins was higher in the pomace (T_1 and T_2) than in the juice (S_1 and S_2).

The predominant anthocyanin in samples S_1 and T_1 was cyanidin-3-*O*-galactoside (0.5008 mg/g and 0.9894 mg/g, respectively). The second most abundant anthocyanin in sample S_1 was delphinidin-3-*O*-glucoside (0.0161 mg/g), followed by pelargonin-3-*O*-glucoside (0.0070 mg/g), and finally cyanidin-3-*O*-glucoside (0.0069 mg /g). On the other hand, the second most abundant anthocyanin in sample T_1 was pelargonin-3-*O*-glucoside (0.1153 mg/g), followed by cyanidin-3-*O*-glucoside (0.0820 mg/g), and delphinidin-3-*O*-glucoside (0.0786 mg/g). The most abundant anthocyanin in S_2 and T_2 samples was cyanidin-3-*O*-galactoside (0.1746 mg/g and 4.3705 mg/g, respectively), followed by pelargonin-3-*O*-glucoside (0.0334 mg/g and 0.7462 mg/g, respectively). The concentration of cyanidin-3-*O*--glucoside in samples S_2 and T_2 was 0.0181 mg/g and 0.4870 mg/g, respectively; while delphinidin-3-*O*-glucoside was not identified in these samples. The obtained results were in accordance with the available literature data. Milenković Anđelković identified cyanidin-3-*O*-galactoside (1.0068 mg/g),

Table 1. Content of anthocyanins determined by HPLC in the investigated samples [mg/g].

Anthocyanins	S_1	T_1	S_2	T_2
Delphinidin-3-*O*-glucoside	0.0161	0.0786	-	-
Cyanidin-3-*O*-galactoside	0.5008	0.9894	0.1746	4.3705
Cyanidin-3-*O*-glucoside	0.0069	0.0820	0.0181	0.4870
Pelargonin-3-*O*-glucoside derivative	*	*	-	-
Pelargonin-3*O*-glucoside	0.0070	0.1153	0.0334	0.7462

*tentative identification

pelargonidin-3-*O*-glucoside (0.3273 mg/g) and delphinidin-3-*O*-glucoside (0.0491 mg/g) in Cornelian cherry fruits from Serbia (37), and Sozanski identified cyanidin 3-O-galactoside (1.23 mg/g) in Cornelian cherry from Poland (38). Diets rich in anthocyanins are associated with a decreased risk of developing neurodegenerative diseases, through direct scavenging of ROS, increasing the activity of antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)), elevating reduced glutathione GSH content, and reducing malondialdehyde MDA (31). Positive findings have been shown for the treatment of Alzheimer's disease with anthocyanin-rich extracts. Anthocyanin consumption by older people at risk for dementia improves memory impairment via increasing neuronal signaling in brain centers mediating this function. Additionally, they can effectively prevent free radicals from damaging the dopamine-producing cells in the brain, thus inhibiting the development of Parkinson's disease. The protective role of anthocyanins on the cardiovascular system is strongly related to their properties reducing/inhibiting oxidative stress. Anthocyanins are well known for their capacity to decrease low-density lipoprotein cholesterol, triglycerides, and blood pressure (39-41). They inhibit platelet aggregation and activation. In addition, they might reduce the risk of myocardial infarction and inflammation in atherosclerosis. On the other hand, they improve high density lipoprotein cholesterol (32, 42). The results of Jayaprakasam suggest that the consumption of Cornelian cherry or other fruits containing anthocyanins has the potential to reduce the risk of diabetes and obesity (43).

Our results obtained by HPLC analysis showed that juice (S_1) and pomace (T_1) from Šavnik had a lower content of cyanidin-3-*O*-glucoside and pelargonin-3-*O*-glucoside compared to juice (S_2) and pomace (T_2) from Danilovgrad. The sample of pomace T_1 also had a lower concentration of cyanidin-3-*O*-galactoside compared to the sample of pomace T_2, while the sample of juice S_1 had a higher content of this compound compared to the sample of juice S_2. Different stages of plant maturity influence the phenolic compound content, as well as their types. Additionally, the growth conditions such as temperature and altitude significantly affect the qualitative and quantitative composition of secondary plant metabolites (24, 34).

Antioxidant Activity

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS), or free radicals, and antioxidant defenses, which may lead to tissue injury (44). When an organism is exposed to high ROS concentrations, the endogenous antioxidant system involved in free radical defenses cannot protect the body against ROS, hence there is a need for exogenous antioxidants supplied through food, nutritional supplements, or pharmaceuticals. Among the most important exogenous antioxidants are phenolic compounds found in medicinal plants (45).

Recent investigations have shown that there is no universal method to evaluate antioxidant activity both quantitatively and accurately (28). In this study, analysis of the antioxidant potential of the investigated samples was performed using two methods—DPPH and FRAP. These methods are distinguished by their mechanism of action, but are complementary in the evaluation of the antioxidant potential of plants (28). The DPPH assay is based on the hydrogen donating capacity to scavenge DPPH radicals, while the FRAP assay is an electron transfer-based test measuring the substance's ability to reduce Fe^{3+} to Fe^{2+} (results were expressed as mmol Fe^{2+}/g of plant material used). Results for the DPPH test in our research were expressed as IC_{50} with the higher IC_{50} values indicating lower antioxidant capacity.

In this research, the IC_{50} value obtained (Figure 4a) for Cornelian cherry juice was 0.183 μg/ml in S_1 and 0.050 μg/ml in S_2, while in the Cornelian cherry pomace was 0.897 μg/ml in T_1 and 1.886 μg/ml in T_2. However, to the best of our knowledge, there is no available peer-reviewed literature the results of which could be compared with our results. Namely, Cosmulescu noted that the antioxidant capacity of methanol extracts of Cornelian cherry fruits from Romania varied from 1.24 mmol Trolox/100 g to 2.71 mmol Trolox/100 g (18), while in Greek Cornelian cherry fruits Tiptiri-Kourpeti found the IC_{50} value of 0.067% (8). Behrangi investigated different Cornelian cherry extracts and depending on the applied extraction procedure, the obtained IC_{50} values varied from 3.95 mg/mL to 9.67 mg/mL (46).

Our results (Figure 4a) revealed that, regardless of the geographical location of the collection sites, the Cornelian cherry juice samples (S_1 and S_2) had higher antioxidant activity measured by the DPPH test than the pomace (T_1 and T_2). Furthermore, juice from Danilovgrad (S_2) had higher antioxidant activity than juice from Šavnik (S_1), while, pomace T_1 had higher antioxidant potential compared than pomace T_2.

Higher antioxidant activity measured by the FRAP test (Figure 4b) was found in Cornelian

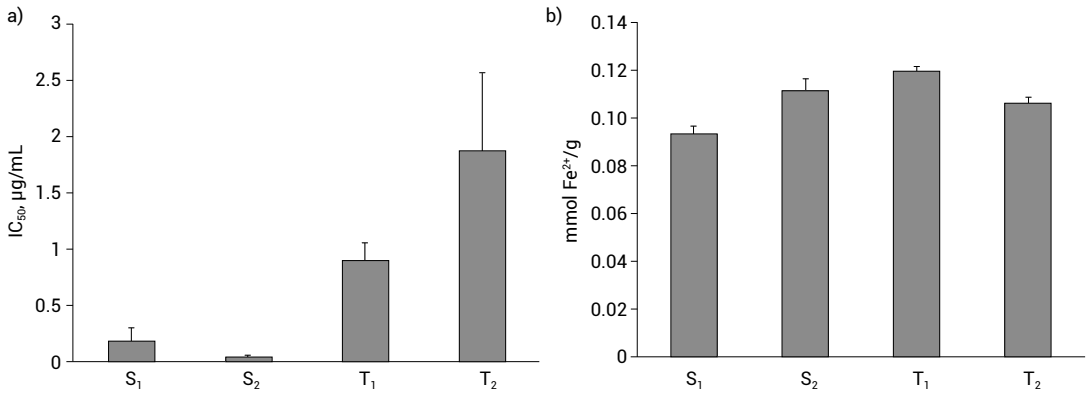

Figure 4. Antioxidant activity in the Cornelian cherry juice and pomace measured by (a) DPPH and (b) FRAP test

cherry juice from Danilovgrad (0.112 mmol Fe^{2+}/g in S_2) than from Šavnik (0.094 mmol Fe^{2+}/g in S_1). On the other hand, pomace T_1 (0.120 mmol Fe^{2+}/g) had higher antioxidant potential than pomace T_2 (0.107 mmol Fe^{2+}/g). De Biaggi found a slightly lower value in Italian Cornelian cherry fruits (0.020 mmol Fe^{2+}/g) (17).

Using the DPPH and FRAP methods, it was observed that Cornelian cherry juice from the central area (S_2) had the highest antioxidant capacity using the DPPH method; while using the FRAP method, the best antioxidant response was obtained from Cornelian cherry pomace from the northern part (T_1) of Montenegro. Antioxidant activity measured by DPPH followed the same trend as the total content of phenolic compounds (TP), while antioxidant activity measured by FRAP followed the same trend as the total content of flavonoids (TF). This is not surprising bearing in mind that the antioxidant activity of phenolic compounds is often attributed to the capacity of scavenging free radicals donating hydrogen atoms, electrons, or chelate metal cations. However, this activity is not only limited to phenolic content. It is necessary to keep in mind that interactions between the phenolics themselves—as well between phenolics and other compounds of the plant matrix—can occur. These interactions can cause a decrease in the antioxidant activity of plant material, but also a synergistic effect can be achieved (47).

Metal Content in Cornelian Cherry Juice, Pomace and Fruits

Although the most attention has been focused on phenolics in relation to the health benefits, investigating the mineral content of different plant parts is crucial, as they are as necessary for the optimal functioning of the human body as essential trace elements. One of their most important roles is as cofactors for enzymes and the reactions constantly occurring in the human body (48). However, minerals can be potentially toxic depending on their concentrations.

We determined the content of eleven elements—macroelements (Mg, Ca, K and Na) and microelements (Fe, Cd, Cu, Mn, Ni, Pb and Zn)—in Cornelian cherry juice, pomace, and fruits (Table 2).

The most abundant essential macroelement in all our Cornelian cherry samples was K. The K concentration in Cornelian cherry juice was 2810.6 mg/kg in S_1 and 2427.8 mg/kg in S_2, while in the pomace was 3276.2 mg/kg in T_1 and 2749.7 mg/kg in T_2. In F_1 and F_2 samples, the K concentration was 3274.2 mg/kg and 2397 mg/kg, respectively. The obtained results were in accordance with the previously reported data for Cornelian cherry. Bijelić found K concentration varied from 1585 mg/kg to 9171 mg/kg in the Cornelian cherry fruits from Serbia (25). Also, Karaaslan noticed similar content of this macroelement (2090.82 mg/kg) in Turkish fruit (23).

The second most abundant essential macroelement in all the Cornelian cherry samples was Ca. The Ca concentration in Cornelian cherry juice was 475.7 mg/kg in S_1 and 361.5 mg/kg in S_2, while in the pomace was 442.5 mg/kg in T_1 and 641.8 mg/kg in T_2. The Ca concentrations in F_1 and F_2 samples were similar (398.05 mg/kg and 366.3 mg/kg, respectively). The obtained Ca content was in accordance with the range recorded by Bijelić in Serbian (from 24.47 mg/kg to 526 mg/kg) (25) and Karaaslan (425.92 mg/kg) in Turkish Cornelian cherry fruits (23).

The Mg concentration in our Cornelian cherry juice was 130.6 mg/kg in S_1 and 97.22 mg/kg in S_2, while pomaces T_1 and T_2 contained 146.5 and 113.3 mg/kg, respectively. The Mg content in F_1 and F_2 samples was 140.6 mg/kg and 95.41 mg/kg, respectively. The obtained Mg amount was similar

Table 2. Macroelement and microelement content in the investigated samples [mg/kg].

	S_1	S_2	T_1	T_2	F_1	F_2
Fe	23.44 ± 2.08	23.53 ± 1.60	33.6 ± 7.10	29.74 ± 0.37	31.7 ± 3.71	37.69 ± 16.97
Zn	5.33 ± 0.48	6.25 ± 0.31	3.32 ± 0.24	3.88 ± 0.18	4.89 ± 0.24	3.14 ± 0.40
Cu	1.59 ± 0.30	1.57 ± 0.31	1.80 ± 0.53	1.50 ± 0.39	1.77 ± 0.22	1.65 ± 0.30
Mn	0.78 ± 0.08	0.60 ± 0.15	0.78 ± 0.23	0.69 ± 0.23	0.78 ± 0.08	0.58 ± 0.05
Ni	-	-	-	-	-	-
Pb	-	-	-	-	-	-
Cd	-	-	-	-	-	-
Ca	475.7 ± 4.39	361.5 ± 0.21	442.5 ± 11.4	641.8 ± 27.8	398.05 ± 8.76	366.3 ± 6.66
Mg	130.6 ± 0.77	97.22 ± 0.24	146.5 ± 2.91	113.3 ± 3.39	140.6 ± 1.66	95.41 ± 0.90
K	2810.6 ± 69	2427.8 ± 49	3276.2 ± 193	2749.7 ± 50	3274.2 ± 53	2397 ± 6.06
Na	11.91 ± 0.63	8.347 ± 1.54	16.59 ± 3.19	9.341 ± 1.99	14.30 ± 1.58	10.01 ± 1.09

to the results obtained by Bijelić for Serbian (from 10.12 mg/kg to 160.90 mg/kg) (25) and Karaaslan for Turkish Cornelian cherry fruits (104.23 mg/kg) (23).

The Na concentration in our Cornelian cherry juice was 11.91 mg/kg in S_1 and 8.347 mg/kg in S_2, while the content of this microelement in pomaces T_1 and T_2 was 16.59 and 9.341 mg/kg, respectively. The Na concentrations in F_1 and F_2 samples were 14.30 mg/kg and 10.01 mg/kg, respectively. The obtained Na concentrations in the Cornelian cherry samples were lower compared to those found by Bijelić (from 40.75 mg/kg to 315.04 mg/kg) (25). Also, Karaaslan recorded higher Na content (2090.82 mg/kg) in Turkish Cornelian cherry (23).

The most abundant essential microelement in all our Cornelian cherry samples was Fe. The Fe concentration in Cornelian cherry juice was 23.44 mg/kg in S_1 and 23.53 mg/kg in S_2, while pomaces T_1 and T_2 contained 33.6 and 29.74 mg/kg, respectively. The Fe concentrations in F_1 and F_2 samples were estimated to be 31.7 mg/kg and 37.69 mg/kg, respectively. The obtained Fe content was higher than the range recorded by Bijelić in Serbian (from 3.10 mg/kg to 9.06 mg/kg) (25) and Karaaslan in Turkish Cornelian cherry fruits (2.78 mg/kg) (23). On the other hand, Randjelovic recorded a significantly higher Fe range (from 123.8 mg/kg to 221.7 mg/kg) in Cornelian cherry from Serbia (49).

The second most abundant essential microelement in all our Cornelian cherry samples was Zn. The Zn concentration in our Cornelian cherry juice was 5.33 mg/kg in S_1 and 6.25 mg/kg in S_2, while T_1 and T_2 was 3.32 and 3.88 mg/kg, respectively. The Zn concentrations in F_1 and F_2 samples were 4.89 mg/kg and 3.14 mg/kg, respectively. Randjelovic recorded a range of Zn from 2.02 mg/kg to 16.54 mg/kg (49), while Bijelić found a range from 0.86 mg/kg to 2.12 mg/kg (25). Karaaslan recorded Zn concentration of 1.34 mg/kg in Turkish Cornelian cherry fruit (23).

The Cu concentration in Cornelian cherry juice was 1.59 mg/kg in S_1 and 1.57 mg/kg in S_2, while in pomace was 1.80 mg/kg in T_1 and 1.50 mg/kg in T_2. The Cu concentrations in F_1 and F_2 samples were 1.77 mg/kg and 1.65 mg/kg, respectively. Bijelić recorded a range of Cu similar to ours (from 0.52 mg/kg to 1.47 mg/kg) (25), while Randjelović recorded slightly higher content (from 2.57 mg/kg to 10.38 mg/kg) (49) in Cornelian cherry fruits.

The Mn concentrations in the investigated samples were 0.78, 0.60, 0.78, and 0.69 mg/kg in S_1, S_2, T_1, and T_2, respectively. The Mn concentrations in F_1 and F_2 samples were 0.78 mg/kg and 0.58 mg/kg, respectively. Bijelić published a range of Mn in Cornelian cherry fruit similar to ours (from 0.24 mg/kg to 1.59 mg/kg) (25), while Randjelović recorded a higher content (from 2.22 mg/kg to 7.99 mg/kg) (49).

Using the ICP-OES method, in all the examined Cornelian cherry samples (S_1, S_2, T_1, T_2, F_1, F_2), the Pb, Cd and Ni concentrations were below the detection limit of the instrument. It is known that Cd and Pb have a high affinity for the thiol groups of enzymes and metal cofactors, which leads to reduced antioxidant enzyme activity (50). Hence, this finding confirmed the safe use of Cornelian cherry fruit, without toxicological risk. Randjelovic also did not detect Ni, Cd and Pb in Cornelian cherry fruits from different areas in Serbia (49).

Furthermore, we noticed that the Cornelian cherry juice and pomace from Šavnik (S_1 and T_1)

had higher amounts of almost all the studied metals (Mg, Na, Fe, Cu, Mn) than the juice and pomace from Danilovgrad (S_2 and T_2). On the other hand, Zn was higher in Cornelian cherry juice and pomace from Danilovgrad (S_2 and T_2). For K and Ca, we observed different distributions. Namely, higher concentrations of both macroelements were recorded in Cornelian cherry juice from the northern area (S_1), while pomace from the same place (T_1) had higher K concentrations but a lower Ca content compared to the corresponding samples from the central area (S_2 and T_2).

Ca, Mg, Na, Cu, Zn, and Mn were higher in Cornelian cherry fruit sample F_1, while K and Fe were higher in fruit sample F_2.

We recorded higher content of almost all the investigated elements in the Cornelian cherry pomace (T_1 and T_2) compared to the juice (S_1 and S_2) regardless of the geographical location of the collection sites.

Dietary Elemental Intake

The calculated RDA and DMI values of essential elements for Cornelian cherry fruits are provided in Table 3. In the case of macroelements, very significant DMI was found for K (7.81% of RDA in F_1 and 5.75% of RDA in F_2). It is known that K takes part in protein synthesis, carbohydrate metabolism, and enzyme activation. K is required for various biochemical and physiological processes in the human body (51).

For Mg, the calculated DMI was 2.38% of RDA in F_1 and 1.79% of RDA in F_2, whereas the calculated DMI for Ca was 2.19% of RDA in F_1 and 1.22% of RDA in F_2. Ca and Mg are necessary for building the skeleton, for nerve and muscle functions, and for cells to function normally (52). For Na, the calculated DMI was 0.117% of RDA in F_1 and 0.081% of RDA in F_2.

In the case of microelements, very significant DMI was found for Fe (10.86% of RDA in F_1 and 12.92% of RDA in F_2). This is an important finding, taking into account that numerous studies have confirmed that in hemoglobin Fe binds to oxygen in the capillaries of the lungs and transports it to cells where the oxygen is released. If Fe levels are low, hemoglobin is not synthesized in sufficient amounts and the oxygen-carrying capacity of red blood cells becomes reduced, resulting in anemia (53).

The calculated DMI for Cu was 8.49% of RDA in F_1 and 7.92% of RDA in F_2, whereas for Zn was 2.346% of RDA in F_1 and 1.506% of RDA in F_2. Zn and Cu reduce oxidative stress by participating in the synthesis of antioxidant enzymes, as well as act as enzyme catalyzers taking part in lipid, carbohydrate, and protein metabolism. They are involved in the synthesis, storage, and release of insulin, which suggests the critical role of this microelement in the progression of type-2 diabetes mellitus, atherosclerosis, and metabolic syndrome (54, 55). Mn is an essential element that is also involved in the synthesis and activation of many enzymes and in the regulation of glucose and lipid metabolism in humans. In addition, Mn is one of the required components for Mn superoxide dismutase (MnSOD), which is mainly responsible for scavenging reactive oxygen species (ROS) in mitochondrial oxidative stress (56). For Mn, the calculated DMI was 1.872% of RDA in F_1 and 1.392% of RDA in F_2.

All the abovementioned elements play essential roles in development, growth, and metabolism, participating in various metabolic processes by acting as cofactors of enzymes or providing structural support to proteins. Deficiencies of these metals impact human health, giving rise to a number of metabolic and neurological disorders.

Table 3. Daily mineral intake [%] of essential elements for Cornelian cherry fruits.

Element	RDA (mg/dan)	DMI (%)	
		F_1	F_2
Fe	14	10.866	12.921
Mn	2	1.872	1.392
Zn	10	2.346	1.506
Cu	1	8.496	7.920
Ca	800	2.388	2.196
Mg	375	1.797	1.221
K	2000	7.857	5.751
Na	575	0.117	0.081

Correlation Between Polyphenolic Compound Content and Mineral Composition and Antioxidant Activity

The goal of the current study was to establish correlations between the chemical composition and antioxidant activity of Cornelian cherry samples (Tables 4, 5, 6).

The correlation between TF content (Table 4) and antioxidant activity measured by the FRAP test was strong (R = 0.96), while the correlation with DPPH assays was very weak (R = -0.05). Numerous studies have confirmed that phenolic compounds contribute significantly to the antioxidant activity of a plant. It increases with the increasing degree of hydroxylation. However, previous findings indicated that different compounds might contribute to antioxidant potential through different mechanisms (28).

The correlation between anthocyanin content (Table 4) and antioxidant activity measured by the DPPH test was strong (R = 0.89), while the correlation with FRAP assays was moderate (R = 0.52). Hence, the very strong correlation found between the identified anthocyanins and DPPH values was not surprising (Table 5). The strongest correlation was for cyanidin-3-*O*-galactoside (R = 0.96), followed by cyanidin-3-*O*-glucoside (R = 0.95) and pelargonin-3-*O*-glucoside (R = 0.94), while delphinidin-3-*O*-glucoside had moderate or weak correlations with both the DPPH and FRAP test (R < 0.7).

Anthocyanins have a higher antioxidant potential compared to other flavonoids, due to their special chemical structure (31). The antioxidant capacity of these compounds can be attributed to chelate metal ions involved in free radical production, thereby reducing metal-induced peroxidation. Additionally, their positive charge, number and position of hydroxyl and methoxy groups, the presence of electron-donating and electron-withdrawing substituents make anthocyanins very effective donors of hydrogen to ROS and free radicals, thereby detoxifying them and preventing further radical formation. This effect protects important biomolecules (proteins, lipids, DNA) from oxidative damage leading to ageing and various diseases (31).

Very weak correlations between TT content (Table 4) and both DPPH and FRAP values in the investigated samples were established (R = - 0.04, R = 0.14, respectively).

These results showed that flavonoids and anthocyanins had strong correlations with the antioxidant activity of Cornelian cherry samples, but the moderate or strong correlations (Table 4) between the total phenol content and both the FRAP and DPPH tests (R = - 0.55 and R = - 0.72, respectively) indicated that in addition to phenolics, there could be other nonphenolic antioxidants contributing to antioxidant potential (57). Namely, this research showed (Table 6) that Ca and Zn had high degrees of correlation with antioxidant activity (R = 0.89 and R = - 0.76, respectively), while Fe and Cu had moderate correlations (R = 0.69 and R = 0.59, respectively). Other macroelements and microelements had moderate weak or very weak correlations with antioxidant activity R < 0.50.

Table 4. Pearson's correlation coefficient (R) between TT, TF, TA and TP and antioxidant activity in the Cornelian cherry samples.

	DPPH	FRAP
TP	R = -0.72	R = -0.55
TF	R = -0.05	R = 0.96
TA	R = 0.89	R = 0.52
TT	R = -0.04	R = 0.14

Table 5. Pearson's correlation coefficient (R) between identified individual anthocyanins and antioxidant activity in the Cornelian cherry samples.

	DPPH	FRAP
Delphinidin-3-*O*-glucoside	R = 0.02	R = 0.57
Cyanidin-3-*O*-galactoside	R = 0.96	R = -0.01
Cyanidin-3-*O*-glucoside	R = 0.95	R = 0.04
Pelargonin-3-*O*-glucoside	R = 0.94	R = 0.04

Table 6. Pearson's correlation coefficient (R) between metals and antioxidant activity in the Cornelian cherry samples.

	DPPH	FRAP
Fe	R = 0.68	R = 0.69
Zn	R = -0.76	R = -0.42
Cu	R = -0.18	R = 0.59
Mn	R = 0.11	R = -0.20
Ca	R = 0.89	R = -0.27
Mg	R = 0.12	R = 0.12
K	R = 0.31	R = 0.35
Na	R = 0.03	R = 0.37

CONCLUSIONS

The results of this research revealed that all the investigated Cornelian cherry samples, regardless of the geographical location of the collection sites, were rich in phenolic compounds and demonstrated good antioxidant activity evaluated by employing two spectrophotometric methods. Using the HPLC method, four anthocyanins in different amounts in the Cornelian cherry juice and pomace were determined. The most abundant was cyanidin-3-*O*-galactoside, present in significant quantities in the investigated samples.

The results of this research revealed that Cornelian cherry samples were rich in all the essential metals. Based on these findings, the Cornelian cherry fruits posed no toxicological risk. The calculated element dietary intakes showed that this fruit could serve as a good dietary source of essential elements, especially Fe (12.92% of RDA), Cu (8.49% of RDA), and K (7.81% of RDA). This is of paramount importance since these essential elements are required for various biochemical and physiological processes in the human body.

These results indicate that, in addition to their traditional use in folk medicine, this plant might represent a valuable source of natural antioxidants, and thus may be considered to have great potential for the food industry, representing feasible alternatives to synthetic additives.

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Conflicts of Interests

The authors declare no conflicts of interests.

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