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# CHEMICAL PROPERTIES AND ANTIOXIDANT CAPACITY OF CORNELIAN CHERRY GENOTYPES GROWN IN CORUH VALLEY OF TURKEY

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**Abstract.** Turkey is rich in terms of cornelian cherry (*Cornus mas* L.) germplasm resources. The country also has traditional cornelian cherry production for a long time. The purpose of this study was to assess the chemical properties, antioxidant activity and anthocyanin content in the fruits of five cornelian cherry genotypes grown in Coruh valley of Turkey. The total phenolics content, total antioxidant activity and the total anthocyanin content (TAC) of cornelian cherry fruit extracts were determined by Folin-Ciocalteu, β-carotene bleaching, and pH-differential method respectively. The individual anthocyanins of cornelian cherry fruits were analyzed by using HPLC. The results showed that three anthocyanins (cyanidin-3-O-rutinozit chloride, delphinidin chloride, peonidin-3-O--glucoside chloride) were found in cornelian cherry fruits. There was a significant difference on total anthocyanin content among genotypes. The highest total anthocyanin content was recorded in genotype 1 (342 mg·100 $ml^{-1}$) whereas genotype 2, 3, 4 and 5 had 276; 271; 239 and 262 mg·100 $ml^{-1}$ total anthocyanin content, respectively. The major anthocyanin in cornelian cherry fruits was cyanidin-3-O-rutinozit chloride followed by delphinidin chloride and peonidin-3-O-glucoside chloride, respectively.

**Key words**: *Cornus mas*, anthocyanins, antioxidant activity, polyphenol, HPLC

## INTRODUCTION

The genus *Cornus* belongs to the family Cornaceae, comprise about 55 species. These plants in general are characterized by brilliant, colorful and attractive flowers and fruits [Yilmaz et al. 2009]. Most species are used as ornamentals and only a few species are grown for their fruits, chief among these is the cornelian cherry (*Cornus mas* L.) [Ercisli et al. 2011].

Cornelian cherry plants range from a shrub to a small tree of about 7–8 m in height, with dark brown branches and greenish twigs. As ornamental, cornelian cherry, with its brilliant leaves and abundant, attractive flowers, is employed with very interesting effect

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in parks and small gardens [Pawlowska et al. 2010]. The fruit is olive shaped, 10–20 mm long, pink, yellow or red external colour. Ripe fruit from cornelian cherry is red and edible directly or dried, but when unripe it is astringent [Yilmaz et al. 2009].

Cornelian cherry fruits have significant amounts of bioactive substances including anthocyanins [Tural and Koca 2008; Rop et al. 2010]. More recently, there has been increased interest by consumers on health benefits of horticultural crops including cornelian cherries. Anticancer, anti-inflammatory and antioxidant effects as well as treatment of diabetes mellitus-related disorders by anthocyanins in cornelian cherry fruits have been reported [Bjorøy et al. 2007]. Anthocyanins, which they are subclass of flavonoids, are polyphenolic pigments. They are responsible for the orange, red, violet, blue and purple colours of many plants [Kong et al. 2003]. Anthocyanins can be used in food, cosmetic and pharmaceutical products. Moreover, attention has been given to the nutraceutical properties of anthocyanins such as their ability of inhibiting free radicals and their capacity of reducing the risk of cardiac diseases and cancer [Tonon et al. 2010]. Anthocyanins have potent antioxidant power and are related to a broad range of beneficial effects in human health and human disease prevention. They can promote healthy vision and dermal health and are proposed to exhibit positive effects on cardiovascular, neuroprotective, anticarcinogenic and antidiabetic properties [Verbeyst et al. 2010]. In Turkey, cornelian cherry fruits are consumed fresh or dried, also used to produce jam, stewed fruit, paste, marmalade, pestil (a locally dried fruit pulp product), syrup, several types of soft drinks and for medicinal purposes [Tural and Koca 2008]. People use cornelian cherry fruit for the medical treatment of gastrointestinal disorder and diarrhea in Turkey as well [Yilmaz et al. 2009]. Extract from the fruits is also used in Europe for cosmetic purposes, replacing synthetic astringent substances, and are claimed to exert a favourable action on the human complexion [Polinicencu et al. 1980].

The purpose of the present study was to determine the total anthocyanins content (TAC) by pH-differential method and determine the antioxidant activity of cornelian cherry fruit from Turkey, by use of β-carotene bleaching assay. Furthermore, the anthocyanins in cornelian cherry fruit extracts were analyzed by high performance liquid chromatography (HPLC).

## MATERIALS AND METHODS

In this study, fruits of five cornelian cherry genotypes belongs to C*ornus mas* L. were used. Commercially ripe fresh fruits were harvested from different mature trees Uzundere town located in Erzurum province of Norteastern Turkey (coordinates: 40º32’11” N 41°32’54” E). All genotypes were grown under the same geographical and cultural conditions. Fruits were transported to the laboratory soon after harvest, where cornelian cherry with defects were discarded. Approximately 8 kg of cornelian cherry fruit was sampled for each genotypes. The fruits were kept at +4°C until analysis. Three replicates were maintained for each analysis.

Total dry matter, total soluble solid (TSS), ash, pH and titratable acidity were determined according to standard AOAC [1995] method. TSS was determined with an Abbe-Zeis refractometer (calibrated using distilled water). Results were reported as °Brix at

21°C. The titrable acidity (TA) was determined by titration to 8.1 with 0.1 M NaOH solution and expressed as g of malic acid per 100 g of juice. The pH measurements were performed using a digital pH meter (ATI ORION 420A) at 20°C. Reducing sugar and colour were determined with the Merck RQflex reflectometer (Merck Company, Darmstadt, Germany) and Minolta CR-400 Chromameter (Minolta Co. Osaka, Japan), respectively.

10 g of the fruit pulp was mixed with 10 ml ethanol and stirred for six hour on a magnetic stirrer. The suspension was filtered through Whatman No. 1 filter paper. Final solutions were used as stock solution for the analysis of antioxidant activity and phenolic compounds and kept at -20º C until analyses.

Total phenolics content in the ethanol extracts of fruits was determined by the Folin–Ciocalteau colorimetric method [Yilmaz et al. 2009] with analytical grade gallic acid as standard. Briefly, one millilitre of the solution (contains 1 mg sample) extract in water was pipetted into a flask. Then 46 ml of distilled water and 1 ml of Folin and Ciocalteu's reagent was added and mixed throughly. The mixture was left to stand for 3 min and 3.0 ml of 2% sodium carbonate were added. After 120 min incubation at ambient temperature with shaking, the resulting absorbance was measured at 760 nm using a spectrophotometer (T60V PG Instruments Ltd). Measurements were carried out in triplicate, the calibration curve was performed with gallic acid, and the results were expressed as μg of gallic acid equivalents per milligram of sample (μg GAE $mg^{-1}$ of sample).

The antioxidant activity in the ethanol extracts of fruits was determined according to the β-carotene bleaching method described by Kaur and Kapoor [2002] with some modifications. Briefly, 4 ml of β-carotene solution (0.1 mg in 1 ml chloroform), 40 mg of linoleic acid and 400 mg of Tween 40 were transferred to a round-bottom flask. The mixture was then evaporated at 50°C by means of a rotary evaporator to remove chloroform. Then, 100 ml of oxygenated distilled water were added slowly to the residue and vigorously agitated to give astable emulsion. Then, 800 μl of extracts were added to 3 ml aliquots of β-carotene/linoleicacid emulsion. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer (T60V PG Instruments Ltd). The mixtures were incubated at 50°C for 100 min. The measurement was carried out at 10 min intervals for 100 min. Water instead of plant extract was used as control. A blank, devoid of β-carotene, was prepared for background subtraction. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as a standard. All samples were assayed in triplicate. Degradation rate (DR) was calculated according to first order kinetics, using the following equation based on:

$$\ln (a/b) \times 1/t = DR_{sample} \text{ or } DR_{standart}$$

Where ln is natural log, $a$ is the initial absorbance (470 nm) at time 0, $b$ is the absorbance (470 nm) at 100 min and $t$ is time. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula:

$$AA = (DR_{control} - DR_{sample \text{ or } standart} / DR_{control}) \times 100$$

For anthocyanin extractions, sample preparation was carried out according to the procedure described by Chandra et al. [2001] with minor modifications. 25 g cornelian cherry pulp was placed into flat-bottom centrifuge tube, then 20 mL portion of 1% HCL/MeOH solution was added. The tube was capped, and the sample was sonicated for 15 min. The sample was vortexed, then centrifuged (Micro 22R Hettich) at 3000 rpm for 10 min at 4°C and the clear supernatant was collected. The residue was further extracted with acidic methanol (3 × 20 mL) and the extracts were collected by centrifugation. The combined supernatants were made up to 100 mL with acidic methanol. The sample was filtered through a 0.45-mm filter (Biocrom MN 718020, Phonex nylon filter 25 mm) prior to injection into the HPLC system.

Total monomeric anthocyanin content was determined according to the pH differential method, as described by Giusti and Wrolstad [2002]. The absorbance (*A*) of the diluted sample was then calculated as follows:

$$A = (A_{\lambda\text{vis-max}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{\lambda\text{vis-max}} - A_{700\text{nm}})_{\text{pH 4.5}}.$$

The monomeric anthocyanin pigment concentration in the original sample was expressed as cyanidin-3-glucoside equivalents according to the following formula:

$$\text{Anthocyanin content (mg L): } = (A \times MW \times DF \times 1000) / (\varepsilon \times 1).$$

Where A is absorbance; MW is molecular weight of cyanidin-3-glucoside (MW = 449.2); *DF* is the dilution factor (DF = 50); 1000 is the factor to convert g to mg and $\varepsilon$ is the molar absorptivity, which equal to 26, 900 for cyanidin-3-glucoside.

Table 1. Anthocyanin fractions evaluated from the chromatograms

| Peak number | Retention time (minute) | Anthocyanin fraction |
|---|---|---|
| 1 | 0.940 | nd[a] |
| 2 | 1.137 | nd[a] |
| 3 | 1.300 | nd[a] |
| 4 | 1.408 | nd[a] |
| 5 | 2.330 | Cyanidin-3-O- rutinozit chloride |
| 6 | 4.626 | Delphinidin chloride |
| 7 | 7.607 | Peonidin 3-*O*-glucoside chloride |

[a] not determinated

The anthocyanin profile was determined by HPLC as described by Pawlowska et al. [2010] with some modifications. A Hewlett Packard 1100 Model HPLC system with isocratic pump and diode array detector was used. Following are the operating conditions used. The column was an Luna C18 5 µm column +guard column with dimensions of 250 × 4.6 mm. The solvents were (A) 0.5% aqueous trifluoroacetic acid (v/v), and (B) water-acetonitrile-lacial acetic acid-trifluoroacetic acid (50:48.5:1.0:0.5, v/v/v/v). The solvent flow rate was 1ml/min, column temperature was 35°C, sample temperature

was ambient and injection volume was 20 µl. Anthocyanins were detected by their absorbance at 525 nm. Anthocyanin peaks were determined by comparing the retention times of the anthocyanin fractions with that of the standards (peonidin 3-*O*-glucoside chloride, delphinidin chloride and cyanidin-3-O- rutinozit chloride) (tab. 1).

Analysis of variance was performed by ANOVA procedures (SPSS 9.0 for Windows). Significant differences between means were determined by Duncan's Multiple Range tests. *P* values < 0.05 were regarded as significant.

## RESULTS AND DISCUSSION

The physicochemical properties of cornelian cherry fruits are given in Table 2. Significant differences ($P < 0.01$) were found among the cornelian cherry genotypes for total dry matter, total soluble solids, pH, titrable acidity, total sugar, *L*, *a* and *b* color value.

Table 2. Chemical properties of cornelian cherry (*Cornus mas* L.) fruits

| Genotype No | Total dry matter (%) | Total soluble solid (%) | Total ash (%) | pH | Titratable acidity (%) | Reducing sugar (%) | External fruit color *L ab* | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 19.63$^{b}$ | 19.50$^{ab}$ | 0.61$^{a}$ | 2.10$^{a}$ | 2.63$^{b}$ | 11.73$^{ab}$ | 23.60$^{d}$ | +25.65$^{c}$ | +11.23$^{c}$ |
| 2 | 17.98$^{c}$ | 16.00$^{c}$ | 0.68$^{a}$ | 2.13$^{a}$ | 2.66$^{b}$ | 11.07$^{b}$ | 28.01$^{a}$ | +27.19$^{b}$ | +11.41$^{c}$ |
| 3 | 19.82$^{b}$ | 17.90$^{bc}$ | 0.67$^{a}$ | 2.02$^{b}$ | 3.21$^{a}$ | 11.04$^{b}$ | 27.03$^{b}$ | +27.21$^{b}$ | +12.28$^{b}$ |
| 4 | 19.84$^{b}$ | 18.50$^{b}$ | 0.80$^{a}$ | 2.14$^{a}$ | 3.17$^{a}$ | 8,64$^{b}$ | 24.02$^{d}$ | +23.22$^{d}$ | +8.74$^{d}$ |
| 5 | 24.34$^{a}$ | 21.00$^{a}$ | 0.81$^{a}$ | 2.11$^{a}$ | 2.82$^{b}$ | 15.24$^{a}$ | 25.10$^{c}$ | +29.27$^{a}$ | +12.93$^{a}$ |

Values in the same column with different lower-case letters are significantly different at $p < 0.01$.

As shown in Table 2, total dry matter content of cornelian cherry genotypes ranged from 17.98 to 24.34%. Total dry matter content are in agreement with those obtained in a previous study on cornelian cherry fruits grown in Turkey [Tural and Koca 2008]. Total soluble solids content ranged between 16.00 and 21.00 °Brix. Our results were coincide with by [Yilmaz et al. 2009] reported between 12.53 and 21.17%. The ash content (%) ranged from 0.61 to 0.81 but the differences weren't statistically significant. The pH values ranged between 2.02 and 2.14. Our results were lower than values observed (3.11–3.53) by Tural and Koca [2008] while our results were in agreement with values (2.50–2.88) reported by Demir and Kalyoncu [2003]. In our study, titrable acidity varied from 2.63 to 3.21% (as malic acid). Similar result were also reported by Yilmaz et al. [2009]. As shown in Table 2, variation in terms of reducing sugar content was observed among the cornelian cherry fruits (8.64–15.24%) and the differences were statistically significant ($P < 0.05$). Previous study has also reported variable ranges of total sugar in *Cornus mas* [Didin et al. 2000]. Color directly affects the appearance and

the consumer acceptability of fruit. As can be seen from Table 2, significant differences among genotypes ($p < 0.01$) were observed for lightness ($L^*$ value), redness ($a^*$ value) and yellowness ($b^*$ value). Significant differences among the cornelian cherry fruits have also been reported [Tural and Koca, 2008] which supports our findings. According to the results, genotype plays an important role in terms of their total dry matter, total soluble solids, pH, titrable acidity and total sugars of the cornelian cherry fruits. Many factors affect the physicochemical properties in fruit including cultivar/genotype, altitude, environmental conditions, etc [Ercisli and Orhan 2008].

Phenolic compounds are plant secondary metabolites and present in food ingredients such as cereals, legumes and vegetables. These sompounds have antioxidant, antimicrobial, anticancer, anti-obesity, antidiabetic, anti-hypertensive and anti-mutagenic properties [Kunyanga et al. 2012].

Table 3 shows the total phenolic content (TPC) of cornelian cherry fruit extracts. The total phenolic contents of cornelian cherries fruits were in the range of 6.53–10.10 μg GAE $mg^{-1}$ fresh sample. Among the studied cornelian cherry genotypes, genotype 5 had the highest total phenolic content (10.09 μg GAE $mg^{-1}$ fresh sample), followed by genotype 1 (8.09 μg GAE $mg^{-1}$ fresh sample), genotype 4 (8.01 μg GAE $mg^{-1}$ fresh sample), genotype 3 (7.64 μg GAE $mg^{-1}$ fresh sample) and genotype 2 (6.50 μg GAE $mg^{-1}$ fresh sample). ANOVA showed significant differences ($p < 0.05$) in total phenolic content among the studied cornelian cherry genotypes. The various factors such as genotype, agronomic practices, maturity level at harvest, postharvest storage, climatic and geographical locations effect the total phenolic content of plant [Kunyanga et al. 2012]. Tural and Koca [2008] showed that the total phenolic contents of cornelian cherries ranged between 2.81 mg $g^{-1}$ and 5.79 mg $g^{-1}$. Total phenolic contents of cornelian cherry genotypes were in the range of 10.97–26.95 mg GAE$g^{-1}$ FW [Hassanpour et al. 2011].

Table 3. Antioxidant activities and total phenolic content of cornelian cherry fruits

| Genotype No | Total antioxidant activity (%) | Total phenolic content μg GAE $mg^{-1}$ fresh sample |
|---|---|---|
| 1 | $85.07^{d}$ | $8.09^{ab}$ |
| 2 | $88.33^{c}$ | $6.53^{b}$ |
| 3 | $94.17^{b}$ | $7.64^{b}$ |
| 4 | $84.68^{d}$ | $8.01^{ab}$ |
| 5 | $88.86^{c}$ | $10.09^{a}$ |
| BHA | $97.96^{a}$ | – |

Values in the same column with different lower-case letters are significantly different at $p < 0.01$

Total antioxidant activity of the five cornelian cherries fruit ethanolic extracts is shown in Table 3. BHA, used as the standard, had a higher antioxidant activity than cornelian cherries fruit extracts. A decrease in absorbance, for all the samples compared with the standard, indicates that all the studied cornelian cherry genotypes possessed lower antioxidant capacity than BHA. Total antioxidant activity of cornelian cherry

genotypes ranged from 84.68 to 94.17%. As shown in Table 3, there were significant differences ($p < 0.01$) among the genotypes. Total antioxidant activity for genotype 3, 5, 2, 1, and 4 were 94.17, 88.86, 88.33, 85.07 and 84.68%, respectively. The results showed that genotype 3 had the highest total antioxidant activity, followed by genotypes 5, 2, 1, and 4. Hassanpour et al. [2011] found that total antioxidant activity of cornelian cherry fruit ranged from 38.98 to 82.37%. The antioxidant effect is mainly due to phenolic compounds, such as flavonoids, phenolic acids, and phenolic diterpenes. Many of these phytochemicals have significant antioxidant activities that may be associated with lower incidence and lower mortality rates of cancer in human. The antioxidant activity of phenolic compounds is due to their redox properties. These components can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Antioxidant compounds can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or produce oxidative chain reactions [Javanmardi et al. 2003].

The contents of the total anthocyanins in the cornelian cherry fruit extracts are presented in Table 4. The results were expressed as cyanidin-3-glucoside equivalents. As shown in Table 4, total anthocyanin content was significantly different among genotypes ($p < 0.01$) and the values ranged from 239 to 342 mg cy-3-glu 100 $ml^{-1}$ FM. Genotype 1 contained significantly higher levels of anthocyanins than other genotypes. The total anthocyanin level in genotype 1, 2, 3, 4, and 5 was 342, 276, 271, 239 and 262 mg 100 $ml^{-1}$, respectively. Similar findings have been published for cornelian cherry fruit grown in Turkey, with anthocyanins values between 1.12 and 2.92 mg $g^{-1}$ [Tural and Koca 2008]. Hassanpour et al. [2011] reported that the content of total anthocyanins of cornelian cherry genotypes ranged from 107 to 442 mg cy-3-glu 100 $g^{-1}$ FW. The results of the current study were in agreement with the previous studies.

Table 4. Total monomeric anthocyanin and individual anthocyanin content of cornelian cherry fruits

| Genotype No | Total anthocyanin (mg 100 $ml^{-1}$) | Delphinidin chloride (mg 100 $ml^{-1}$) | Peonidin-3-O-glucoside chloride (mg 100 $ml^{-1}$) | Cyanidin-3-O- rutinozit chloride (mg 100 $ml^{-1}$) |
|---|---|---|---|---|
| 1 | $342^{a}$ | $9.96^{ab}$ | $6.85^{a}$ | $11.59^{a}$ |
| 2 | $276^{b}$ | $12.37^{a}$ | $8.60^{a}$ | $10.08^{a}$ |
| 3 | $271^{c}$ | $6.98^{bc}$ | $5.92^{a}$ | $8.93^{a}$ |
| 4 | $239^{e}$ | $6.95^{bc}$ | $4.96^{a}$ | $7.07^{a}$ |
| 5 | $262^{d}$ | $4.20^{c}$ | $2.82^{a}$ | $6.83^{a}$ |

Values in the same column with different lower-case letters are significantly different at $p < 0.01$

Three anthocyanins were identified as peonidin-3-O-glucoside chloride, delphinidin chloride, and cyanidin-3-O-rutinozit chloride (tab. 4). The predominant anthocyanins in cornelian cherry fruits was cyanidin-3-O-rutinozit chloride ranged from 6.83 to 11.59 mg 100 $ml^{-1}$ FW and followed by delphinidin chloride, which ranged from 4.20 to

12.37 mg 100 $ml^{-1}$ FW, peonidin-3-O-glucoside chloride, which ranged from 2.82 to 8.60 mg 100 $ml^{-1}$ FW, respectively (tab. 4). Variations were found, between genotypes in anthocyanin composition. As shown in Table 4, cyanidin-3-O-rutinozit chloride content was significantly ($P < 0,05$) different among genotype. Tural and Koca [2008] and Pawlowska et al. [2010] found three anthocyanins in cornelian cherry. The differences in the chemical properties of the fruits, could be due to the growing conditions, such as soil, geographical and environmental conditions, degree of maturity, and genetic factors [Pawlowska et al. 2010]. The major anthocyanins found in fruits are cyanidin, peonidin, petunidin, malvidin, delphinidin and pelargonidin [Leong and Oey 2012]. Anthocyanins are important components in fruit and they can prevent cardiovascular, atherosclerosis and neurodegenerative diseases and cancer [Sun et al. 2009].

## CONCLUSIONS

In the present study, all genotypes of cornelian cherry contained high phenolic compounds and possessed antioxidant activity. The total phenolic content and antioxidant activity varied greatly among cornelian cherry genotypes suggesting that they have beneficial phytochemicals and a natural new sources for human health. Thus, results of the present study supported the antioxidant and nutraceutical potential of this plant species. However, further studies of the antioxidative components of cornelian cherry fruits are required, especially identification and quantification of individual phenolic compounds.

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## CECHY CHEMICZNE ORAZ ZDOLNOŚĆ ANTYOKSYDACYJNA GENOTYPÓW DERENIA JADALNEGO HODOWANYCH W DOLINIE CORUH W TURCJI

**Streszczenie.** Turcja ma bogate zasoby materiału genetycznego derenia jadalnego (*Cornus mas* L.). Kraj ten od dawna produkuje dereń. Celem niniejszego badania była ocena cech chemicznych, działania antyoksydacyjnego oraz zawartości antocyjan w owocach

pięciu genotypów derenia hodowanych w dolinie Coruh w Turcji. Całkowitą zawartość fenoli, całkowite działanie antyoksydacyjne oraz całkowitą zawartość antocyjan (TAC) w ekstrakcie z derenia jadalnego ustalono, odpowiednio, metodą Folina-Ciocalteu'a, metodą BCB oraz spektrofotometryczną metodą różnicową. Poszczególne antycyjany w owocach derenia analizowano przy użyciu HPCL. Wyniki wykazały obecność trzech antocyjan (chlorek 3-O-rutynozydu cyjanidyny, chlorek delfinidyny, chlorek 3-O-glukozydu peonidyny) w owocach derenia. Istniały istotne różnice w całkowitej zawartości antocyjan między genotypami. Największą całkowitą zawartość antocyjan zaobserwowano w genotypie 1 (342 mg·100 $ml^{-1}$) natomiast genotypy 2, 3, 4 i 5 miały, odpowiednio, 276; 271; 239 i 262 mg·100 $ml^{-1}$ całkowitej zawartości antocyjan. Głównym antocyjanem w owocach derenia był chlorek 3-O-rutynozydu cyjanidyny, a następnie chlorek delfinidyny i chlorek 3-O-glukozydu peonidyny.

**Słowa kluczowe**: *Cornus mas,* antocyjany, aktywność antyoksydacyjna, polifenol, HPLC

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