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BIOACTIVE COMPONENTS AND ANTIOXIDANT, ANTIPROLIFERATIVE, AND ANTIHYPERGLYCEMIC ACTIVITIES OF WILD CORNELIAN CHERRY (*CORNUS MAS* L.)

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The contents of polyphenolic components (total polyphenols, flavonoids, and monomeric anthocyanins) and vitamin C, and the bioactive potential (antioxidant, antiproliferative, and antihyperglycemic activities) of wild cornelian cherry were determined. Samples were collected from four different locations in Bosnia and Herzegovina. Sample CC_3 from Drinić had the highest monomeric anthocyanin content (1.40 mg CyGE/g FW) and the highest inhibition of free radicals (IC_{50}^{DPPH} = 262.19 mg/ml; IC_{50}^{ABTS} = 76.78 mg/ml; $IC_{50}^{OH^{\cdot}}$ = 102.31 mg/ml) and inhibition of breast adenocarcinoma cell line growth ($IC_{50}^{MCF\text{-}7}$ = 1.37 mg/ml). Sample CC_4 from Drvar showed the highest total polyphenol (55.92 mg GAE/g DW) and vitamin C (88.74 mg/g FW) contents. Sample CC_4 significantly inhibited the growth of cervix epithelioid carcinoma (IC_{50}^{HeLa} = 0.62 mg/ml) and lung adenocarcinoma (IC_{50}^{A549} = 0.48 mg/ml) cell lines, and α-glucosidase (IC_{50}^{AGHA} = 0.466 mg/ml). Wild cornelian cherry could be used as a functional food with beneficial pro-health properties.

Key words: cornelian cherry; polyphenolic compound; vitamin C; bioactivity

БИОАКТИВНИ КОМПОНЕНТИ И АНТИОКСИДАЦИСКО, АНТИПРОЛИФЕРАТИВНО И АНТИХИПЕРГЛИКЕМИЧНО ДЕЈСТВО НА ДРЕНКИ (*CORNUS MAS L.*)

Определена е содржината на полифенолни состојки (вкупни полифеноли, флавоноиди и мономерни антицијани) и витамин С, како и биоактивниот потенцијал (антиоксидациското, антипролиферативното и антигликемичното дејство) на дренки. Примероците беа собрани од различни локации во Босна и Херцеговина. Примерокот CC_3 од Дриниќ имаше најголема содржина на мономерен антоцијанин (1,40 mg CyGE/g FW) и највисока инхибиција на слободни радикали (IC_{50}^{DPPH} = 262,19 mg/ml; IC_{50}^{ABTS} = 76,78 mg/ml; $IC_{50}^{OH^{\cdot}}$ = 102,31 mg/ml) како и инхибиција врз растот на клетки на аденокарцином на дојка ($IC_{50}^{MCF\text{-}7}$ = 1,37 mg/ml). Примерокот CC_4 од Дрвар покажа највисока содржина на полифеноли (55,92 mg GAE/g DW) и на витамин С (88,74 mg/g FW). Примерокот CC_4 значително го спречуваше растот на цервикалниот епителоиден карцином (IC_{50}^{HeLa} = 0,62 mg/ml) и клетките од белодробниот аденокарцином (IC_{50}^{A549} = 0,48 mg/mL, како и на α-глукозидаза (IC_{50}^{AGHA} = 0,466 mg/ml). Дренките можат да се користат како функционална храна со корисни здравствени својства.

Клучни зборови: дренка; полифенолни соединенија; витамин С; биоактивност

1. INTRODUCTION

Wild fruits, especially berries, are good sources of various components such as biologically active substances. Recently, consumer interest in fruit has been growing due to its pleasant flavor and specific composition [1]. Cornelian cherry (*Cornus mas* L.) is the most important fruit of the family *Cornaceae* and grows mostly in the Balkan Peninsula, central Europe, and southwest Asia [2, 3]. Bosnia and Herzegovina is abundant in wild edible plants. Uncultivated cornelian cherry is widespread in this area and its use for the traditional production of beverages and jams dates back centuries. Many studies indicate the nutritional value and high content of polyphenolic components, organic acids, vitamins and other bioactive compounds in cornelian cherry fruit [4–7]. Vitamin C stimulates the immune system and protects the body from infection. It affects the increased number of lymphocytes important for the formation of antibodies. It is known for its antioxidant activity, keeping ferrous and cuprous ions levels low, and its preventive role in cardiovascular diseases, hypertension, and diabetes [4–7]. Phenolic compounds, as secondary metabolites of plants that protect plant cells against oxidative stress caused mainly by free radicals, are important in the human diet because of their antioxidant properties. All these compounds play a part in the wide range of biological activity of cornelian cherry fruits, such as their antimicrobial, antihyperglycemic, anti-inflammatory, anti-atherosclerotic, antiproliferative, hepatoprotective, cardioprotective, and antioxidant activities [8–10]. Antioxidant activity is a substantial factor in the evaluation of the nutritional value of fruit and is influenced by the content of bioactive components and the mechanism and kinetics of their reactions [11, 12]. Therefore, different antioxidant assays, including DPPH, ABTS, FRAP, PRAC, $OH^{\cdot}$, and $NO^{\cdot}$ radical scavenging activities, are used for cornelian cherry [4–6]. An appropriate level of antioxidants in fruit reduces the risk of cancer. Studies performed *in vitro* and *in vivo* on different human cancer cell lines indicate the antiproliferative activity of cornelian cherry [13–15]. The inhibitory effect of cornelian cherry on the enzyme α-glucosidase, responsible for the hydrolysis of carbohydrates, has been reported [2]. The aim of this study was to determine the contents of polyphenolic compounds and vitamin C in wild cornelian cherry from Bosnia and Herzegovina. In order to investigate the bioactivity of these samples, antioxidant, antiproliferative, and antihyperglycemic activities were determined. The possible contribution of the contents of biologically active compounds to the obtained effects was calculated by Pearson correlation.

2. MATERIALS AND METHODS

2.1. *Plant material*

Wild cornelian cherry (*Cornus mas L.*) fruit samples were collected at the beginning of September 2019 at the stage of full maturity from four different locations in Bosnia and Herzegovina (B&H):

- Ljubomir (sample CC_1: altitude: 666 m; latitude: 42°47′9.09″; longitude: 18°21′54.32″; average temperature: 15 °C; average precipitation: 1600 l/m^2; average number of sunny days: 260).
- Koravlica (sample CC_2: altitude: 556 m; latitude: 42°50′40.28″; longitude: 18°28′16.29″; average temperature: 13 °C; average precipitation: 1700 l/m^2; average number of sunny days: 250).
- Drinić (sample CC_3: altitude: 559 m; latitude: 44°21′53.75″; longitude: 16°22′25.51″; average temperature: 9 °C; average precipitation: 1350 l/m^2; average number of sunny days: 150).
- Drvar (sample CC_4: altitude: 789 m; latitude: 44°31′1.30″; longitude: 16°28′30.71″; average temperature: 11 °C; average precipitation: 1100 l/m^2; average number of sunny days: 160).

2.2. *Preparation of fruit extracts*

Sample extraction was performed according to the standard method described by Jazić *et al.* [16]. Samples were crushed and homogenized after removal of the stones from the fruits. Fruit samples (200 g) were extracted with 250 ml of 80 % ethanol (v/v) using a Soxhlet extractor. After 6 h of extraction, the samples were evaporated to dryness in a rotary vacuum evaporator at temperatures up to 50 °C. The drying of the samples was continued in a vacuum desiccator for 7 days. The obtained extracts were kept at –18 °C until analysis.

2.3. *Total polyphenol, flavonoid, and monomeric anthocyanin contents*

Content of polyphenolic compounds was measured by UV–vis spectrophotometry (Lambda 25, PerkinElmer, USA).

The total polyphenol content (TPC) of extracts was determined according to the Folin-Ciocalteu method, described by Orsavová *et al.*

[12] with some modifications. Folin-Ciocalteu solution (1.5 ml of stock Folin-Ciocalteu solution dissolved with water at 1:10 ratio) and 1.5 ml of 7.5 % $NaHCO_3$ were added to 0.2 ml of diluted extract. The mixture was kept for 30 min in the dark at room temperature, and the absorbance then measured at 765 nm. The results were expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g DW) using a calibration curve ($y = 0.0043x - 0.0295$; $R^2 = 0.9993$).

The total flavonoid content (TFC) was determined according to the method described by Ordoñez *et al*. [17]. A mixture of 2 % ethanolic $AlCl_3$ solution (1 ml) and diluted extract (1 ml) was kept for 30 min in the dark at room temperature. The absorbance was measured at 420 nm.

The results were expressed as mg quercetin equivalent per gram of dry extract (mg QE/g DW) using a calibration curve ($y = 0.0408x - 0.0339$; $R^2 = 0.9981$).

Total monomeric anthocyanins content (TMAC) was determined by the pH differential method described by Popović *et al*. [6]. Sample (10 g) was extracted with HCl/ethanol (85:15 % v/v) for 24 h at 0 °C. The extract was filtered through filter paper and 0.5 ml of extract was mixed with 9.5 ml of KCl buffer, pH 1.0 and 9.5 ml NaOAc buffer, pH 4.5. Absorbance was measured at 510 nm and 700 nm after 15 min incubation at room temperature. The absorbance was calculated as:

$$A = (A_{510} - A_{700})\text{pH}_{1.0} - (A_{510} - A_{700})\text{pH}_{4.5}.$$

The anthocyanin content of each fruit sample was calculated from the following equation:

$$\text{TMAC} = (A \times M \times \text{DF} \times 100/\varepsilon),$$

where A is absorbance, M is the molecular weight (449.2 g/mol), DF is the dilution factor (40), ε is the molar absorptivity of cyanidin-3-glucoside (26,900 l/mol cm). The results were expressed as mg cyanidin-3-glucoside equivalent per gram of fresh weight (mg CyGE/g FW).

2.4. *Vitamin C content*

Vitamin C was determined according to the EN 14130:2003 method and expressed as content of ascorbic acid (the sum of ascorbic acid and its oxidized form dehydroascorbic acid). The extraction of vitamin C from fresh fruit samples (within 24 h of harvesting the fruit) was performed using metaphosphoric acid solution. A reducing solution was used to transform dehydro-L(+) ascorbic acid to L(+) ascorbic acid. Homogenized sample (10 g) was extracted with 100 ml of metaphosphoric acid (20 g/l) and 20 ml of the sample extract solution stabilized with 10 ml of L-cysteine solution (40 g/l). The pH was adjusted to 7.0–7.2 by adding trisodium phosphate solution (200 g/l) and stirring for exactly 5 min. The pH was then reduced to 2.5–2.8 by adding metaphosphoric acid solution (200 g/l). The prepared samples were adjusted to a volume of 50 ml with ultrapure water, homogenized, and filtered through 0.45-μm syringe filters (Macherey-Nagel, 25 mm × 0.45 μm, regenerated cellulose) into the vial. Vitamin C was determined by HPLC (Ultimate 3000, Dionex, Germering, Germany) with a PAD 3000 photodiode array detector and Ascentis RP Amide column (5 μm, 250 × 4.6 mm). Two mobile phases, solvent A (50 mM phosphoric acid) and B (methanol) were eluted in isocratic mode for 10 min for chromatographic separation. The prepared sample (20 μl) was injected into the column at 25 °C. The time of analysis was 10 min, the flow rate 1 ml/min, and the chromatograms were registered at 245 nm. The results were expressed as mg vitamin C per 100 g of fresh weight.

2.5. *HPLC analysis of individual polyphenolic compounds*

Identification and quantification of the polyphenolic compounds in the cornelian cherry extracts was performed by HPLC (Shimadzu Prominence, Shimadzu, Kyoto, Japan), consisting of an LC-20AT binary pump, CTO-20 A thermostat and SIL-20 A automatic dispenser connected to an SPD-20AV UV–vis detector. Chromatograms were recorded at different wavelengths for individual compounds: 280 nm for hydroxybenzoic acids, ellagic acid, catechin, and epicatechin, 320 nm for hydroxycinnamic acids, and 360 nm for flavonols. The separation was performed on a Luna C-18 RP column, 5 μm, 250 × 4.6 mm with a C18 guard column, 4 × 30 mm (both from Phenomenex, Torrance, CA, USA). Two mobile phases, A (acetonitrile) and B (1 % formic acid) were used at flow rates of 1 ml min^{-1} with the following gradient profile: 0–10 min from 10 to 25 % B; 10–20 min linear increase up to 60 % B, and from 20 min to 30 min linear rise up to 70 % B, followed by 10 min return to initial 10 % B with additional 5 min of equilibration time. Polyphenolic compounds were identified by matching the retention time and their spectral characteristics against those of standards. The external standard method was used for quantification. For each compound, a stock solution was

made by weighing accurately standard commercial polyphenolic compounds followed by dissolution in 50 % methanol. Solutions used for calibration were prepared by dilution of the stock solutions. Peak areas of chromatograms were plotted against known concentrations of standards. Equations generated via linear regression were used to establish concentrations of polyphenolic compounds in samples.

2.6. *Antioxidant activity determination*

2.6.1. *DPPH test*

For assessment of antioxidant activity of fruit extracts, evaluation of free radical scavenging effect on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used, according to the method described by Liyana-Pathiranan and Shahidi [18]. DPPH solution in methanol (1 ml, 0.135 mM) was mixed with 1 mL of the sample (solution of extract in methanol at a concentration of 100–500 μg/ml). After stirring, the reaction mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 515 nm by UV–vis spectrophotometry (Lambda 25, PerkinElmer, USA). The antioxidant activity of samples was quoted as IC_{50} (mg/ml) and also as mmol Trolox equivalent per 100 g of dry extract (mmol TE/100 g DW) using a calibration curve ($y = 8.0778x + 1.3959$; $R^2 = 0.9994$).

2.6.2. *ABTS test*

The antioxidant activity of extracts was determinate by ABTS radical cation (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) decolorization assay [19]. ABTS stock solution was prepared freshly by reacting 7 mM ABTS with 2.45 mM potassium persulfate and storing in the dark at room temperature for 16 h. The working solution was obtained by diluting the ABTS radical cation stock solution with methanol to obtain an absorbance of 0.7 ± 0.02 at 734 nm. The working solution was mixed with 1 ml of the sample (solution of extract in methanol at a concentration of 40–200 μg/ml). After stirring, the reaction mixture was left in the dark at room temperature for 6 min. The absorbance was measured at 734 nm by UV–vis spectrophotometry (Lambda 25, PerkinElmer, USA). The antioxidant activity of samples was quoted IC_{50} value (mg/ml) and also as mmol Trolox equivalent per 100 g of dry extract (mmol TE/100 g DW) using a calibration curve ($y = 18.039x + 0.1882$; $R^2 = 0.9977$).

2.6.3. *OH radical neutralization*

The method described by Jazić *et al*. [16] was used for measuring 2-deoxy-D-ribose level degradation under the influence of hydroxyl radical. Hydroxyl radical solution (generated by mixing 10 mM $FeSO_4$, 0.0147% H_2O_2, 0.05 M deoxyribose and 0.067 M phosphate buffer (pH 7.4)) was mixed with 25 μl of the sample (solution of extract in methanol at a concentration of 50–250 μg/ml). Following incubation at 37 °C for 60 min, 0.0372 g/ml EDTA and 1.0 ml TBA were added to the reaction mixture, which was then heated at 100 °C for 10 min. The absorbance was measured at 532 nm by UV–vis spectrophotometry (Lambda 25, PerkinElmer, USA). The antioxidant activity of samples was shown as IC_{50} values (mg/ml) and also as mmol butylated hydroxytoluene equivalent per 100 g of dry extract (mmol BHTE/100 g DW) using a calibration curve ($y = 1.131x + 16.879$; $R^2 = 0.9945$).

2.7. *Antiproliferative activity determination*

2.7.1. *Cell lines and samples preparation*

Colorimetric MTT assay was used for the determination of the antiproliferative activity of cornelian cherry extracts on human cancer cell lines (ATCC, USA): MCF-7 (breast adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), HeLa (cervix epithelioid carcinoma) and A549 (lung adenocarcinoma). Cell lines were grown in a medium containing DMEM (PAA Laboratories GmbH, Pashing, Austria) with 2 mM glutamine, supplemented with 10% heat-inactivated fetal calf serum (PAA Laboratories GmbH, Pashing, Austria), 100 IU/ml of penicillin, and 100 μg/ml of streptomycin (ICN Galenika, Belgrade, Serbia). All investigated cell lines were incubated at 37 °C in culture flasks (Costar, 25 cm^3 in an atmosphere of 100 % humidity and 5 % CO_2 (Heraeus), and subcultured twice per week. Single-cell suspensions were obtained using 0.5 % trypsin (Serva, UK) with 0.1 % EDTA-PBS.

Extracts were dissolved in distilled water and further diluted in DMEM, without fetal calf serum, to obtain five working concentrations (100–2000 μg_{dw}/ml). The final concentrations of extracts were obtained by mixing 10 μl solution of working concentration with 90 μl of culture medium.

2.7.2. *MTT test*

Cell lines were harvested and seeded in quadruplicate 96-well microtiter plates at there

were $5 \cdot 10^3$ cells in 90 µl of medium. Plates with seeded cells were left in a thermostat at 37 °C, with 5 % CO_2 for the next 24 h. At the end of the incubation, 10 µl of cornelian cherry extracts of appropriate concentration was added to all wells except the control (cells in the medium) and the incubation continued under the same conditions for 48 h. The MTT solution ([2,5-diphenyltetrazolium bromide and 3-(4,5-dimethyl thiazolyl)], Sigma, USA), prepared immediately before addition, was added to all wells in the plate at 10 µl/well and incubation continued for the next 3 h (in a thermostat at 37 °C, with 5 % CO_2). After 3 h, 100 µl of 0.04 M HCl in isopropanol was added to each well. The absorbance was read immediately after incubation on a microtiter plate reader (Multiscan, MCC/340, Fisher Scientific, Pittsburgh, USA) at a test wavelength of 540 nm and a reference wavelength of 690 nm. The inhibition rates got measured in accordance with the following formula:

$$RI = (1 - A_s/A_k) \times 100,$$

where RI is rate of inhibition, A_s is the absorbance value of the sample with the extract, and A_k is the control sample's absorbance value.

The antiproliferative activity of samples was shown as IC_{50} values (mg/ml).

2.8. *Antihyperglycemic activity determination*

Antihyperglycemic activity (AHGA), presented as α-glucosidase inhibitory potential, was determined using the method reported by Tumbas Šaponjac *et al*. [20], where each well contained 100 µl of 2 mmol/l 4-nitrophenyl-α-D-glucopyranoside in 10 mmol/l potassium phosphate buffer (pH 7.0) and 20 µl of the sample diluted in the buffer. The reaction was initiated by the addition of 100 µl of the enzyme solution (56.66 mU/ml) and the plates incubated at 37 ºC for 10 min. The absorbance of 4-nitrophenol released from 4-nitrophenyl-α-D-glucopyranoside at 405 nm was measured by UV–vis spectrophotometry (Lambda 25, PerkinElmer, USA). The antihyperglycemic activity is shown as IC_{50} value (mg/ml).

2.8. *Statistical analysis*

All measurements were carried out in triplicate and presented as mean value with standard deviation (SD). All results were subjected to a one-factor analysis of variance (ANOVA). Duncan's test was performed to estimate the significance of differences between mean values at $P < 0.05$, using Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA). The correlation coefficient was assessed by Pearson's correlation coefficient.

3. RESULTS AND DISCUSSION

3.1. *Determination of polyphenolic and vitamin C contents*

Results of total polyphenols, total flavonoids, total monomeric anthocyanins, and vitamin C content in cornelian cherry fruit samples are presented in Table 1.

Sample CC_4 showed statistically higher ($P \leq 0.05$) TPC compared to other samples. The highest TMAC and vitamin C contents were in samples CC_3 and CC_4 with statistically significant differences ($P \leq 0.05$) from samples CC_1 and CC_2.

T a b l e 1

Content of total polyphenol, total flavonoid, total monomeric anthocyanin, and vitamin C contents in cornelian cherry fruit extracts

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TMAC (mg CyGE/g FW)	Vitamin C (mg/100 g FW)
CC_1	49.84 ± 1.76^b	1.47 ± 0.01^a	0.89 ± 0.01^c	50.33 ± 1.56^b
CC_2	47.19 ± 0.85^b	1.03 ± 0.01^d	0.80 ± 0.01^d	49.76 ± 1.20^b
CC_3	50.27 ± 0.19^b	1.21 ± 0.01^b	1.40 ± 0.06^a	87.44 ± 3.28^a
CC_4	55.92 ± 2.88^a	1.19 ± 0.01^c	1.08 ± 0.01^b	88.74 ± 3.59^a

The results are expressed as mean value ($n = 3$) ± SD.
Mean values with different superscript letters in the same column have a statistically significant difference with 95% probability ($P < 0.05$).
TPC – total polyphenol content; TFC – total flavonoid content.
TMAC – total monomeric anthocyanin content.

Higher TPC, TMAC, and vitamin C content were found in samples CC_3 and CC_4 from locations with lower average precipitation, lower average number of sunny days and lower average temperature relative to samples CC_1 and CC_2. Based on these results, it can be assumed that climatic conditions and the regions of growth affect the contents of these components in cornelian cherry. A similar conclusion was reached by Yilmaz *et al*. [23]. Unlike the TPC, TMAC, and vitamin C content, the TFC was the highest in sample CC_1 and the lowest in sample CC_2 (Table 1). Samples CC_3 and CC_4 had higher ($P \leq 0.05$) TFCs than sample CC_2 and lower ($P \leq 0.05$) TFCs than sample CC_1. The results are comparable to those obtained in cornelian cherry from Romania [11]. The TPCs of the investigated samples (Table 1) were lower than the TPC of cornelian cherry fruit from southeast Serbia [21]. A much lower TPC was found in cornelian cherry from Poland [22] and from northern Serbia [2, 6]. These differences can be explained by the use of different solvents and extraction conditions but also by genotypes, geographical region and environmental factors [23]. The differences in TMAC (Table 1) among cornelian cherry samples were significant ($P \leq 0.05$). Similar values for the content of these components were obtained by Tural and Koca [24] and Hassanpour *et al*. [3]. The results of vitamin C content presented in Table 1 are similar to that in cornelian cherry from Montenegro obtained by Martinović and Cavoski [4]. Higher vitamin C content was found in cornelian cherry fruit from east Azerbaijan [3]. Cetkovská *et al*. [25] found that cornelian cherries from Azerbaijan, Greece, and Turkey were richer in vitamin C than the same fruit from other countries, such as Serbia, Slovakia, and the Czech Republic.

3.2. *Determination of individual polyphenolic compounds*

Individual flavan-3-ols catechin and epicatechin were detected in relatively high levels in all cornelian cherry extracts (Table 2). Sample CC_3 had significantly lower ($P \leq 0.05$) content of epicatechin than other samples. The lowest content of catechin was found in sample CC_2. Samples CC_1 and CC_2 had higher content of flavonoids (rutin) than phenolic acids, and samples CC_3 and CC_4 showed an opposite ratio. Samples CC_3 and CC_4 had higher total polyphenolic content (total-PC) than samples CC_1 and CC_2 (Table 2). Contents of ferulic and coumaric acids were significantly higher ($P \leq 0.05$) in samples CC_3 and CC_4, while the contents of elagic acid were significantly higher ($P \leq 0.05$) in samples CC_1 and CC_2. The highest contents of caffeic and chlorogenic acids were found in sample CC_3. Contents of rutin and kaempferol were significantly higher ($P \leq 0.05$) and the contents of quercetin significantly lower ($P \leq 0.05$) in samples CC_1 and CC_2 compared to the other two samples. Drkenda *et al*. [26] found a significant influence of growing region on the contents of individual polyphenolic compounds.

T a b l e 2

Content of individual phenolic compounds (mg/g DW) in cornelian cherry fruit extracts identified and quantified by HPLC

Compound	CC_1	CC_2	CC_3	CC_4
Epicatechin	0.960 ± 0.039^a	0.946 ± 0.041^a	0.557 ± 0.019^b	0.962 ± 0.042^a
Catechin	3.195 ± 0.148^a	2.394 ± 1.183^a	3.862 ± 0.180^a	3.672 ± 1.791^a
Caffeic acid	0.139 ± 0.005^a	0.141 ± 0.031^a	0.151 ± 0.004^a	0.101 ± 0.003^b
Ferulic acid	0.051 ± 0.002^c	0.052 ± 0.002^c	0.243 ± 0.011^a	0.173 ± 0.006^b
Coumaric acid	0.026 ± 0.001^c	0.025 ± 0.001^c	0.032 ± 0.001^b	0.036 ± 0.001^a
Chlorogenic acid	0.077 ± 0.003^b	0.075 ± 0.002^b	0.143 ± 0.003^a	0.040 ± 0.001^c
Elagic acid	0.053 ± 0.001^b	0.081 ± 0.003^a	0.022 ± 0.001^c	0.010 ± 0.001^d
Rutin	0.233 ± 0.009^b	0.250 ± 0.008^a	0.131 ± 0.006^c	0.087 ± 0.003^d
Quercetin	0.036 ± 0.001^c	0.035 ± 0.001^c	0.091 ± 0.004^a	0.050 ± 0.002^b
Kaempferol	0.111 ± 0.005^a	0.113 ± 0.004^a	0.012 ± 0.004^b	0.007 ± 0.001^b
Total-PC	4.882	4.113	5.242	5.138

The results are expressed as mean ($n = 3$) ± SD.
Mean values with different superscript letters in the same row have a statistically significant difference with 95% probability ($P < 0.05$).
Total-PC – total polyphenolic content.

A similar relationship can be seen in this study, and it can be assumed that climatic conditions affect the content of individual polyphenolic compounds in cornelian cherry. Similar flavan-3-ol, phenolic acid and flavonol profiles were described by Moldovan *et al*. [9]. Ellagic acid and kaempferol were detected at lower levels and coumaric acid in a similar range in results published by Blagojević *et al*. [2]. Quercetin was detected in the examined samples in contrast to results published by Blagojević *et al*. [2] for different genotypes of cornelian cherry from Serbia.

3.3. *Antioxidant activity*

The antioxidant activity of the four cornelian cherry fruit extracts was determined by three different methods: DPPH test, ABTS test and OH radical neutralization (Table 3).

All samples showed strong antioxidant activity. The results of antioxidant activity (DPPH test) were in a range similar to results obtained for cornelian cherry from Turkey [24], but higher compared to those from Serbia [2, 21]. Šamec and Piljac-Žegarac [27] obtained lower antioxidant activity (ABTS test) for cornelian cherry from northern Croatia, and Szczepaniak *et al*. [28] obtained higher antioxidant activity for cornelian cherry from Poland, compared to results obtained in this study. Antioxidant activity established by OH radical neutralization in this study is higher according to results presented by Popović *et al*. [6]. Sample CC_3 showed significantly higher ($P \leq 0.05$) antioxidant activity (DPPH, ABTS, and OH radicals) compared to other samples and had the highest chlorogenic acid, caffeic acid, and quercetin contents. The literature data shows that these phenolic compounds are the main active compounds and significantly influence the antioxidant properties of plant material [29].

T a b l e 3

Antioxidant activity of cornelian cherry fruit extracts

Sample	IC_{50} (mg/ml)			TE (mmol/100g DW)		BHTE (mmol/100g DW)
	DPPH	ABTS	$OH^{\cdot}$	DPPH	ABTS	$OH^{\cdot}$
CC_1	289.83 ± 4.3^{b}	83.38 ± 2.18^{c}	183.21 ± 6.58^{b}	6.37 ± 0.07^{a}	7.93 ± 0.15^{d}	83.94 ± 0.45^{a}
CC_2	280.56 ± 1.75^{c}	103.66 ± 0.10^{b}	201.50 ± 5.33^{a}	6.14 ± 0.03^{b}	8.60 ± 0.08^{c}	83.97 ± 0.40^{a}
CC_3	262.19 ± 3.82^{d}	76.78 ± 2.10^{d}	102.31 ± 4.24^{d}	6.36 ± 0.07^{a}	9.44 ± 0.19^{a}	74.71 ± 0.22^{b}
CC_4	309.07 ± 5.55^{a}	109.15 ± 1.03^{a}	121.71 ± 3.01^{c}	5.78 ± 0.08^{c}	9.18 ± 0.07^{b}	72.03 ± 0.34^{c}

The results are expressed as mean value ($n = 3$) ± SD.
Mean values with different superscript letters in the same column indicate a statistically significant difference with 95% probability ($P < 0.05$).
$OH^{\cdot}$ – hydroxyl radical; TE – Trolox equivalent (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).
BHTE – butylated hydroxytoluene equivalent.

Very strong significant correlation ($P \leq 0.05$) was found between DPPT test and OH radical neutralization ($r = 0.978$). Correlation between DPPH test and OH radical neutralization was strong ($r = 0.683$) and weak between ABTS test and OH radical neutralization ($r = 0.132$). Depending on the reaction system, antioxidants may act via multiple mechanisms or by a different single mechanism. No single antioxidant test can reflect precisely all antioxidants in a complex system because of multiple reaction characteristics and mechanisms [6].

3.4. *Antiproliferative and antihyperglycemic activity*

Results of antiproliferative and antihyperglycemic activity are presented in Table 4. Extract CC_3 showed the strongest and CC_2 statistically the weakest antiproliferative activity ($P \leq 0.05$) toward the MCF-7 cell line. Extract CC_4 showed the strongest activity toward the HeLa and A549 cell lines ($P \leq 0.05$).

Extract CC_1 had a lower ($P \leq 0.05$) IC_{50} for the MDA-MB-231 cell line in relation to other samples (Table 4), which indicates its stronger antiproliferative activity. To our knowledge, there are no data for the inhibition of MDA-MB-231 cell line proliferation by cornelian cherry. Lower IC_{50} values were obtained by Yousefi et al. [14] for the MCF-7 and A549 cell lines and by Blagojević *et al*. [30] for the MCF-7 cell line. The potential of cornelian cherry samples shown in this study for antiproliferative activity toward the MCF-7 cell line was higher than that obtained by Tiptiri-

Kourpeti *et al*. [15]. Šavikin *et al*. [13] obtained strong antiproliferative activity toward the HeLa cell line (lower IC_{50} values) by cornelian cherry leaves and flowers. According to Šavikin *et al*. [13], ursolic and gallic acids show the highest activity against HeLa cells. The activity of ellagic acid and rutin was weaker than that of ursolic acid. The lowest contents of ellagic acid and rutin were found in the CC_4 sample, which showed the highest antiproliferative effect toward the HeLa cell line, which is in line with the results from other authors [13]. Studies indicate the connection between the contents of polyphenolic compounds such as total phenols, flavonoids, and anthocyanins and the antiproliferative activity of plant material [1]. Extracts CC_3 and CC_4 had the highest TPCs and TMACs, and CC_1 the highest TFC, compared to other samples (Table 1).

T a b l e 4

Antiproliferative and antihyperglycemic activity of cornelian cherry fruit extracts

Sample	IC_{50} (mg/ml)				
	Antiproliferative activity				Antihyperglycemic activity
	MCF-7	MDA-MB-231	HeLa	A549	
CC_1	1.97 ± 0.09^b	1.01 ± 0.17^b	2.43 ± 0.14^a	0.98 ± 0.03^b	0.600 ± 0.021^b
CC_2	17.71 ± 2.37^a	1.42 ± 0.09^a	0.67 ± 0.03^c	2.03 ± 0.14^a	0.707 ± 0.030^a
CC_3	1.37 ± 0.13^b	1.47 ± 0.12^a	0.94 ± 0.03^b	0.74 ± 0.13^c	0.570 ± 0.019^b
CC_4	1.51 ± 0.20^b	1.49 ± 0.22^a	0.62 ± 0.05^c	0.48 ± 0.11^d	0.466 ± 0.016^c

The results are expressed as mean value ($n = 3$) ± SD.
Mean values with different superscript letters in the same column indicate a statistically significant difference with 95 % probability ($P < 0.05$).

Plant material with a high polyphenol content has a positive effect on the inhibition of α-glucosidase, which is responsible for the breakdown of starch and disaccharides to glucose. Inhibition of α-glucosidase in humans reduces blood glucose levels and protects the body from the risk of type 2 diabetes [31]. Extract CC_4 showed the highest ($P \leq 0.05$) antihyperglycemic activity compared to the other three extracts (Table 4). Higher α-glucosidase inhibition of cornelian cherry from Serbia (IC_{50} = 0.19–0.37 mg/ml) was obtained by Blagojević *et al*. [2]. Fruits, such as berries, rich in anthocyanins are known to be effective α-glucosidase inhibitors. Results for the antihyperglycemic activity of cornelian cherry fruits in this study were in the range reported by Popović *et al*. [1] for the antihyperglycemic activity of blackthorn fruits. In other studies, quercetin was found to be one of the strongest α-glucosidase inhibitors [32, 33], and samples CC_3 and CC_4, with higher antihyperglycemic activities, had higher quercetin contents compared to samples CC_1 and CC_2, which is in line with literature data.

3.5. *Correlations*

The results of correlations (Pearson's correlation coefficient) between the content of polyphenolic compounds and bioactive effects of the investigated cornelian cherry samples are presented in Table 5.

T a b l e 5

Correlations between contents of polyphenolic components and vitamin C and antioxidant, antiproliferative, antihyperglycemic activities

	IC_{50}^{DPPH}	IC_{50}^{ABTS}	$IC_{50}^{OH^\cdot}$	$IC_{50}^{MCF\text{-}7}$	$IC_{50}^{MDA\text{-}MB\text{-}231}$	IC_{50}^{HeLa}	IC_{50}^{A549}	IC_{50}^{AHGA}
TPC	0.562	0.308	**–0.598***	**–0.593***	0.173	–0.227	**–0.767***	**–0.828***
TFC	0.137	–0.561	–0.010	**–0.685***	**–0.706***	**0.915***	–0.488	0.270
TMAC	–0.431	–0.503	**–0.925***	**–0.622***	0.398	–0.249	**–0.648***	–0.508
Vitamin C	0.025	–0.008	**–0.961***	**–0.605***	0.510	–0.510	**–0.769***	**–0.786***

*Significant at $P \leq 0.05$.
Very strong ($r < –0.7$), strong ($r < –0.5$), moderate ($r < –0.3$) and weak ($r > –0.3$) correlations.

TPC had strong (r = –0.598) and TMAC had very strong correlation (r = –0.925) with OH radical neutralization. Vitamin C content also had a very strong correlation (r = –0.961) with antioxidant activity obtained by OH radical neutralization. Pantelidis *et al*. [5] found a similar correlation between hydroxyl radical inhibition and polyphenol and anthocyanin contents, but an opposite correlation with vitamin C content compared to this study. Antioxidant activity (ABTS test) were strongly, but not significantly ($P \geq 0.05$) correlated with TFC (r = –0.561) and TMAC (r = –0.503). An inverse correlation was found between antioxidant activity (DPPH test) and TPC (r = 0.562). A positive correlation between antioxidant activity and TPC has been reported by other authors [3, 6, 21, 25]. Correlation between TMAC and DPPH test (r = –0.431) was not significant ($P \geq 0.05$), which is in accordance with the literature data [3]. Strong significant correlation ($P \leq 0.05$) was found between cell growth inhibition (MCF-7) and phenolic compound and vitamin C contents (Table 5). A very strong correlation was seen between TFC and MDA-MB-231 cell line inhibition (r = –0.706). HeLa cell line inhibition was strongly, but not significantly ($P \geq 0.05$), correlated (r = –0.510) with vitamin C content. An inverse correlation was found between the antiproliferative activity (HeLa cell line) and TFC (r = 0.915), which means that samples with lower TFCs had higher antiproliferative activity. Samples CC_2 and CC_4, with lower TFCs (Table 1), showed a stronger antiproliferative activity toward HeLa cell lines (Table 4) compared to samples CC_1 and CC_3 with higher TFCs. Antiproliferative activity (A549 cell lines) showed significant ($P \leq 0.05$) correlation with TPC (r = –0.767), TMAC (r = –0.648) and vitamin C content (r = –0.769).

Very strong and significant ($P \leq 0.05$) correlation was found between α-glucosidase inhibition TPC (r = –0.828) and vitamin C content (r = –0.786), whereas α-glucosidase inhibition was strongly but not significantly correlated with TMAC.

4. CONCLUSIONS

This investigation showed that the content of polyphenolic components and vitamin C varied among the samples from different growth locations. Results of the antioxidant, antiproliferative, and antihyperglycemic activity determination indicated the high bioactive potential of wild cornelian cherry. Pearson correlation coefficient values indicated a strong impact of total monomeric anthocyanin and vitamin C contents on OH radical neutralization. The growth inhibition of cell line MDA-MB-231 was significantly influenced by the total flavonoid content, and the total polyphenol and vitamin C contents significantly affected the A549 cell line and α-glucosidase inhibition. Wild cornelian cherry could be considered a good source of natural antioxidants with beneficial pro-health properties.

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