Determination of phenolic compounds content and antioxidant capacity of Cornelian cherry (*Cornus mas L.*) and gooseberry (*Ribes uva-crispa L.*)

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Introduction

Cornelian cherry (*Cornus mas L.*) and Gooseberry (*Ribes uva-crispa L.*) belong to the group of wild fruit species that are not commonly used in everyday human diet. However, they are beginning to gain increased importance as they are considered an important source of antioxidants and bioactive compounds. The content of nutritive and non-nutritive compounds (e.g. polyphenols) depends on type of variety, cultivation methods and weather conditions. Today's lifestyle which is full of stress, requires the consumption of food rich in antioxidants that helps the human organism to fight free radicals.

Aims

The aim of the study was to determine the content of phenolic compounds and antioxidant capacity of Cornelian cherry (*Cornus mas L.*) and gooseberry (*Ribes uva-crispa L.*) and to find out whether these neglected fruit species, that naturally grow in wild flora of Bosnia and Herzegovina, can help in the fight against oxidative stress.

Material and methods

In experimental part of this study content of total phenolic (flavonoids and non flavonoids) in investigated samples was determined by means of three spectroscopic methods (Folin-Ciocalteu method, vanilin-HCl method and colorimetric method with aluminum (III) chloride), as well as their antioxidant capacity using different in vitro test (DPPH radical assay, FRAP assay, TEAC assay and total antioxidant capacity according to Prieto et all. The samples were prepared in the same manner: 5 g of the fresh whole fruit was homogenized and extracted with 25 ml of distilled water, filtered and used for the further analysis.
Total phenolic content was determined using Folin-Ciocalteu spectrophotometric method: 0.1 mL of the Folin-Ciocalteu reagent and 1.58 mL of distilled water were added to sample aliquots (20 µL). After 8 minutes 0.3 mL of aqueous sodium bicarbonate solution (20 %) was added and samples were termostated for 30 minutes at 40 0C. The apsorbance was measured at 765 nm and the content of total phenolic was calculated from the previously generated calibration curve (range 100-1000 mg/L TAE) and the results were expressed as TAE (tannic acid equivalent, mg of tannic acid /L or mg of tannic acid/g w.w.).
The same method was used to estimate concentration of flavonoids and non-flavonoids, after addition of 100 mg of PVPP (polyvinyl poly pyrrolidone) to 2 mL of samples.
The total flavonoids content was estimated by using the slightly modified colorimetric method based on formation of metal complexes with Al^{+3}, which give stable complexes with C-4 ketone group and C-3 or C-5 hydroxyl group of flavons or flavanols. Additionally, aluminum chloride forms labile complexes (in acidic medium) with ortho dihydroxyl groups in A or B ring of the flavonoids. Using this analytical method glycolized or non-glycolized flavonoids can be determined.
The proanthocyanidin content in analyzed samples was determined by vanilin-HCl method, and content was expressed as concentration of catechin (hydroxy flavan-3-ol). The results were calculated from the calibration curve (range 20-1000 mg/L) and expressed as mg of catechin/L or mg of catechin/g w.w.

DPPH radical assay

Sample: 50 µl of sample + 2 ml of DPPH solution (concentratiion 5 $\cdot 10^{-5}$ mol/l)
After 16 minutes (960 s) absorbances were measured at 517 nm, and the percentage of inhibition were calculated using equation:

% of inhibition = $[(A_{c.s.\ (0)} - A_{s\ (960s)}) / A_{c.s.\ (0)}] \times 100$

$A_{c.s.\ (0)}$- apsorbance of the control solution (blank) measured after 0 seconds
$A_{s\ (960s)}$ – apsorbance of the sample measured after 960 seconds

After addition of PVPP percentage of inhibition of non-flavonoids was measured and calculated in the same manner as for the samples.
Catechin, ascorbic acid and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as pure standards ($5x10^{-5}$ mol/L). For all the analyzed samples and trolox, effective concentrations (EC_{50}), were calculated and expressed as v/v % (relative to volume of the DPPH solution).

FRAP assay

FRAP reagent :

300 mM acetic buffer pH 3,6
(3,1 g sodium acetate trihydrate + 16 ml of glacial acetic acid, destiled water to 1 L)
10 mM TPTZ in 40 mM HCl
20 mM $FeCl_3$ x 6 H_20

FRAP reagent is mixed just before the use in 10:1:1 ratio.

50 µl of sample is mixed with 1,5 mL of FRAP reagent before measuring the absrobance at 593 nm.

The absorbance is measured in 0 and 4 minutes, and for the calculation the mean value is used.

For the preparation of the calibration curve standard solution of Fe (II) sulfate x7 H_2O is used (concentration range 250-3500 µM, and results were expressed as FRAp values (mM Fe^{+2}) and mmol Fe^{2+}/kg wet weight. Ascorbic acid, catechin and trolox were used as pure standards.

Total antioxidant capacity

This method is based on reduction of Mo(VI) to Mo(V) by the antioxidants, which causes formation of the green phosphate/Mo(V) complex.

The intensity of the colour is measured spectrophotometricaly at 695 nm.

An aliquot of 0.1 mL was mixed with 1 mL of previously prepared reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate), and termostated for 90 minutes at 95 °C.

The absorbance was measured at 695 nm relative to blank (0,3 mL of methanol and 3 mL of reagent solution).

Calibration curve was constructed with the standrad solution of the ascorbic acid in the range 10-500 mg/L.

Results were expressed as ascorbic acid equivalent, e.g. mg ascorbic acid/L and also as mg ascorbic acid/kg wet weight.

TEAC assay

Method is based on generation of the stable radical cation ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Blue/green $ABTS^{*+}$ chromophore is generated in reaction between ABTS and potassium persulfate. Equal aliquotes of 7 mM. ABTS and 2.45 mM $K_2S_2O_8$. were mixed and kept in the dark for 14h, and diluted prior use with ethanol in order to adjust the absorbance at λ=734 nm to 0,7±0,02.

The calibration curve is constructed with the standard ethanolic solution of trolox in the concentration range 0-15 µmol/L.

0.5 mL of each standard or 0.02 mL of sample was mixed with 1 mL of ABTS solution.

The results were expressed as Trolox equivalent (TE, mmol trolox/L) or mmol trolox/kg wet weight.

TE (Trolox equivalent) were calculated using the area under the curve (AUC), were % on inhibition of the absorbance and time were plloted. The absorbance was measured at every 10 second (15 minutes in total).

Results

Table 1. Content of total phenolic compounds, non flavonoids and flavonoids in samples, expressed as mg of tannic acid (TA)L^{-1} or mg TA g^{-1} wet weight (w.w.)

Sample	Total phenolic content*		Non flavonoids*		Flavonoids*	
	mg TA L^{-1}	mg TA g^{-1} w.w.	mg TA L^{-1}	mg TA g^{-1} w.w.	mg TA L^{-1}	mg TA g^{-1} w.w.
1	244.6±2.685	1.223±0.013	191.4±0.895	0.957±0.004	53.17±3.581	0.266±0.018
2	724.7±0.448	3.623±0.002	641.3±1.969	3.207±0.009	83.36±1.522	0.416±0.008

1-gooseberry; 2-cornelian cherry
*results are expressed as mean value ± STD

Table 2. Content of total flavonoids and proanthocyanidins in samples

Sample	Total flavonoids*		Proanthocyanidins content*	
	mg of quercetin mL^{-1}	mg of quercetin g^{-1}w.w.	mg of catechin L^{-1}	mg of catechin g^{-1}w.w.
1	18.16±0.105	0.091±0.001	257.2±1.302	1.286±0.007
2	19.92±0.052	0.100±0.000	480.6±0.434	2.403±0.002

1-gooseberry; 2-cornelian cherry
*-results are expressed as mean value ± STD

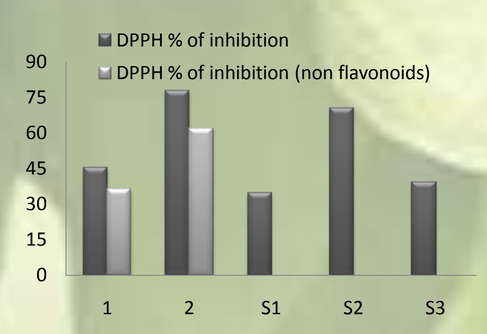

Figure 1. Antioxidant activity of samples determined by DPPH assay (1-gooseberry; 2-cornelian cherry; S1-ascorbic acid; S2-catechin; S3-trolox)

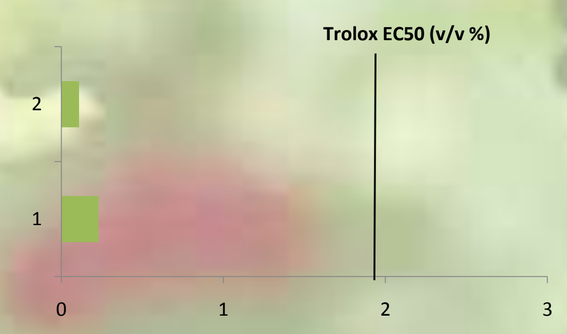

Figure 2. DPPH EC50 (v/v %) of samples (1-gooseberry; 2-cornelian cherry) comparing to EC50 (v/v %) of standard substance (trolox)

Table 3. Total antioxidant capacity, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) of fruit extracts

Sample	Total antioxidant capacity		FRAP		TEAC	
	mg AA L^{-1}	mg AA g^{-1} w.w.	mmol $Fe^{2+}L^{-1}$	mmol Fe^{2+} kg^{-1} w.w.	mmol trolox L^{-1}	mmol trolox kg^{-1} w.w.
1	124.2	0.621	38.89	19.44	10.91	54.53
2	403.7	2.018	79.94	39.97	12.09	60.45
ascorbic acid			24.30			
catechin			27.87			
trolox			28.26			

1-gooseberry; 2-cornelian cherry; AA-ascorbic acid

Conclusions

In general, wide variety of methods for the determination of the phenols and phenolic compounds were presented in this study. As expected, investigated samples showed high content of phenolic, as well as antixodant capacity comparable to pure standards.
Our results emphasized, the need for the more detailed evaluation of the fruit species, than are not consumed frequently in our country, but have obvious antioxidant potential, such as cornelian cherry and gooseberry.