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Original Article

Antioxidant Capacity, Total Phenols, Total Flavonoids and Colour Component of Cornelian Cherry (*Cornus mas* L.) Wild Genotypes

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Abstract

A number of selected cornelian cherry wild genotypes were investigated regarding antioxidant capacity, total phenols, total flavonoids and colour component. The total phenolic, total flavonoids and antioxidant activity of methanol extract of cornelian cherry fruits were determined using Folin-Ciocalteu, colorimetric and DPPH methods, respectively. Total phenolic content ranged from 163.69 (S1) to 359.28 (H2) mg GAE 100 g^{-1} FW. Genotypes H2 and H3 had the highest total phenolic content (359.28 and 343.50 mg GAE 100 g^{-1} FW, respectively), total flavonoids (54.26 and 64.48 mg QE 100 g^{-1} FW, respectively) and antioxidant capacity (2.39 and 2.71 mmolTrolox100g^{-1} FW, respectively). Significant differences of yellow/blue color component (b*), red/ green (a*) color component, values of lightness (L*) and chroma (C) were obtained for extracts from fruits. The present study shows the potential of certain cornelian cherry genotypes as valuable source of natural antioxidants and gene stock for breeding programs.

Keywords: antioxidant activity; cornelian cherry; spontaneous flora; total flavonoids; total phenols

Introduction

Cornus mas L. (cornelian cherry) is a species from *Cornaceae* family, native to southern Europe and south western Asia. In Romania it is part of the spontaneous flora and is found in the forests spread on the plains and hills (Cornescu and Cosmulescu, 2017). Commonly, the most frequent use of cornelian cherry fruits is to produce different drinks, syrups, gels, jams and other traditional products. *C. mas* fruits have been used for centuries as traditional cuisine and folk medicine in various countries of Europe and Asia (Dinda *et al*., 2016). Plants are valuable sources of bioactive compounds with an important role in nutrition and human health. Among the natural compounds, phenols and flavonoids have been found to be an important part of human diet and are considered as active principles in many herbs. There are investigations regarding the chemical properties of cornelian cherry fruits. *C. mas* fruits collected in wild flora are a valuable source of natural antioxidants and deserve attention in preservation of genetic and biological diversity (Bajic-Ljubicic *et al*., 2018). A high number of bioactive compounds have been identified in *C. mas* fruits, among which flavonoids exert favorable health effects, especially by acting as potent antioxidants (Moldovan and David, 2017). Cornelian cherry cultivars have high biological efficiency-antioxidant activity, total phenolic content and content of ascorbic acid (Rop *et al*., 2010; Hassanpour *et al*., 2012). According to Pawlowska *et al*. (2010), it looks like the fruits of cornelian cherry revealed the presence of considerable amounts of flavonoids. Cyanidin-3-rhamnosylgalactoside, cyanidin-3-galactoside and delphinidin-3-galactoside, were isolated and identified from the berries of *C. mas* by Du and Francis (1973). There is a variety of cornelian cherry genotypes with different physical-chemical characteristics. The investigation made by Popovic *et al*. (2012) show that the fruits are a significant source of phenolic compounds and anthocyanins with high antioxidant activity, which varied greatly among the genotypes. Significant variability has also been found in other constituents as well, such as vitamin C, total sugars, organic acid, flesh lipids, linoleic acid in the seed (Brindza *et al*., 2007). In most cornelian cherry fruits, studied by Kucharska *et al*. (2015) differences of origin do affect the quantitative but not the qualitative composition and pelargonidin 3-galactopyranoside was dominant.

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Romania is rich in cornelian cherry populations, and as a result it is important to select spontaneous flora of genotypes with important characteristics, whose commercial cultivation could be successfully practiced. The objective of this research was to examine the antioxidant activity, total phenols and total flavonoids of six cornelian cherry that originate from the spontaneous flora of Romania, in order to identify valuable genotypes as a source of natural antioxidants and gene stock for breeding programs.

Materials and Methods

Materials

Materials were obtained from fruits of six genotypes of cornelian cherry selected from spontaneous flora in the following areas in Romania: Strimba-Jiu (S1) and Calaparu (C1 and C2) in the town of Turceni, Gorj county (44°40'0"N 23°23'0"E) and from the village Hartagani (H1, H2 and H3), Baita village in Hunedoara county (46°01'52"N 22°53'34"E). During 2017, fruits of the above genotypes were picked at the stage of full maturity (consumption ripeness). Each sample consisted of 500 g of fruits per genotype. All samples were frozen at -20 °C until use.

Extraction procedure

Pulp (50 g) from each cultivar have been finely chopped and then homogenized (using an IKA T-25 ULTRA-TURRAX mixer-homogenizer); 2 g of product have been combined with 20 mL methanol, with 2% hydrochloric acid and put in sealed polyethylene flasks; and then were kept for 60 minutes at a temperature of 30 °C under the ultrasounds action.

Determination of total phenol content

The method described by Cosmulescu *et al*. (2015) was used to determine total phenolics content in the extracts. The absorbance of mixture was measured at 765 nm using an Evolution 600 UV/VIS spectrophotometer (Thermo Scientific, USA). Gallic acid was used to prepare the standard curve (0-250 mg/L). Gallic acid was used as reference standard and results were expressed as gallic acid equivalents in milligrams per 100g fresh weight (mg GAE $100g^{-1}$ FW).

Total flavonoids content

Determination of flavonoids was done by aluminum nitrate colorimetric method described by Cosmulescu *et al*. (2015). Quercetin was used for preparing the standard curve (0-100 mg/L). The results were expressed as milligrams of quercetin equivalents per 100g fresh weight (mg QE $100g^{-1}$ FW).

Antioxidant activity

The scavenging capacity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to the method reported by Cosmulescu *et al*. (2015). Antioxidant capacity was expressed in $mmolTrolox100g^{-1}$.

Phenolic compounds

HPLC analyses were performed on a Finningan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA, USA) including a vacuum degasser, a Surveyor Plus LCPMPP pump, a Surveyor Plus ASP autosampler, a PDA5P diode array detector with 5 cm flow cell and a Chrom Quest 4.2 system manager as data processor. Separation was performed using a reversed-phase Hypersil Gold C18 column provided by Thermo Electron Corporation (USA). Chromatographic conditions were similar to those described by Nour *et al*. (2013), Cosmulescu *et al*. (2017). Phenolic compounds were expressed in mg/100g fresh weight (mg $100\ g^{-1}$ FW).

*Measurement of color by the CIEL*a*b* system*

Absorbance of undiluted samples was measured in quartz cuvettes with a path length of 0.1 cm from 350 to 850 nm using an Evolution 600 spectrophotometer (Cosmulescu *et al*., 2014). L* (lightness), a* (red/green colour component), b* (yellow/blue colour component) were determined using the color analysis program VL-ColorCalc. Chroma (C) or saturation and hue angle (H) or tone was determined considering McGuire (1992): $C = (a^{*2}+b^{*2})^{1/2}$; H = arctangent of b^*/a^*.

Statistical analysis

Results were expressed as mean ± standard error of three samples analyzed separately. Statistical comparisons between samples were performed with Duncan t-test for independent observations (using Statgraphics Centurion XVI software). Differences were considered significant at $p < 0.05$.

Results and Discussion

Total phenolics, total flavonoid, individual phenolic compounds and antioxidant capacity

The results obtained have indicated significant differences ($p < 0.05$) in antioxidant capacity, total phenols and total flavonoids content between the genotypes analyzed (Table 1). Total phenolic content ranged from 163.69 (S1) to 359.28 (H2) mg GAE 100 g^{-1} FW. A content of 315.3 mg $100g^{-1}$ FW phenolics was found by Hashempour *et al*. (2010) in cornelian cherry genotypes from Iranian native populations. A variation of the content in total phenolic content was also found by Rop *et al*. (2010) in 12 cultivars of cornelian cherry (2.61 to 8.11 g GAE kg^{-1} FM). These differences may be due to genetic factors and different ability to synthesize secondary metabolites of genotypes. In this study, the content of polyphenols is higher than the content found by Cosmulescu *et al*. (2017) in genotypes previously analyzed. According to the results obtained by Cosmulescu *et al*. (2017), total polyphenols (mg GAE $100g^{-1}$ FW) of cornelian cherry (*Cornus mas*) are lower (184.693), than those of *Rosa canina* (727.292), *Rubus fruticosus* (412.385), *Hippophae rhamnoides* (343.572), *Crataegus monogyna* (203.011), or *Prunus spinosa* (192.602). A content of 2.81-5.79 mg g^{-1} total phenolics was found by Tural and Koca

(2008) in the cornelian cherry fruits. In a study by Pawlowska *et al*. (2010), the fruits of cornelian cherry revealed the presence of considerable amounts of flavonoids, which supports the antioxidant and nutritional potential of this plant species. In our study, the content of flavonoids differs from one genotype to another and the highest content of total flavonoid was determined in H3 genotype (64.48 mg QE $100g^{-1}$) followed by H2 genotype (54.26 mg QE $100g^{-1}$). A high content of total flavonoids (669 mg catechin 100 g^{-1} FW) was found by Hassanpour *et al*. (2011) in the cornelian cherry genotypes of Iran's spontaneous flora. The DPPH radical scavenging effects of cornelian cherry fruit extracts are given in Table 1. Among six cornelian cherry types that have different results, the results have a variation between 1.24 and 2.71 mmolTrolox$100g^{-1}$ FW. The variability of total antioxidant activity was equally high (from 3.30 to 9.54 g AAE kg^{-1} FM) to 12 cornelian cherry genotypes cultivated in Czech Republic (Rop *et al*., 2010). According to Table 1, the highest antiradical activity was found H3 genotype (2.71) followed by H2 (2.39). Popovic *et al*. (2012) believe that cornelian cherry fruits possess high antioxidant activity and that there is a huge variation among *C. mas* genotypes in terms of antioxidant activity. Also, some authors have reported potential antioxidant activity of cornelian cherry leaves and flowers (Stankovic *et al*., 2014; Hassanpour *et al*., 2011; Islamovic *et al*., 2014). Comparing the antioxidant activity of *Cornus* vs *Crataegus* extract, antioxidant activity was found to be significantly higher (1.5-1.7 times) for *Cornus* (Babalica-Petrescu *et al*., 2014). Among the individual phenolic compounds (Table 2), gallic acids were determined in higher amounts (14.49 mg/100g), followed by coumaric acid (13.79 mg/100g), ellagic acid (5.71mg/100g), salicylic acid (1.43 mg/100g), ferulic acid (1.25 mg/100g) and synaptic acid (0.19 mg/100g). Milenkovic-Andjelkovic *et al*. (2015) reported that ellagic acid was predominant phenol acid in cornelian cherry fruit and leaf extracts, followed by chlorogenic and gallic acids. Other authors also reported the presence of pelargonidin 3-O-galactoside, cyanidin 3-O-galactoside and delphinidin 3-O-galactoside (Pyrkosz-Biardzka *et al*., 2014). Also, myricetin (26.54mg/100g) and rutin (3.07 mg/100g) have been determined in cornelian cherry fruit. Rudrapaul *et al*. (2015) reported one new β-hydroxychalcone, 4-acetoxy-5,2′,4′,6′,β-pentahydroxy-3-methoxychalcone, one new flavanone, 7,3′-dihydroxy-5,4′-dimethoxyflavanone and seven known compounds, 2R, 3R-trans-aromadendrin, naringenin-7-O-methylether , myricetin, quercetin-3-O-rutinoside, ursolic acid, gallic acid and d-glucose in methanolic fruit extract of *Cornus mas*. A wide variability was recorded between genotypes within the same phenolic compound. The variation limits were quite high, indicating that the genotype and environmental factors make their mark on fruit composition (Table 2).

*Measurement of color by the CIEL*a*b* system*

To investigate color of the extracts obtained from the fruits of cornelian cherry, lightness and color components were measured. Table 3 presents the results of color evaluation of the fruits extracts using the CIEL*a*b* color system. Significant differences of yellow/blue color component (b*), red/ green (a*) color component, values of lightness (L*) and chroma (C) were obtained for extracts from fruits. The maximum for L (lightness) is 100, representing a perfectly reflecting diffuser and the minimum is zero which represents black. The a* and b* axes have no specific numerical limits, where the positive a* is red and the negative a* is green. In the present study, the a* values were in range of 12.05 to 21.8, which is higher than those reported by Tural and Koca (2008) (6.25 – 15.59). Positive and negative b* are yellow and blue, respectively (Mohebbi *et al*., 2009). Values b* ranged from 10.95, for samples H2, to 4.15, for samples C2. In other studies b* values are in range from 6.64 to 8.12 (Islamovic *et al*., 2014), 1.22 to 11.35 (Drkenda *et al*., 2014) and from 3.46 and 6.64 (Tural and Koca, 2008).

Table 1. Antioxidant activity, total phenolics content and total flavonoids content of wild cornelian cherry fruits

Genotypes	Total phenolic content (mg GAE/ 100 g FW)	Total flavonoid content (mg QE/100 g FW)	Antioxidant activity (mmolTrolox /100g FW)
C1	262.02±2.92^{b}	25.80±0.24^{b}	1.64±0.03^{ab}
C2	299.47±7.86^{c}	33.40±1.87^{c}	2.21±0.10^{c}
H1	171.94±7.44^{a}	13.77±0.14^{a}	1.24±0.01^{a}
H2	359.28±9.57^{d}	54.26±1.67^{d}	2.39±0.13^{c}
H3	343.50±7.33^{d}	64.48±0.81^{de}	2.71±0.05^{d}
S1	163.69±0.04^{a}	12.14±0.01^{a}	1.28±0.01^{a}
Mean	266.65±6.84	33.97±1.74^{c}	1.91±0.25^{ab}

*Data expressed as means ± SE of three samples analyzed separately. * Different superscript letters within the same column indicate significant differences ($P < 0.05$)

Table 2. Descriptive statistics for individual phenolic compounds in wild cornelian cherry fruits genotypes (mg/100 g FW)

Phenolic compounds	Gallic acid	Coumaric acid	Ferulic acid	Synapic acid	Salicylic acid	Rutin	Ellagic acid	Myricetin
Mean	14.49	14.49	1.25	0.19	1.43	3.07	5.71	26.54
Standard Deviation	8.46	12.43	0.89	0.12	0.92	2.68	5.96	3.70
Sample Variance	71.62	154.64	0.80	0.02	0.85	7.22	35.58	13.76
Minimum	5.29	0.42	0.12	0.05	0.40	1.18	0.15	19.80
Maximum	37.17	41.87	2.72	0.43	3.43	10.85	17.47	32.33
Confidence Level(95.0%)	5.68	8.35	0.60	0.08	0.61	1.80	4.00	2.49

Table 3. CIEL*a*b* parameters for three differently extracts in wild cornelian cherry fruits

Genotypes	Parameters				
	L*	a*	b*	C=(a*2+b*2)1/2	Ho
C1	48.8±1.61[a]	13.55±1.95[d]	6.20±0.59[d]	14.9	24.58
C2	44.45±0.69[d]	12.05±1.29[e]	4.15±1.24[e]	12.74	19.00
H1	47.5±1.05[b]	16.65±0.47[c]	7.75±0.30[c]	18.36	24.95
H2	48.45±2.12[a]	21.80±0.89[a]	10.95±0.74[a]	24.39	26.66
H3	46.1±1.35[c]	19.80±0.65[b]	9.15±0.94[b]	21.81	24.80
S1	46.9±0.56[c]	16.60±1.23[c]	7.20±1.03[c]	18.09	23.44

*All data are expressed as average value±standard deviation. Different superscript letters within the same column indicate significant differences ($P < 0.05$) among genotypes.

Table 2 shows that lightness (L*) has values between 44.45 and 48.8. L* is the indicator of lightness-darkness and the higher it is, the lighter the extracts. Indicators a* and b* varies from one genotype to another, the values are positive, thus pointing out a greater contribution of red and yellow components. Together the three parameters lightness (L*), saturation (C*), hue angle or tone (Ho) all contribute tothe color observedin each sample. Intensity or saturation (C) is higher in the H2 genotype (24.39) and hue angle (Ho) at the same genotype (26.66). Hue angle presented values between 19.00-26.66° from red purple (0°) to yellow (90°). A value of 39.7 and 20.7, respectively, was reported by Gunduz *et al*. (2013) to cornelian cherries fruit for chroma (C) and tone (Ho) in the stage of dark red.

Conclusions

C. mas fruits collected in wild flora are a valuable source of natural antioxidants and gene stock for breeding programs. The present study shows the potential value of the cornelian cherry germplasm in Romania.

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