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Analysis of antioxidant activity of different species of wild cherry (*Prunus avium L.*)

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The results of determination of antioxidant capacity of methanolic extract of wild cherry (*Prunus avium L.*), cultivars B and R, are presented in this paper. Content of total phenols determined with modified Folin-Ciocalteu method is 107.56 ± 5.09 mg GAE/g of dry extract for R cultivar and 139.78 ± 3.85 mg GAE/g of dry extract for B cultivar. Content of flavones determined with method to respect to Kumaran and Karunakaran is 22.09 ± 0.2 mg Q/g of dry extract for R cultivar and 9.50 ± .073 mg Q/g of dry extract for B cultivar. Content of flavonoids determined with method to respect to Ordoñez is 51.36 ± 1.18 mg Q/g of dry extract for cultivar R and 48.55 ± 0.31 mg Q/g of dry extract for cultivar B. Total and monomerous anthocyanins content is 13.66 mg/g of fresh sample for cultivar B and 0.5276 mg/g of fresh sample for cultivar R. The results of antioxidant activity of methanolic extract of wild cherry obtained using DPPH, FRAP and ABTS methods show that the significantly larger antioxidant activity of cultivar B compared to cultivar R is probably a consequence of larger content of anthocyanins in cultivar B.

Keywords: wild cherry, *Prunus avium L.,* total phenols*, phlavons, phlavonoids,* anthocyanin, antioxidant activity

PREFACE

It is a well-known fact that reactive oxygen species (ROS), as well as other pro-oxidant species arise as byproducts of very important chemical processes of aerobic organisms and as very reactive intermediates result in oxidant tissue damage. Every type of molecule can be damaged in this way. The build-up of these species result in not only aging of organisms but in serious illnesses as well, e.g. cardiovascular, rheumatic, inflammatory illnesses and cancer.

Protective mechanisms which hindered the occurrence or decompose already occurred pro-oxidant species, repair or replace damaged molecules, have been developed over the course of evolution. In normal conditions, the production of pro-oxidant species is in balance with antioxidant protection of the organism in question. However, under the influence of different endogenous and exogenous factors which affect in stressful manner, this balance can be ruined. This can result in larger production of pro-oxidants and decrease in anti-oxidant protection of organism. This state is called oxidant stress and it is a cause or accompanying factor in pathology of states illnesses (Halliwell et al., 1992; Knight 1995; Bauerova and Bezek 1999; Visioli et al., 2000; Finkel and Holbrook 2000).

Given that human endogenous anti-oxidant system of protection is not adequate to completely prevent excessive production of free reactive oxygenic species and oxidative

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stress, it is necessary to intake additional amount of anti-oxidants.

Lately, following the proof of toxic effect, the usage of synthetic anti-oxidants is being abandoned. As a regular example, synthetic anti-oxidants as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are being stated, known for their ability to stop chain reaction of lipidous peroxidation for which has been found that they are cancerogenous and cause liver damage (Ito et al., 1985). This is the basic reason why the interest in application of natural anti-oxidant is constantly on the rise. Consumption of food rich in natural anti-oxidants is being particularly recommended. In this way, wanted anti-oxidative state is being secured and helps preventing the illnesses in which oxidative stress is the primal cause. Numerous researches that point to positive correlation between consumption of fruit and vegetable and prevention of development of different illnesses are in support of these claims (Kang et al., 2003; Kim et al., 2005; Ruxton et al., 2006; He et al., 2007; Marcason 2007).

It is known that fruit and vegetable, as well as nutrients (Esti et al., 2002), contains a significant amount of phytochemicals as well: phenolic acids (derivate of hydroxybenzoic acid and p-hydroxybenzoic acid), stilbenes, carotenoids, coumarins, chalcones, lignins, flavonoids (fava's, flavonols, dihydroflavonols, anthocynanins) and their glycosides (Mazza and Gao 1995; Prior et al., 1998; Mozetič et al., 2002; Chaovanalikit and Wrolstad 2004; Serrano et al., 2005; Nicoué et al., 2007; Ferretti et al., 2010). It is believed that the biological activity of these compounds is conditioned with their anti-oxidant capacity (Visioli et al., 2000; Finkel and Holbrook 2000; Ito et al., 1985; Kang et al., 2003; Kim et al., 2005), even though the relationship between phytochemicals and their contribution to antioxidant capacity is still not very clear (Shipp et al., 2010).

Other chemical matters in plants (proteins, carbohydrates, vitamins and fibres) also give their contribution to anti-oxidant activity. This claim goes in favour of believes that the relationship between chemical compound of plants and anti-oxidant effects of plants is much more complex, and thus it should not come as a surprise the fact that there are too few literary data that would explain this relationship (Serrano et al., 2005; Melicháčová et al., 2010; Prvulović et al., 2011).

Compared to some other species, cherries contain larger amounts of phytochemicals, polyphenols especially, whose biological activity was studied in few different experimental model. However, there are large deviations in cherries when it comes to different species. Reasons for that are numerous: genetic predispositions, climatic and agro ecological conditions of cultivating, as well as the level of matureness can have an effect to total chemical compound of the yield (Esti et al., 2002; Mazza and Gao 1995 Mozetič et al., 2002; Chaovanalikit and Wrolstad 2004; Nicoué et al., 2007; Prvulović et al., 2011; Ballistreri et al., 2013).

Even though different authors have applied different methodological approaches, results they have obtained for cherries are comparable to results obtained from some other types of fruit (Mazza and Gao 1995; Prior et al., 1998; Nicoué et al., 2007; Heinonen et al., 1998; Rimpapa et al., 2007). However, there are too comparative data on individual components obtained by application of same analytical methods of chemical compound of cherries because there is no fundamental research with which the range of every component in chemical compound of cherries would be determined (Chaovanalikit and Wrolstad 2004).

Taking all this into account, emphasis is placed on samples of wild cherry in this paper, all the more because the genotype of wild cherry has different nutritional values compared to cultivated types, which are relatively larger in darker types of cherries compared to lighter ones.

Today, over 150 different species of wild cherry are known to the world. Literary classification of cherry is not fully carried out. This was the main reason why types (Figure 1. and Figure 2.) named as "cultivar R (cultivar *Red*)" and "cultivar B" (cultivar *Black*) have been examined in the paper.

The goal of this paper was determining the total content of different phenolic compounds and anti-oxidant activity of two types of wild cherries coming from the same habitat and having the same ripening time and the comparison of results obtained.

MATERIALS AND WORKING METHODS

Chemicals

All chemicals used in this work are of p.a. purity: *Folin-Ciocalteu,* 2, 2-diphenyl-1-picrylhydrazyl, 2,4,6-Tri(2-pyridinyl)-1,3,5-triazine and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (Sigma-Aldrich, St. Louis, USA); gallic acid, aluminium chloride and quercetin hydrate (Acros Organics, New Jersay, USA); sodium hydrogen carbonate and sodium acetate-3-hydrate, methanol, hydrochloric acid, iron sulfate-7-hydrate and sodium acetate (Lach-Ner, s.r.o., Czech Republic); 96% ethanol (Vrenje a.d., Beograd, Serbia), acetic acid (MP Hemija d.o.o., Beograd, Serbia); potassium chloride (Alkaloid, Skopje, FYRM) and potassium persulfate (Merck, Darmstadt, Germany).

Equipment

Scale (Mettler H54AR), hand blender (Bosch MSM7150), ultrasonic bath (U300, Ultrawave Limited), magnetic stirrer

Figure 1. *Prunus avium L,cultivar B*

Figure 2. *Prunus avium L,cultivar R*

(ARE, Velp Scientifica), vortex (Velp Scientifica), rotary evaporator (Devarot, Elektromedicina), spectrophotometers (Jenway 6315 and Milton Roy Spectronic 1201).

Sample preparation

Samples of two wild cherries needed for this paper were picked in full consumption ripeness on 6th of July 2013 in Romanovci, municipality Laktaši, Bosnia and Herzegovina. They were transported to the laboratory being protected from the sunlight and in the laboratory they were store in the dark on 2-4°C during 48hours.

Seeds were removed from the fruit and then 40*g* of seedless samples were mashed using hand blender.

Polyphenols extraction

Fresh and mashed samples (40*g*) were extracted into 160*mL* of 80% methanol (*v/v*), treated during 3x10 minutes in ultrasonic bath and 30 more minutes in magnetic stirrer.

Filtrates obtained using *Büchner* funnel on vacuum pump were diluted with 80% methanol of total volume of 250*mL* and stored in dark on 2-4°C during 24 hours. Samples prepared using this method were steamed until dry on rotary evaporator on 40°C under lower pressure and the rest was dried during next 3 days in darkness and under vacuum.

Solution of convenient concentration were prepared in 70% methanol using the dried extracts.

Determination of total phenols

Content of total phenols in extracts was determined using modified *Folin-Ciocalteu* method (27) which is based on coloured reaction of phenols with *Folin-Ciocalteu* reagent. Aliquot (0.2*mL*) of diluted extract (100 *µg*/*mL*) is mixed with 1.5*mL* of *Folin-Ciocalteu* reagent (previously diluted with water 1:10, *v/v*) and 1.5*mL* of $NaHCO_3$ (75 *g*/*L*). Obtained mixture is stirred vigorously using vortex and is left during 30 minutes in dark on room temperature. Absorbency is read using spectrophotometer on 765*nm*.

Standard diagram is obtained in the same way with a difference that instead of sample solution, an aliquot (0.2*mL*) of gallic acid (50-250 *µg*/*mL*) in a mixture of water and methanol (50:50, *v/v*) is taken, and the results are presented graphically.

Results are expressed as total phenols equivalent to gallic acid, *mg* GAE/*g* of extract, using obtained equation of calibration curve of gallic acid: y=0.003x-0.0226 (R^2=0.9975), where y is the absorbency and x is corresponding concentration of gallic acid.

Determination of flavonols

Content of flavonol in extract is determined using method developed by *Kumaran* and *Karunakaran* (Kumaran and Joel 2007).

Aliquot (1 *mL*) extract solution (500 *µg*/*mL*) is mixed with 1 *mL* of 2% solution of $AlCl_3$ in pure ethanol and with 1.5 *mL* of Na-acetate (50 *g*/*L*), stirred vigorously using vortex and left during 2.5 hours in dark and on room temperature. Absorbency is read using spectrophotometer on 440*nm*.

Standard diagram is obtained in the same way with a difference that instead of solution, an aliquot (1 *mL*) of solution of quercetin hydrate (20-100 *µg*/*mL*) in methanol and the results are presented graphically.

Results are expressed as flavones equivalent to quercetin, *mg* Q/*g* of extract, using obtained equation of calibration curve of quercetin: y=0.0214x+0.004 (R^2=0.9993), where y is the absorbency and x is corresponding concentration of quercetin.

Determination of flavonoids

Content of flavonoids in extract is determined using Ordoñez method (Ordoñez et al., 2006).

Aliquot (1 *mL*) extract solution (500 *μg*/*mL*) is mixed with 1 *mL* of 2% solution of $AlCl_3$ in pure ethanol, stirred vigorously using vortex and left during 1 hour in dark and on room temperature. Absorbency is read using spectrophotometer on 420 *nm*.

Standard diagram is obtained in the same way with a difference that instead of solution, an aliquot (1 *mL*) of solution of quercetin hydrate (20-100 *μg*/*mL*) in methanol and the results are presented graphically.

Results are expressed as flavonoids equivalent to quercetin, *mg* Q*/g* of extract, using obtained equation of calibration curve of quercetin: y=0.0368x-0.135 (R^2=0.9996), where y is the absorbency and x is corresponding concentration of quercetin.

Determination of total and monomerous anthocyanins

Quantitative determination of total and monomerous anthocyanins is based on a property of anthocyanins which change their structure reversibly when the pH value of the medium is changed, wherein absorbing spectrum is changed.

Total and monomerous anthocyanin content is determined spectrophotometrically, using modified "single" pH and differential pH method developed by *Sun* and associates (Sun et al., 1998). Fresh sample (20 *g*) is extracted into 20 *mL* extracting solution (85 *mL* of 95% ethanol + 15 *mL* 1.5 *mol/L* HCl) during 24 hours on 0°C temperature, after which the obtained extracts are profiltered.

To every 0.5 *mL* of obtained extract (1 *g/mL*) is to be added 9.5 *mL* of pH 1 buffer (400 *mL* 1 *mol*/*L* CH_3COONa + 240 *mL* 1 *mol*/*L* HCl + 360 *mL* deionised H_2O). After 15 minutes read absorbency is read using spectrophotometer on 500 *nm* and 700 *nm* on both buffers.

Concentration of total and monomerous anthocyanins in the extract is calculated using formulas [1], [2], [3] and [4]:

$$A_{tot} = (A_{500} - A_{700})_{pH_1} \quad [1]$$

$$A_{mon} = (A_{500} - A_{700})_{pH_1} - (A_{500} - A_{700})_{pH_2} \quad [2]$$

$$c_{tot}\left(\frac{mg}{L}\right) = \frac{A_{tot} \times M \times R \times 1000}{\varepsilon \times l} \quad [3]$$

$$c_{mon}\left(\frac{mg}{L}\right) = \frac{A_{mon} \times M \times R \times 1000}{\varepsilon \times l} \quad [4]$$

where:

M = 449.2 *g*/*mol* (molecule mass of cyanidin 3-glucoside),
R = 20 (dilution factor),
ε = 26900 $Lcm^{-1}mol^{1}$ (molar coefficient of absorption of cyanidin 3-glucoside),
l = 1 *cm* (thickness of cuvette).

Based on calculated concentrations of total and monomerous anthocyanins ([3], [4]), content of anthocyanins is to be expressed as equivalent of cyanidin 3-glucoside, *mg* cyanidin 3-glucoside/*g* of fresh sample.
Degradation index is determined as concentration ratio of total and monomerous anthocyanins using a formula [5]:

$$ID = \frac{c_{tot}}{c_{mon}} \quad [5]$$

Determination of antioxidant activity

FRAP method

Total antioxidant activity was determined using modified FRAP (*Ferric Reducing Antioxidant Power*) method based on reduction of Fe^{3+} ions to Fe^{2+} ions in the presence of antioxidants. Resulting Fe^{2+} in the presence of TPTZ reagent (2, 4, 6 tri (2-pyridine)-S-triazine) create coloured complex which attains absorption maximum at 593 *nm*. (Benzie and Strain 1996).

Prepared content: 300 *mmol/L* pH 3.6 acetate buffer (3.1 g CH3COONa + 16 mL CH3OOH + distilled water until total volume of 1 *L*), 10 *mmol/L* solution of TPTZ reagent in 40 *mmol/L* HCl and 20 *mmol/L* solution of FeCl3·6H2O.

FRAP working solution is obtained my mixing of 25 *mL* of acetate buffer, 2.5 *mL* of TPTZ reagent solution and 2.5 *mL* of FeCl3·6H2O solution.

Aliquot (0.2 *mL*) of extract solution (100 *μg*/*mL*, 1000 *μg*/*mL*) is mixed with 1.8 *mL* of FRAP working solution, stirred vigorously with magnetic stirred and left to incubate during 10 minutes on room temperature of 37°C. Absorbency is read using a spectrophotometer on 593 *nm*.

Standard diagram is obtained in the same way with a difference that instead of solution, an aliquot (0.2 *mL*) of solution of $FeSO_4 \cdot 7H_2O$ (0.2-1.0 *μmol*/*L*), and the results are presented graphically.

Results are expressed as flavonoids equivalent to *mmol*/*L* Fe(II)/*g* of extract, using obtained equation of calibration curve of $FeSO_4 \cdot 7H_2O$: *y=3.1038x+0.0495 (R^2=0.9665),* where y is the absorbency and x is corresponding concentration of $FeSO_4 \cdot 7H_2O$.

Obtained results for extracts of wild cherry are compared to common compounds: BHA and BHT.

DPPH method

DPPH method is based on examination of the ability of antioxidants to neutralize DPPH radical. Transformation of violet coloured, stable DPPH radical (1, 1-diphenyl-2-pycrylhydrazine) into a reduced, yellow coloured form of

DPPH-H is being observed at the same time. (Liyana-Pathiranana et al., 2005).

Aliquot (1 *mL*) of extract solution (100-2000 *μg/mL*) is mixed with 1 *mL* of formerly prepared solution of DPPH radical (0.135 *mmol/L*) in pure methanol. Obtained mixture is stirred vigorously using a vortex and is left during 30 minutes in dark on a room temperature. Absorbency is read using spectrophotometer on 517 *nm*.

Shutdown power of DHHP radical is calculated using following formula:

$$I_{\%} = \frac{A_{control} \times A_{sample}}{A_{control}}(100) \qquad [6]$$

where:

I% - percentage of inhibition of DPPH radical
$A_{control}$ – absorbency of mixture of solution of DPPH radical (1 *mL*) and methanol (1 *mL*)
A_{sample} – absorbency of mixture of solution of DPPH radical (1 *mL*) and extract solution (1 *mL*)
Obtained results for inhibition percentage of DPPH radical are presented using a diagram of I% in function of concentration of extract solution. From the plotted diagram, IC_{50} is determined graphically.IC_{50} is solution concentration which inhibits 50% of free DPPH radical (*μg/mL*). Based on IC_{50} value, the antioxidant activity index (AAI) is determined and is calculated using the following formula:

$$AAI = \frac{c_{DPPH}^{final}}{IC_{50}} \qquad [7]$$

where:

c_{DPPH}^{final} - final concentration of DPPH radical in measure cuvette (μg/ml)
Values of IC_{50} and AAI for wild cherry are compared to values of IC_{50} and AAI of common compounds: BHA and BHT.

ABTS method

To determine antioxidant capacity the ABTS test is uses modified method developed by *Re* and associates (Re and Pellegrini 1999).

ABTS method is based on oxidation of ABTS reagent (2, 2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) of diammonium salt) using potassium persulfate during which green-blue ABTS radical cation ($ABTS^+$) is created, which is reduced using antioxidant. With this reduction $ABTS^+$ is transformed back to colourless ABTS form.

Formerly prepared 7 *mmol/L* solution of ABTS reagent and 2.4 *mmol/L* solution of potassium persulfate are mixed in equivalent ratio (1:1, *v/v*) and are left to react in dark on room temperature during 12 hours.

1 *mL* of reacted mixture (solution of $ABTS^+$ radical) is diluted with 60 *mL* of methanol and absorbency is read using spectrophotometer on 734 *nm*, which has to be 0.706 ± 0.001.

Solution having absorbency 0.706 ± 0.001 will be called a working solution of $ABTS^+$ in the following part of the manuscript.

Aliqout (1 *mL*) of extract solution (1-800 *μg/mL*) is mixed with 1 *mL* of $ABTS^+$ working solution and after 7 minutes the absorbency is read using a spectrophotometer on 734 *nm*.

Shutdown power of $ABTS^+$ radical is calculated using the following formula:

$$I_{\%} = \frac{A_{control} \times A_{sample}}{A_{control}}(100) \qquad [8]$$

where:
I% - percentage of inhibition of $ABTS^+$ radical
$A_{control}$ – absorbency of mixture of solution of $ABTS^+$ radical (1 *mL*) and methanol (1 *mL*)
A_{sample} – absorbency of mixture of solution of $ABTS^+$ radical (1 *mL*) and extract solution (1 *mL*)
Obtained results for inhibition percentage are presented using I% diagram in function of concentration of extract solution. From the plotted diagram, IC_{50} is determined graphically.IC_{50} is solution concentration which inhibits 50% of free $ABTS^+$ radical. Values of IC_{50} for wild cherry are compared to values of IC_{50} of common compounds: BHA and BHT.

RESULTS AND DISCUSSION

Determination of total phenols, flavonols, flavonoids and anthocyanins

Total phenols, flavones flavonoids were determined in dry methanol extract of wild cherries whose seeds were removed and total anthocyanin content was determined in fresh wild cherries also seedless. Results are presented in tables 1. and 2.

The results show that total phenols content in wild cherries is significant and that it is somewhat larger in cultivar B (139.78 mg GAE/g $_{\text{of dry extract}}$) than in cultivar R (107.56 mg GAE/g $_{\text{of dry extract}}$). On the other side, total flavonol and flavonoid content is larger in cultivar R 22.09 and 51.36 mgQ/ g $_{\text{of dry extract}}$) than in cultivar B (9.50 and 48.55 mgQ/ g $_{\text{of dry extract}}$) respectively.

Results for total phenols are presented in table 1. in accordance with literary ones. Namely, total phenol content in different genotypes of cherries are in wide range, ranging from 0.41 to 13.09 mg GAE/gdw (Kim et al., 2005; Nicoué et al., 2007; Prvulović et al., 2011; Serra et al., 2011). This is in accordance with earlier work which

Table 1. Total phenols, flavones and phlavonoids content in wild cherry extract

	Cultivar B	Cultivar R	
Total phenols	139.78 ± 3.85*	107.56 ± 5.09	(*mg GAE****/*g* of dry extract)
Flavonols	9.50 ± 0.73	22.09 ± 0.22	(*mg Q*****/*g* of dry extract)
Flavonoids	48.55 ± 0.31	51.36 ± 1.18	(*mg Q*****/*g* of dry extract)

* Average value in three different meas. ± st. dev, ** Galic acid, *** quercetin

Table 2. Total and monomerous anthocyanins content in wild cherry fruits without seed (FW)

	Cultivar B	Cultivar R	
Anthocyanins**	13.66	0.528	*mg(C3G*) /*g_{FW}
Total anthocyanins **	683.32	26.38	*mg(C3G)/L*
Monomerous anthocyanins **	579.77	12.36	*mg(C3G)/L*
Degradation index	1.191	2.134	

*C3G – cyanidin-3-glucoside, ** average value of two different meas.

indicated that the difference in total phenol content in different genotypes of cherries, of the same age and growing under identical ecological conditions was conditioned by genetic variations (Serrano et al., 2005; Gonzales-Gomez et al., 2010).

Phenols are bioactive components that are most concentrated in cherry scarfskin, and also in meat and dimple. They contribute to sensory and organoleptic properties of fruit as well as to taste and astringency. (Ferretti et al., 2010). Likewise, it is known that in plants, phenols have a defensive role, e.g. to neutralise reactive oxygen compounds and in that way to prevent degradation of biomolecules and it is know that they have antimicrobial and anti-inflammatory activity. (Prvulović et al., 2011).

It was determined that total flavonoid content also depends on different genotypes. Flavonoids are present in many plants and represent active components being used in medicine (Cooper-Driver 2001). In literature, total flavonoid content is in range from 0.42 to 1.66 mg of routines /gdw (Prvulović et al., 2011), Flavonoids are known to reduce oxidative stress in biological conditions due to their antioxidant capacity (Kim et al., 2005). Many flavonoids are present in nature in form of O- or C-glycosides. Glycosylation of flavonoids is significant because it reduces its reactivity and increases solubility in water which in return prevents their degradation in cytoplasm and provides a possibility in vacuoles. (Cuyckenes and Claeys 2005), The reason of reactivity reduction is most probably due to glycoside part changing coplanarity of flavonoid molecule and reduces the possibility of electron delocalisation which induces reduction of antioxidant activity of flavonoids. (Heim et al., 2002).

From table 2 can be seen that anthocyanin content in wild cherry denoted as cultivar B (total anthocyanin and monomerous anthocyanins content, 13.66 mg C3G/gfw or 683.32 and 578.77 C3G/L) far larger than in cultivar R(0.528 mg C3G/gfw or 26.38 and 12.36 C3G/L).

These results are in accordance with literary results obtained where it was determined that the largest anthocyanin content is present in indigenous cherries. (Gonzales-Gomez et al., 2010).

Anthocyanin content is ranging in wide range in different cherry genotypes and with different authors. Some authors say that the anthocyanin concentration in various cherries ranges from 0.028 to 0.63 gC3G/gfw (Mozetič et al., 2002), while other authors say that that range is from 0.01 to 1.09. (Ferretti et al., 2010).

Anthocyanins are one of the most important flavonoid groups and are widespread in nature, in various flowers, fruits and vegetables and give it its intensive colour. (Mazza and Gao 1995), Anthocyanin build-up is closely related to fruit ripeness and its colour. (Serradilla et al., 2011). Fruit ripeness is followed by change from starting

Table 3. Determination of antioxidant capacity of methanolic extract of wild cherry

	FRAP $\frac{mmolFe(II)}{g(dryextract)}$	DPPH		ABTS IC_{50} (µg/mL)
		IC_{50} (µg/mL)	AAI	
Cultivar B	**7.95 ± 0.85**	**224.56± 11.5**	**0.11**	**4.99±0.20**
Cultivar R	**0.54 ± 0.106**	**1073.12 ± 36.12**	**0.024**	**48.64± 0.80**
BHA	**147.28 ± 13.87**	**6.38 ± 0.38**	**3.96**	**1.88±0.08**
BHT	**16.64 ± 0.30**	**23.16 ±0.102**	**1.15**	**7.21±0.16**

green to red, violet or black like colour, which is a consequence of anthocyanin build-up and chlorophyll degradation. (Serrano et al., 2005), Cherry colour usually depends on anthocyanin concentration and distribution in scarfskin. (Esti et al., 2002).

Antioxidant capacity determination

One method is not enough for precise determination of antioxidant activity because many factor influence the determination. Also, it is necessary to perform multiple measurements and to take into account different mechanisms of antioxidant activity. Antioxidant activity of methanolic extracts of wild cherry was determined using ABTS, FRAP and DPPH tests and the results were compared to referent compounds such as BHA and BHT. (Table 3)

ABTS test is a common one in determination of fruit extract antioxidant activity. Extracts of wild cherries have a capability of inhibition of ABTS radicals and this was used for determination of their antioxidant activity. Activity is presented in form of IC_{50}, or concentration of wild cherry extract needed for inhibition of 50% of present stable radical, Results imply that cultivar B shows significantly higher antioxidant activity, only 2.6 times lower activity than BHT and 1.44 times higher activity than BHA. On the other side, cultivar R showed significantly lower activity (25.8 times and 6.7 times lower than BHA and BHT respectively. Antioxidant activities differ mutually and cultivar B has 10 times higher activity than cultivar R.

Total antioxidant capacity, determined using FRAP method, showed relatively good activity of wild cherries compared to reference compound, provided that cultivar B has 15 times stronger effect than cultivar R.

These results are in accordance with literary data where different authors found large content of anthocyanins and high antioxidant activity in certain cheery genotypes. (17, 23, 36, 41).

Towards stable DPPH radical, wild cherries show quite weak effect. IC_{50} is 224 224.56 $\mu g_{dry\ extract}$/mL for cultivar B and 1073.12 $\mu g_{dry\ extract}$/mL for cultivar R, or 0.11 and 0.024 of AAI values for cultivar B and R respectively. These values show weak effect of wild cherry on stable DPPH radical taking into account Scherrer classification. (Scherer and Godoy 2009). Although wild cherry has a weak antioxidant effect on DPPH radical, from the results can be seen that cultivar B has 5 times more prominent effect than cultivar R.

Based on results in table 3, it can be seen that wild cherry is a quite good electron donor which allows shutdown of $ABTS^+$ radical and ferri-complex reduction. On the other side, modest results in reaction of cherry extracts with stable DPPH results show that this extract is relatively weak donor of hydrogen atoms.

CONCLUSION

High phenol, flavonoid, flavonol and anthocyanin content values were found in examined samples of wild cherry. High values for antioxidant activity of wild cherry denoted as cultivar B are most probably influenced by high anthocyanin content. Conclusion can be made that wild cherry, cultivar B especially, is a significant source of phenolic compounds and can be considered as a good source of antioxidants.

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