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 ± 0.73 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 monomorous 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 hinder 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
stress, it is necessary to intake additional amount of anti-
odants.

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-
odative 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, anthocyanins) 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-
odant 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; Melichácová 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; Ballisteri 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 Jersey, 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), magnetic stirrer
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 40g of seedless samples were mashed using hand blender.

Polyphenols extraction

Fresh and mashed samples (40g) were extracted into 160mL 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 250mL 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 (0.2mL) of diluted extract (100 µg/mL) is mixed with 1.5mL of Folin-Ciocalteu reagent (previously diluted with water 1:10, v/v) and 1.5mL of NaHCO3 (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 765nm.

Standard diagram is obtained in the same way with a difference that instead of sample solution, an aliquot (0.2mL) 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 (R²=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 AlCl3 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 440nm.

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 (R²=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 AlCl₃ in pure ethanol, stirred vigorously using vortex and left during 1 hour in dark and on room temperature. Absorbeny 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: \( y = 0.0368x - 0.135 \) (R²=0.9996), where \( y \) is the absorbeny 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 profiltered.

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 CH₃COONa + 240 mL 1 mol/L HCl + 360 mL deionised H₂O), after 15 minutes read absorbeny 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]:

\[
A_{tot} = (A_{500} - A_{700})_{pH_1} - (A_{500} - A_{700})_{pH_2}
\]

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

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

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

where:

\[
M = 449.2 \text{ g/mol (molecule mass of cyanidin 3-glucoside)},
\]

\[
R = 20 \text{ (dilution factor)},
\]

\[
\varepsilon = 26900 \text{ Lcm}^{-1} \text{mol}^{-1} \text{ (molar coefficient of absorption of cyanidin 3-glucoside)},
\]

\[
l = 1 \text{ cm (thickness of cuvette)}.
\]

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]:

\[
ID = \frac{c_{tot}}{c_{mon}}
\]

Determination of antioxidant activity

FRAP method

Total antioxidant activity was determined using modified FRAP (Ferric Reducing Antioxidant Power) method based on reduction of Fe³⁺ ions to Fe²⁺ ions in the presence of antioxidants. Resulting Fe²⁺ 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. Absorbeny 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 FeSO₄·7H₂O (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 FeSO₄·7H₂O: \( y = 3.1038x + 0.0495 \) (R²=0.9665), where \( y \) is the absorbeny and \( x \) is corresponding concentration of FeSO₄·7H₂O.

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:

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

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 1% 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:

\[ AAI = \frac{c_{final}^{DPPH}}{IC_{50}} \]  \[ (100) \]  \[ [7] \]

where:

\( c_{final}^{DPPH} \) - 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 diaminoniammonium 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.

Aliquot (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:

\[ I_\% = \frac{A_{control} \times A_{sample}}{A_{control}} \times 100 \]  \[ [8] \]

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 1% 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 of dry extract) than in cultivar R (107.56 mg GAE/g of dry extract). On the other side, total flavonol and flavonoid content is larger in cultivar B (22.09 and 51.36 mgQ/ g of dry extract) than in cultivar B (9.50 and 48.55 mgQ/ g 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

<table>
<thead>
<tr>
<th></th>
<th>Cultivar B</th>
<th>Cultivar R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols</td>
<td>139.78 ± 3.85*</td>
<td>107.56 ± 5.09</td>
</tr>
<tr>
<td>(mg GAE/**g of dry extract)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonols</td>
<td>9.50 ± 0.73</td>
<td>22.09 ± 0.22</td>
</tr>
<tr>
<td>(mg Q/***g of dry extract)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>48.55 ± 0.31</td>
<td>51.36 ± 1.18</td>
</tr>
<tr>
<td>(mg Q/***g of dry extract)</td>
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</table>

* 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)

<table>
<thead>
<tr>
<th></th>
<th>Cultivar B</th>
<th>Cultivar R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins</td>
<td>13.66</td>
<td>0.528</td>
</tr>
<tr>
<td>mg(C3G*)/gFW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total anthocyanins</td>
<td>683.32</td>
<td>26.38</td>
</tr>
<tr>
<td>mg(C3G)/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monomerous</td>
<td>579.77</td>
<td>12.36</td>
</tr>
<tr>
<td>anthocyanins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degradation index</td>
<td>1.191</td>
<td>2.134</td>
</tr>
</tbody>
</table>

*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 (Mozetić 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
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.

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.

REFERENCES


