Antioxidant capacities and antioxidants of strawberry, blackberry and raspberry leaves

Buricova, Lucie; Andjelkovic, Mirjana; Cermakova, Anna; Reblova, Zuzana; Jurcek, Ondrej; Kolehmainen, Erkki; Verhe, Roland; Kvasnicka, Frantisek

2011

Please cite the original version:
Antioxidant Capacity and Antioxidants of Strawberry, Blackberry, and Raspberry Leaves

LUCIE BUŘIČOVÁ¹, MIRJANA ANDJELKOVIC², ANNA ČERMÁKOVÁ¹, ZUZANA RÉBLOVÁ¹, ONDŘEJ JURČEK³⁴⁵, ERKKI KOLEHMAINEN⁴, ROLAND VERHÉ² and FRANTIŠEK KVASNIČKA⁶

¹Department of Food Chemistry and Analysis, ²Department of Chemistry of Natural Compounds and ³Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic; ⁴Department of Organic Chemistry, Ghent University, Ghent, Belgium; ⁵Department of Chemistry, Laboratory of Organic Chemistry, University of Jyväskylä, Jyväskylä, Finland; ⁶Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Isotope Laboratory, Prague, Czech Republic

Abstrakt


The total phenolic content (Folin-Ciocalteu method), free radical scavenging ability expressed as DPPH value, ferric reducing antioxidant capacity (FRAP), and oxygen radical absorbance capacity (ORAC) were determined in water extracts of leaves from Rosaceae family plants (Fragaria vesca L., Rubus fruticosus L., and Rubus idaeus L.). The antioxidant capacities of the extracts (in the order of the above mentioned methods) were 73.6–88.9%, 60.1–71.4%, 49.7–78.0% respectively, and 45.3–66.5% of that of green tea water extract. Further, the presence of 15 compounds (gallic acid, rutin, ellagic acid, caffeic acid, p-coumaric acid, quercetin, kaempferol, myricetin, quercetin-3-d-glucoside, ascorbic acid, (+)-catechin, (-)-epicatechin, epicatechin gallate, epigallocatechin, procyanidin B1) was studied by HPLC-ECD and their antioxidant capacities were compared to the antioxidant capacity of the extracts. Out of the compounds studied, mostly (+)-catechin, ellagic acid, and (-)-epicatechin participated in the antioxidant capacities of the studied plant leaves water extracts. The antioxidant capacity of leaves infusions (determined by DPPH method) was lower than those of red wines and tea infusions, but comparable to the antioxidant capacities of white wines and fruit beverages.

Keywords: DPPH; Folin-Ciocalteau method; Fragaria vesca; Rubus fruticosus; Rubus idaeus; FRAP; HPLC; ORAC

In the screening study comparing the antioxidant capacities (AC) (measured by DPPH method) of seventeen Czech medicinal plants, the leaves of strawberry (Fragaria vesca L.), blackberry (Rubus fruticosus L.) and raspberry (Rubus idaeus L.) belonged to the most efficient plants tested (Buřičová & Rěblová 2008). The AC of their extracts were comparable to and in some cases even higher than AC of the studied plants from the family Lamiaceae (oregano, sweet balm, thyme, dead-

nettle, and mint), which are known as rich sources of antioxidants (Dorman et al. 2004; Capecka et al. 2005). Good AC of the listed plants from the Rosaceae family was also confirmed by other articles. Katalinic et al. (2006) determined the AC (ferric reducing antioxidant capacity (FRAP)) and phenolic content of 70 medicinal plants. The leaves of raspberry, blackberry, and strawberry were among eleven of the most effective plants. In another study (Wang & Lin 2000), the AC (oxygen radical absorbance capacity (ORAC)) of leaves and berries of strawberry, blackberry, and raspberry were compared. The leaves were a richer source of antioxidants and had a greater content of phenolic substances than berries.

However, for the estimation of the possible antioxidant effect in vivo it is important to know not only the AC of herb extracts, but also the contents of particular antioxidants and the participation of these compounds in the AC of extracts. A lot of compounds possessing AC were identified in the leaves of strawberry, raspberry, and blackberry as procyanidin B1, epigallocatechin, (+)-catechin, procyanidin B2, (−)-epicatechin, astragalin, epicatechin-3-gallate, piceid, quercetin-4-glucoside, trans-resveratrol (Mudnic et al. 2009), ellagic acid (Gudej & Rychlínská 1996; Gudej & Tomczyk 2004; Skupien & Oszmianski 2004; Hukkanen et al. 2007), p-coumaric acid (Gudej 2003; Skupien & Oszmianski 2004; Hukkanen et al. 2007), quercetin, kaempferol (Gudej & Rychlínska 1996; Gudej 2003; Gudej & Tomczyk 2004; Skupien & Oszmianski 2004), myricetin (Skupien & Oszmianski 2004), gallic acid, agri-moninii, casuaricin, lambertianin C, potentiin, sanguin H-10, nobotanin A (Hukkanen et al. 2007), ascorbic acid (Wang 1999), quercetin-3-O-β-d-glucopyranoside, quercetin-3-O-β-d-galactopyranoside, quercetin-3-O-α-L-arabinopyranose, kaempferol-3-O-β-d-galactopyranoside, kaempferol-3-O-α-L-arabinopyranose (Gudej & Rychlínska 1996; Gudej 2003), quercetin-3-O-glucoside, rutin, quercetin glucuronide (Venskutonis et al. 2007), and some other phenylethanol derivatives of phenylpropanoid glucosides (Hanhinea et al. 2009). However, the contents of these compounds in water extracts and infusions have not been studied sufficiently, as well as their participation in the antioxidant capacities of the respective plants.

With respect to the previous text, the aim of this research was to validate the high AC of strawberry leaves, blackberry leaves, and raspberry leaves water extracts using four different methods applied to the same samples. For the evaluation of the possible effects of the extracts in vivo, quantification of the selected phenolic compounds (gallic acid, rutin, ellagic acid, caffeic acid, p-coumaric acid, quercetin, kaempferol, myricetin, quercetin-3-d-glucoside, ascorbic acid, (+)-catechin, (−)-epicatechin, epicatechingallate, epigallocatechin, procyanidin B1) in the studied extracts was done and the participation of these compounds in AC of the extracts was calculated. Further, the AC (measured by DPPH method) of leaves water infusions were compared with AC of other sources of antioxidants in human diet such as green and black teas, white and red wines, and fruit (vegetable) beverages.

**MATERIAL AND METHODS**

**Materials.** Three leaf samples of each of the medicinal plants studied, i.e. strawberry leaves (Fragaria vesca L.), blackberry leaves (Rubus fruticosus L.), and raspberry leaves (Rubus idaeus L.), were purchased from a Czech producer and medicinal herbs distributor (Natura, Děčín, Czech Republic), from pharmacies (respectively), the plants having been grown in the area of the Czech Republic. Green and black teas, red and white wines, fruit and vegetable beverages were purchased in ordinary Czech shops or specialised tea shops. All the samples were used within the recommended consumption period.

All standards used for HPLC, Folin-Ciocalteu reagent, 2,4,6-tripyridyl-5-triazine, ferrous sulphate, Trolox, 2,2-diphenyl-1-picrylhydrazyl, and formic acid, and all chemicals for electrophoresis were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Sodium carbonate, AAPH, and fluorescein were purchased from Acros (Geel, Belgium), sodium chloride was from Lachema Neratovice, Czech Republic. Organic solvents were of analytical grade, acetonitrile was of HPLC grade. All solvents were purchased from Merck (Darmstadt, Germany).

**Samples preparation.** For the water extracts preparation, the leaves of the medicinal plants (or green tea) were ground and 1 g of the ground leaves was left in 50 ml of deionised water for extraction during 20 minutes. The temperature of the water was 98°C.

Water infusions of medicinal plants and green and black teas were prepared as recommended by
the producers. The amount of the sample used for infusion and its duration were adjusted according to the plant type in the following manner: 1.5–2 g of the medicinal plants per 250 ml water with infusion period of 15 min; 2 g of tea per 150 ml or 250 ml water with infusion period of 2–4 min (green tea) or 3–5 min (black tea).

After the preparation, the extracts and infusions were diluted with the respect to the detection method used and were analysed shortly after the preparation. Red and white wines and fruit and vegetable beverages were diluted with respect the detection method used.

**Total phenolics assay (Folin-Ciocalteu method, TPC).** Total phenolics content was measured using the Folin-Ciocalteu colorimetric method described by Singleton et al. (1999). The absorbance was measured at 760 nm with Varian UV-Visible Spectrophotometer (Cary 50 BIO, Mulgrave, Australia). The results were expressed as mg of gallic acid equivalents per 1 g of dry sample.

**Ferric reducing antioxidant capacity (FRAP).** The ferric reducing ability was determined using the assay described by Benzie and Strain (1996). The absorbance was measured every 10 s during 30 min reading (spectrophotometer Cary 100 Bio Varian, Palo Alto, USA). The results were expressed as mmol of FeSO₄ per litre.

**Oxygen radical absorbance capacity (ORAC).** The determination of oxygen radical absorbance capacity was based on the method used by Huang et al. (2002). The measurements were carried out on a microplate fluorimeter SPECTRAMax Gemini XS (Molecular Device Corporation, Sunnyvale, USA) using the 490 nm excitation and 515 nm emission filters. The fluorescence was recorded every minute after the addition of AAPH until its value was < 5% of the initial reading. The results were expressed as micromole Trolox equivalents per 1 g of dry sample.

**Free radical scavenging ability by the use of a stable DPPH radical.** Total antioxidant capacity was measured using DPPH method, which presents radical scavenging ability of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. This method was described previously by Gadow et al. 1997, and was modified in detail by Buřičová and Réblová (2008). The absorbance at 522 nm was measured by a Varian UV-Visible Spectrophotometer (Cary 50 BIO, Mulgrave, Australia). The results were expressed as mg of ascorbic acid per 1 g of dry plant material or as mg of ascorbic acid per 100 ml of the beverage.

**Determination of selected antioxidants.** The extracts were analysed by HPLC-RP-ECD, specifically with an amperometric detector HP 1049A (Hewlett Packard, Avondale, USA) equipped with a glassy-carbon electrode (operating at a potential of +0.8 V), a reference Ag/AgCl electrode and a platinum counter electrode, and an isocratic non-steel pump LCP 4020.31 (ECOM, Prague, Czech Republic). The data were recorded using a Clarity 2.6.4.402 chromatography system (DataApex, Prague, Czech Republic). The compounds were separated on a RP C18 column (Hypersil ODS: 4.0 mm × 250 mm, 5 μm; Agilent, USA) maintained at the room temperature. Isocratic elution was performed at the flow rate of 1 ml/min, using a mixture of acetonitrile and 0.13% (m/m) formic acid containing sodium chloride (0.005 mol/l) as a mobile phase. The proportion of acetonitrile in the mobile phase depended on the compound of interest (5% acetonitrile (ACN): gallic acid; 10% ACN: caffeic acid, p-coumaric acid, (+)-catechin, epigallocatechin, (−)-epicatechin, procyanidin B1; 20 % ACN: rutin, elagic acid, quercetin-3-d-galactoside, quercetin-3-d-glucoside, (−)-epicatechin gallate; 30% ACN: quercetin, kaemferol, myricetin; 0% ACN and changed detection potential to + 0.3: ascorbic acid). The injection volume was 20 μl. The samples, standards, and spiked samples were analysed. The peaks identification was performed by the comparison of the retention times with those of reference standards.

**Isolation and confirmation of (+)-catechin.** The extract of strawberry leaves was purified using SPE C18 (Supelco, USA), where water was used for purification and methanol for the elution of the sample from the column. The purified extract was concentrated with a vacuum evaporator. The subsequent isolation of catechin was done by HPLC-RP-DAD technique using UV/VIS photodiode array detector SPD-M20A (Shimadzu, Kyoto, Japan), an automatic sampler injector SIL-10AP (Shimadzu, Kyoto, Japan), a preparative LC pump unit LC-8A (Shimadzu, Kyoto, Japan), a fraction collector module FRC-10A (Shimadzu, Kyoto, Japan), and LC workstation LabSolutions/LC solution version 1.2. The compounds were separated on a RP C18 column (Hypersil ODS: 4.0 mm × 250 mm, 5 μm; Agilent, Santa Clara, USA) and 10% acetonitrile contained 0.13% (m/m) formic acid was used as a mobile phase. The absorbance was read at 280 nm.

For the confirmation of catechin, MS and NMR analyses were used. The mass spectra were recorded.
on a Bruker micromass LCT. The samples were dissolved in methanol to an appropriate concentration, ionised in a positive and negative ESI modes, and analysed with TOF detector. NMR spectra were measured in the samples diluted in deuterated water at 303, the solvent signal was suppressed. \(^1\)H NMR, proton decoupled (waltz-16) \(^13\)C NMR, PFG \(^1\)H, \(^13\)C HMQC, and HMBC measurements (detailed acquisition and processing parameters are available on request) were carried out on a Bruker Avance DRX 500 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) equipped with an inverse detection 5 mm probehead and z-gradient accessory.

Enantioseparation of catechin was carried out on a Hewlett-Packard 3DCE capillary electrophoretic system (HPST Ltd., Prague, Czech Republic) equipped with a diode array detector (Konfik et al. 2007). The separation was performed in a fused silica capillary of the total length of 750 mm (665 mm effective length, 50 μm i.d.), the constant voltage applied to the capillary was +30 kV. The samples were injected by pressure (25 mbar for 10 s) and the separated compounds were detected at 280 nm. The electrolyte contained 63.4mM H\(_3\)BO\(_3\) + 9.3mM Na\(_2\)B\(_4\)O\(_7\).H\(_2\)O (pH 8.5) and chiral selector maltosyl-β-cyclodextrin (0.5mM).

RESULTS AND DISCUSSION

Antioxidant capacity of the plants water extracts determined using different methods

The AC of the water extracts of the plants studied as obtained using four selected methods (TPC, DPPH, FRAP, ORAC) are shown in Table 1. The AC generally depends on the method used and even the results obtained by the same method may not be reproducible in different laboratories (for example depending on the pH of the reaction medium, solvents used, calibration standards or way of the results expression) (Huang et al. 2005; Roginsky & Lissi 2005). Due to this, it is difficult to interpret the data of AC. Therefore, the AC of the studied leaves water extracts were compared with the AC of green tea, which is known as a rich source of antioxidant (Lin et al. 1996; Kris-Etherington et al. 2002). The AC of water plant extracts were 73.6–88.9% (TPC), 60.1–71.4% (DPPH), 49.7–78.0% (FRAP), and 45.3–66.5% (ORAC) of the AC of green tea extract. It is obvious that water extracts of strawberry, blackberry, and raspberry leaves show very good AC independently of the method used.

Compounds participated on the AC of the plant extracts studied

The contents of the selected antioxidants in the studied extracts (together with the participation of these compounds in the AC of the extracts) is showed in Table 2. Not all of the compounds studied (i.e. gallic acid, rutin, ellagic acid, caffeic acid, \(p\)-coumaric acid, quercetin, kaempferol, myricetin, quercetin-3-\(p\)-glucoside, ascorbic acid, (+)-catechin, (−)-epicatechin, epicatechingallate, epigallocatechin, procyanidin B1) were identified in the plant water extracts. The main reason for this disproportion can be the way of extraction. The studied compounds were identified in the previous studies in the extracts prepared usually by using organic solvents as benzene, chloroform (Gudej & Rychlínska 1996; Gudej 2003), acetone (Hukkanen et al. 2007), methanol (Gudej & Tomczyk 2004) or ethanol (Venskutonis et al. 2007). However, from the nutritional point of

Table 1. AC of medicinal plants water extracts determined using four methods and their comparison with the antioxidant capacity of green tea water extract

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>DPPH</th>
<th>FRAP</th>
<th>ORAC</th>
<th>TPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td>110.1 ± 16.6</td>
<td>23.3 ± 1.4</td>
<td>1062.0 ± 143.9(^a)</td>
<td>62.4 ± 1.0</td>
</tr>
<tr>
<td>Blackberry</td>
<td>125.2 ± 2.9(^a)</td>
<td>36.7 ± 1.1</td>
<td>1304.3 ± 232.4(^a)</td>
<td>75.4 ± 1.2</td>
</tr>
<tr>
<td>Raspberry</td>
<td>105.2 ± 12.9</td>
<td>26.6 ± 1.0</td>
<td>888.0 ± 106.8(^a)</td>
<td>68.9 ± 5.6</td>
</tr>
<tr>
<td>Green tea</td>
<td>175.2 ± 20.9(^b)</td>
<td>47.0 ± 2.4</td>
<td>1628.6 ± 62.8</td>
<td>84.8 ± 1.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (\(n = 3, ^{a}n = 4, ^{b}n = 6\)); DPPH in mg ascorbic acid/g of dry sample; FRAP in mmol FeSO\(_4\)/l; ORAC in μmol Trolox/g of dry sample; TPC in mg gallic acid/g of dry sample.
On the contrary, for the first time, in this study (-)epicatechin, (+)-catechin, and procyanidin B1 were detected in both raspberry and blackberry leaves while epicatechingallate was present only in blackberry leaves. These compounds were previously detected only in strawberry leaves (Mudnic et al. 2009).

The contents of the compounds varied in the studied extracts and were dependent on the tested plants and even on the individual tested samples of the same plant. Even so, the AC (DPPH) of the individual extracts prepared from different samples of the same plant did not largely deviate (Table 2). Ellagic acid occurred in significant amounts in the extracts of all samples (of all tested plants). Its content ranged from 10 to 35 mg/l, which corresponds to 0.5–1.8 mg/g of dry leaves. In the extracts of strawberry leaves, but not in those of other leaves studied, (+)-catechin was also present in significant amounts ranging from 29 mg/l to 99 mg/l, which corresponds to 1.5–4.9 mg/g of dry leaves. This is more than in a previous study (Mudnic et al. 2009), where (+)-catechin was determined in the amount

<table>
<thead>
<tr>
<th>Medicinal plant/compound</th>
<th>c (mg/l)</th>
<th>% AC</th>
<th>c (mg/l)</th>
<th>% AC</th>
<th>c (mg/l)</th>
<th>% AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.4</td>
<td>&lt; 1</td>
<td>1.1</td>
<td>&lt; 1</td>
<td>1.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>22.7 ± 2.1</td>
<td>8.6</td>
<td>15.7 ± 1.6</td>
<td>5.0</td>
<td>14.0 ± 1.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Quercetin-3-d-glucoside</td>
<td>6.2</td>
<td>&lt; 1</td>
<td>23.7</td>
<td>&lt; 1</td>
<td>21.8</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>(+)-Catechine</td>
<td>-</td>
<td>-</td>
<td>2.6</td>
<td>&lt; 1</td>
<td>1.2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>(--)-Epicatechine</td>
<td>6.8 ± 1.9</td>
<td>1.4</td>
<td>70.9 ± 4.5</td>
<td>11.6</td>
<td>4.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Epicatechingallate</td>
<td>0.8</td>
<td>&lt; 1</td>
<td>3.6</td>
<td>&lt; 1</td>
<td>1.8</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>8.5 ± 0.0</td>
<td>1.8</td>
<td>2.8</td>
<td>&lt; 1</td>
<td>3.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Total</td>
<td>&gt; 11.8</td>
<td>&gt; 15.6</td>
<td>&gt; 11.8</td>
<td>&gt; 15.6</td>
<td>&gt; 4.7</td>
<td></td>
</tr>
<tr>
<td>Blackberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.4</td>
<td>&lt; 1</td>
<td>1.1</td>
<td>&lt; 1</td>
<td>1.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>22.7 ± 2.1</td>
<td>8.6</td>
<td>15.7 ± 1.6</td>
<td>5.0</td>
<td>14.0 ± 1.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Quercetin-3-d-glucoside</td>
<td>6.2</td>
<td>&lt; 1</td>
<td>23.7</td>
<td>&lt; 1</td>
<td>21.8</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>(+)-Catechine</td>
<td>-</td>
<td>-</td>
<td>2.6</td>
<td>&lt; 1</td>
<td>1.2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>(--)-Epicatechine</td>
<td>6.8 ± 1.9</td>
<td>1.4</td>
<td>70.9 ± 4.5</td>
<td>11.6</td>
<td>4.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Epicatechingallate</td>
<td>0.8</td>
<td>&lt; 1</td>
<td>3.6</td>
<td>&lt; 1</td>
<td>1.8</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>8.5 ± 0.0</td>
<td>1.8</td>
<td>2.8</td>
<td>&lt; 1</td>
<td>3.6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Total</td>
<td>&gt; 11.8</td>
<td>&gt; 15.6</td>
<td>&gt; 11.8</td>
<td>&gt; 15.6</td>
<td>&gt; 4.7</td>
<td></td>
</tr>
<tr>
<td>Raspberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.2</td>
<td>&lt; 1</td>
<td>1.9</td>
<td>&lt; 1</td>
<td>1.5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>16.4 ± 0.9</td>
<td>7.8</td>
<td>19.7 ± 4.6</td>
<td>11.0</td>
<td>10.8 ± 0.1</td>
<td>7.7</td>
</tr>
<tr>
<td>(+)-Catechine</td>
<td>0.6</td>
<td>&lt; 1</td>
<td>0.3</td>
<td>&lt; 1</td>
<td>0.5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>(--)-Epicatechine</td>
<td>3.6</td>
<td>&lt; 1</td>
<td>1.9</td>
<td>&lt; 1</td>
<td>1.5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>3.8</td>
<td>&lt; 1</td>
<td>3.7</td>
<td>&lt; 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>&gt; 7.8</td>
<td>&gt; 11.0</td>
<td>&gt; 7.8</td>
<td>&gt; 11.0</td>
<td>&gt; 7.7</td>
<td></td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SD (n = 3 for compounds with % AC > 1); *Antioxidant capacities (DPPH) of leaves water extracts are expressed as means ± SD (n = 3), mg ascorbic acid/l.
of 0.5 mg/g of a dry sample of strawberry leaves. The contents of other antioxidants were generally lower than 10 mg/l (0.5 mg/g of dry leaves) with the exceptions of (–)-epicatechin (71 mg/l) in one extract of blackberry leaves and procyanidin B1 (12 mg/l) in one extract of strawberry leaves. (+)-Catechin was detected as the dominant compound present in the extracts of strawberry leaves (and in very small amounts in other extracts). Therefore, it may be used as a marker for the distinction of strawberry leaves preparations from other related plants preparations (of blackberry and raspberry leaves). Consequently, (+)-catechin (Figure 1) was confirmed in these extracts by MS and NMR after the isolation. ESI-TOF MS m/z (%): 289.08 (100), 578.98 (56), 868.95 (10), 145.02 (12), 245.11 (11). $^1$H NMR ($D_2$O, δ ppm): 2.36 (1H, dd, H-4a), 2.75 (1H, dd, H-4b), 3.96 (1H, m, H-3), 4.47 (1H, d, H-2), 5.85 (1H, s, H-8), 5.95 (1H, s, H-6), 6.73 (1H, d, H-5), 6.82 (1H, d, H-6), 6.84 (1H, s, H-2); $^{13}$C NMR ($D_2$O, δ ppm): 26.89 (C-4), 66.75 (C-3), 80.96 (C-2), 95.08 (C-8), 95.94 (C-6), 100.56 (C-10), 115.09 (C-2'), 116.26 (C-5), 120.11 (C-6'), 130.35 (C-1'), 144.07 (C-4'), 144.34 (C-3'), 154.83 (C-9), 155.07 (C-5), 155.14 (C-7). These NMR results were confirmed by NMR data predictor (software ACD/ChemSketch C+H NMR Predictors and DB (Product version 10.04)) and by comparison with the published results (Mendoza-Wilson & Glossman-Mitnik 2006). Enantiomer of (+)-catechin was confirmed by chiral electrophoresis.

The contents of the compounds were also expressed as % of their participation in DPPH antioxidant capacity in the respective extracts (Table 2). DPPH method was used for this purpose because it appeared to suffer the least synergism interferences in comparison to other methods (Murakami et al. 2003). The studied compounds in the leaves of Rosaceae plants moderately contributed to the antioxidant capacity of their water extracts. Future research should enlighten the remaining fractions of the antioxidant capacities to offer a comprehensive overview of the health and nutritional values of these plants.

Of the compounds studied, ellagic acid (mainly), (+)-catechin, and (–)-epicatechin participated in the AC of the water extracts of strawberry, raspberry, and blackberry leaves. Ellagic acid is assumed to possess antioxidant, anti-mutagenic, anti-inflammatory, and cardioprotective activities (Priyadarshini et al. 2002). Catechin and epicatechin are antioxidants which affect plasma antioxidant biomarkers and energy metabolism (Williamson & Manach 2005). Provided that these compounds have been detected in this study, the above mentioned may imply a possible effect of water Rosaceae plant extracts or infusions in vivo.

Comparison of the antioxidant capacities of herbal infusions with other sources of antioxidants

Antioxidants are recommended to be primarily consumed as part of a daily diet (Halliwell 2006). Therefore, the AC of herbal infusions were compared to the capacities of other sources of antioxidants in the forms in which they are taken in a common diet (Figure 2). The selected beverages like tea, wine, vegetable, and fruit beverages are preferred among consumers and significantly contribute to the total intake of antioxidants (Svilas et al. 2004; Pellegrini et al. 2007). The method with the stable DPPH radical was used for the determination of the AC. In Figure 2, a very high variability of the AC inside the group of wine, tea, and fruit beverage samples can be observed. This variability of beverages reflects the differences in the type of the plant material, type of wine, localities and growing conditions, industry procedures of beverages preparation, portion of raw material in fruit and vegetable beverages etc. Moreover, some antioxidants are added to some fruit beverages during the production which may contribute to the final result.

The AC (radical scavenging capacities) of the plants infusions studied are typically three times lower than the capacity of green tea infusions

Figure 1. Structure of (+)-catechin
and two times lower than the capacity of black tea infusions. As mentioned above, green tea is probably the most efficient source of antioxidants in our diet (Lin et al. 1996; Kris-Etherton et al. 2002; Farhoosh et al. 2007). However, green tea as well as black tea contains caffeine (Farhoosh et al. 2007) and an excessive intake of caffeine can be related to hypertension, dehydration, anxiety, or insomnia (Gardner et al. 2007), whereas no adverse effect has been observed on drinking beverages made from leaves of strawberry, blackberry, and raspberry. Therefore, the studied plants may be seen as an alternative source of antioxidants for the group of consumers consciously selecting the intake of higher levels of antioxidants without caffeine.

The results of AC in wine showed that red wine had higher AC than white wine. This agrees with the report of Katalinic et al. (2004). Moreover, besides green tea, red wine is seen as one of the most significant sources of antioxidants (Katalinic et al. 2004; de Lange et al. 2007; Logan et al. 2008). The AC of the studied plant infusions were comparable to the AC of white wine and were, on average, four times lower than that of red wine. However, in the case of “monitoring dribbling” (when daily no more than 100–200 ml of wine is recommended), two cups of the tested medicinal plants infusion give us the same amount of antioxidants like red wine.

The AC of the studied plant infusions were higher or comparable to the capacities of different fruit (vegetable) beverages. However, according to some authors, fruit and vegetable beverages contribute to the total intake of antioxidants through the diet to a smaller extent than wine, tea, or coffee (Svilaas et al. 2004; Pellegrini et al. 2007).

**CONCLUSION**

The AC (TPC, DPPH, ORAC, FRAP) of the studied water extracts of strawberry, blackberry, and raspberry leaves were determined to be in the range from 49.7% to 89.9% of the antioxidant capacity of green tea water extract, and good antioxidant capacities of the studied herbs infusions (determined by DPPH method) were observed in comparison with those of tea infusions, wines, and other beverages.

For the first time (–)-epicatechin, (+)-catechin, and procyanidin B1 were detected in raspberry leaves, and all these compounds together with epicatechingallate in blackberry leaves, whereas biologically active (+)-catechin, ellagic acid, and (–)-epicatechin significantly participated in the AC of the leaves water extracts of all plants studied. Therefore, the analysed medicinal plants can be considered to be a good source of antioxidants. They can be used for direct consumption as various kinds of beverages or as extracts of antioxidants to increase the nutritional value of different foods and diets.

**References**


of old tea leaves and black tea wastes (Camellia sinensis L.). Food Chemistry, 100: 231–236.


Skupien K., Oszmianski J. (2004): Comparison of six cultivars of strawberries (Fragaria × ananassa Duch.) grown in northwest Poland. European Food Research and Technology, 219: 66–70.


Corresponding author:
Ing. Lucie Buřičová, Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analyzy potravin, Technická 5, 166 28 Praha 6, Česká Republika
tel.: + 420 220 445 120, e-mail: lucie.buricova@vscht.cz