The aim of this study was to determine the anthocyanins, ascorbic acid content and the antiradical activity in less common fruit species native in the territory of Slovakia (black mulberries, cornelian cherries, blackberries, blackthorn, rowanberries) and honeyberries (Lonicera kamtschatica) originated from Kamtschatka. Anthocyanins were evaluated after extraction from solid materials with acidified ethanol or after direct solution of liquid samples with HCl in ethanol solution by spectrophotometer measurement. To evaluate the antiradical activity, a spectrophotometer method based on the reaction of antioxidants with a stable radical 2,2- diphenyl- 1- picrylhydrazyle (DPPH•) in methanol solution was used. The reduction properties of ascorbic acid are used in the measurement. The highest value of anthocyanin pigments was found out in honeyberries (Lonicera kamtschatica) of all analysed clones. Black mulberries of the genotype M104 and cornelian cherries of the genotypes 55 a 44 are the most significant sources of ascorbic acid among analysed less common fruit species. It is an interesting fact that blackthorns (Prunus spinosa) containing the lowest value of ascorbic acid (10.31±1.016 mg.100 g⁻¹) and medium value of anthocyanins (1.71±0.08 g.kg⁻¹) shows the highest level of antiradical activity (461.25±3.69 % ≈ EC₅₀= 0.1084 ± 0.0101). A similar phenomenon was observed in honeysuckles, too. According to these results we can suppose that another flavonoids (flavonols, flavons and flavanols) can also significantly contributed to antiradical activity of the blackthorns and edible honeysuckles. In summary, analysed less known fruit species are important sources of nutritive compounds, particularly anthocyanins, ascorbic acid and show significant free radical scavenging ability.

Keywords: Berries. Anthocyanins. Vitamin C. DPPH
Introduction

Currently, increasing attention has been paid by consumers to the lesser known fruits which have unusual flavours and qualities, and many of which are rich in antioxidants and anthocyanins (ERCISLI et al., 2007; OZGA et al., 2007). Detailed information about the health – promoting components of lesser known fruit species could led to a better understanding of the health – beneficial effects and an increased consumption of these fruits, including their utilization in functional foods and as ingredients in nutraceuticals, medicine and pharmaceuticals (OZGA et al., 2007; YILMAZ et al., 2009).

Anthocyanins are a group of widespread natural phenol compounds in plants. As a major sub-group of flavonoids, anthocyanins are water-soluble plant pigments responsible for the blue, purple and red colour of many plant tissues (MAZZA and MINIATI, 1993). Anthocyanins have been shown to be strong antioxidants, and may exert a wide range of health benefits through antioxidant or other mechanisms (PRIOR, 2004).

Dietary supplements such as vitamin C have become popular for their perceived ability to enhance the body's antioxidant defences. Reactive oxygen species (ROS) have been shown to cause a broad spectrum of damage to biological systems. Scavenging of ROS is part of a healthy, well-balanced, antioxidant defence system (LEONARD et al., 2002). A high scavenging activity of berry extracts toward chemically generated ROS has been describes in several studies (KÄHKÖNEN et al., 2001; WANG and JIAO, 2001; WANG and JIAO, 2000).

In this work the content of anthocyanins, vitamin C and the antiradical activity of selected less common fruit species were evaluated.

Materials and Methods

The subjects of our measurement we can divide to the following groups:

1. older fruit species represented by neglected and under valued Morus nigra
2. well – known wild fruit of interest for in further breeding process – Cornus mas, Sorbus aucuparia, Rubus fruticosus
3. fruit native in Slovakia without interest in researching them – Prunus spinosa
4. another lesser - known foreign like edible honeysuckle from Russia that can be considered as very promising because of its non – demanding cultivation and a high nutritional value of its fruit.

All samples have the same origin - Slovak Agricultural University in Nitra. The analysed samples were harvested in phase of consumption ripeness and stored in frozen form.
1. Evaluation of anthocyanins content

*Principle of measurement:*
Anthocyan pigments were evaluated after extraction from solid materials with acidified ethanol or after direct solution of liquid samples with HCl in ethanol solution by spectrophotometer measurement of absorption in absorption maximum (the method by Fuleki and Francis, 1968).

2. Evaluation of antiradical activity- DPPH method

*The Principle of measurement:*
To evaluate the antiradical activity, a spectrophotometer method based on the reaction of antioxidants with a stable radical 2,2'-biphenyl-1-picrylhydrazyle radical (DPPH•) in methanol solution was used. The decrease of absorbance in the course of time at characteristic wavelength 515.6 nm is the evidence of reaction of the antioxidants from extracts with DPPH•, which signifies the evidence of antioxidants activity of extracts (the method by Sanchéz – Moreno et al., 1998, Brand–Williams et al., 1995).

3. Evaluation of vitamin C content

*Principle of measurement:*
The reduction properties of ascorbic acid are used in the measurement. A basic solution is made from the chopped sample with phosphoric acid, which is filtered. Then Fe (III) ions are added, which are reduced to Fe (II)-ions by ascorbic acid. Thereinafter dipridyl reagent is added to the Fe (II)-ions, which results a red complex. The content of this complex is measured by spectrophotometer (a method by Lásztity and Törley, 1987).

**Results and Discussion**

The analyzed less common fruit species used in this study are good sources of anthocyanins, vitamin C and also show significant free radical scavenging activity. All results of our evaluation are shown in Table 1. In general, according to the anthocyanin content *Lonicera kamtschatica* and their 3 clones (LKL - 2, LKL – 18, LKL – 19) had a distinguishable position among less common fruit species reached up the highest content of mentioned plant pigments with the leading position of LKL – 19 (19.18±1.02 g/kg). Otherwise, they had the lowest values of antiradical activity (30.03±0.24 – 33.00±0.22 %). Values of ascorbic acid can be considered as medium, but ascorbic acid content in the edible honeysuckles is predominantly influenced by climatic conditions as it has been previously reported by (PLEKHANOVA, 1998; JURÍKOVÁ – MATUŠKOVIČ, 2007). As 2005 can be characterised as a drought year with lack of precipitation, it was reflected in
lower values of ascorbic acid (PETROVA, 1987). On the other hand GAZDÍK et al. (2008) found out that the antiradical activity in the samples of the edible honeysuckles more correlated with the content of polyphenolic compounds than with ascorbic acid content. Because the lowest value of anthocyanin content in *Lonicera kamtschatica* showed the highest value of antiradical activity (33.00 ±0.22%) among the analyzed honeyberry species we can suppose that another flavonoids (flavonols, flavons and flavanols) can also significantly contributed to antiradical activity of the edible honeysuckle. Similarly, this fact is evident in blackthorn (*Prunus spinosa*), which contents the lowest value of ascorbic acid (10.31±1.016 mg.100 g⁻¹) and medium value of anthocyanins (1.71±0.08 g.kg⁻¹) but shows the highest level of antiradical activity (461.25±3.69 % ≈ EC₅₀= 0.1084 ± 0.0101). Another species of less common fruit species according to their free radical scavenging ability can be included to the following order: cornelian cherries of the genotype 13 (88.85±5.58 %) > cornelian cherries of the genotype 44 (84.56±0.27 %) > mulberries of the genotype M 410 (81.85±0.99 %) > cornelian cherries of the genotype 55 (77.59±0.25 %) > mulberries of the genotype M 104 (77.25±1.47 %) > rowanberries of the variant ‘Sladkoplodá Moravská’ (76.84±1.22 %) > blackberries of the variant ’Thornfree’(62.80±1.73 %) > honeyberries and their clones (30.03±0.24 - 33.00±0.22 %). Achieved results are in agreement with studies of TURAL – KOCA (2008) pointed to high levels of natural antioxidants and antioxidant activity in cornelian cherries. Our results show higher values of antiradical activity in comparison with TURAL – KOCA (2008) (29 – 69 %). OZGEN et al. (2009) demonstrating superior traits of mulberries with a high antioxidant capacity that confirmed our studies, too.

According to ascorbic acid content less common fruit species can be put in the following order: *Morus nigra* of the genotype M 104 > *Cornus mas* of the genotype 44 > *Cornus mas* of the genotype 55 > *Lonicera kamtschatica* of the clone LKL - 2 >*Rubus caesius* of the variant ’Thorn free’ > *Lonicera kamtschatica* of the clone LKL - 19 > *Cornus mas* of the genotype 13 > *Lonicera kamtschatica* of the clone LKL - 18 > *Morus nigra* of the genotype M 410 > *Lonicera kamtschatica* > *Sorbus aucuparia* of the variant ‘Sladkoplodá Moravská’ > *Prunus spinosa*.

According to ERCISLI – ORHAN (2008) the content of ascorbic acid in mulberries is moderate, ranging from 15.10 – 18.7 mg.100g⁻¹ that is in conflict with our studies with the highest level of ascorbic acid in genotype 104. The following order also confirmed that except for climatic conditions the genotype also play a very important role in ascorbic acid content as it has been reported by (JURÍKOVÁ - MATUŠKOVIČ, 2007). Our study showed that cornelian cherries are rich in ascorbic acid content ranged between (40.86 – 67.69 mg /100g). Similar results have been reported by other researchers (16.41 – 78.58 mg/100g) (GULERYUZ et al., 1998, BRINDZA et al., 2005). The content of anthocyanins reached 0.11 – 0.34 g/kg that represented a lower values reported by JU – HSIEH (2004) (0.34 – 1.41 g/kg). *Cornelian cherries* of the genotype 44 have the second position in antiradical activity and ascorbic acid content.
The content of anthocyanins, vitamin C and the antiradical activity of selected less common fruit species

<table>
<thead>
<tr>
<th>Species of less common fruits</th>
<th>Genotypes</th>
<th>Content of anthocyanins (mg.kg⁻¹)</th>
<th>Content of vitamin C (mg.100 g⁻¹)</th>
<th>Antiradical activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black mulberries (Morus nigra)</td>
<td>M 104 M 410</td>
<td>2.60±0.09 1.59±0.08</td>
<td>70.53±13.07 39.47±4.93</td>
<td>77.25±1.47 81.85±0.99</td>
</tr>
<tr>
<td>Cornelian cherries (Cornus mas)</td>
<td>genotype 55</td>
<td>1.11±0.03 0.34±0.02 0.29±0.06</td>
<td>67.69±8.41 69.96±9.76 40.86±9.85</td>
<td>77.59±0.25 84.56±0.27 88.85±5.58</td>
</tr>
<tr>
<td>Blackberries (Rubus caesius)</td>
<td>Thorn free</td>
<td>2.47±0.07</td>
<td>44.01±4.95</td>
<td>62.80±1.73</td>
</tr>
<tr>
<td>Blackthorns (Prunus spinosa)</td>
<td></td>
<td>1.71±0.08</td>
<td>10.31±1.016</td>
<td>461.25±3.69  461.25±3.69  EC₅₀ = 0.1084 ± 0.0101</td>
</tr>
<tr>
<td>Rowanberries (Sorbus aucuparia)</td>
<td>Sladkopoldá Moravská</td>
<td>0.34±0.099</td>
<td>22.84±1.75</td>
<td>76.84±1.22</td>
</tr>
<tr>
<td>Honeyberries (Lonicera kampschatica)</td>
<td>Lonicera kampschatica</td>
<td>8.58±0.025 12.88±0.98 14.36±0.998 19.18±1.02</td>
<td>35.05±3.21 49.65±3.56 39.91±3.21 42.29±4.25</td>
<td>33.00±0.22 30.9±0.54 30.03±0.24 32.9±0.74</td>
</tr>
</tbody>
</table>

EC – express the 50% ability of plant material to scavenge free radical. This indicator was used to explain the antiradical activity due to the fact that the “percent of inhibition” reach too high values and it was impossibly to explain it via %.

**Conclusions**

Our results demonstrate notable antioxidant activity of all evaluated samples of less common fruit species. The richest sources of anthocyanins are honeyberries among analysed materials. In spite of the low value of vitamin C in the samples of blackthorns their free radical scavenging ability was more significant than the antiradical activity of other analysed less common fruit species. The highest vitamin C content was evaluated in mulberries and cornelian cherries from analysed species of fruit.
We can say that analysed less common fruit species are important sources of nutritive compounds. However, more detailed biological and pharmacological studies are needed for better understanding of the health benefits of antioxidants. However, more detailed biological and pharmacological studies are needed for better understandings of the health benefits of antioxidants.

References


