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Are there the differences in antioxidant potential of local and imported *Rubus idaeus* and *Rubus fruticosus* fruits?

[¿Hay diferencias en el potencial antioxidante entre los frutos locales e importados de *Rubus idaeus* y *Rubus fruticosus*?]

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Abstract: The study focused on the biological activity of ethanol extracts from the fruits of *Rubus idaeus* and *Rubus fruticosus*. The fruits of local origin were compared with those from the organic store - imported. The antioxidant activities were analyzed using the superoxide anion radical scavenging activity and the DPPH• method. The total phenolic content and dry matter (DM) were determined. The DM ranged from 2.9 to 4.2 mg/mL. A significantly higher DM content was found in extracts from fruits of local origin. The total phenolic content ranged from 9.4 to 13.6 mg/g. Extracts from imported fruits were richer in polyphenols. The radical scavenging activity of superoxide anions was 45.4% and 47.5%. The DPPH• activity was 48.5% and 53.1%. No significant differences were found. The DPPH• value of IC_{50} was 40.0-43.1 μg/mL. No significant differences in antioxidant activity were found between the local and imported berries.

Keywords: Antioxidants; Antiradical activity; Oxidative stress; Plant extracts; Polyphenols.

Resumen: El estudio se enfocó en la actividad biológica de extractos etanólicos de *Rubus idaeus* y *Rubus fruticosus*. Los frutos de origen local se compararon con frutos importados de una tienda de productos orgánicos. La actividad antioxidante se analizó mediante los métodos de rastreo de actividad de neutralizar el anión radical superóxido y DPPH•. Se determinó el contenido fenólico y la materia seca (DM). La DM tuvo un rango de entre 2,9 a 4,2 mg/mL. Se encontró un contenido significativamente mayor en los extractos de frutos de origen local. El rango de los contenidos fenólicos totales fue de 9,4 a 13,6 mg/g. Los extractos de frutos importados fueron más ricos en polifenoles. La actividad de eliminación de aniones superóxidos fue de 45,4% y 47,5%. La actividad DPPH• fue de 48,5% y 53,1%. No se encontraron diferencias significativas. El valor DPPH• de IC_{50} fue 40,0 - 43,1 μg/mL. No se encontraron diferencias significativas en la actividad antioxidante entre frutos locales e importados.

Palabras clave: Antioxidantes; Actividad antiradical; Estrés oxidativo; Extractos de plantas; Polifenoles

INTRODUCTION

Berries of *Rubus spp*. (*Rosaceae*) are valued by consumers not only for their high nutritional value, but also for their physical and mental health benefits (Tavares *et al.*, 2012; Ivanovic *et al.*, 2014, Huang *et al.*, 2023). The species are famous for their edible fruits, which are traded worldwide due to their delicious taste, pleasant aroma and nutritional profile (Van de Velde *et al.*, 2016). The beginning of the trading with blackberries and raspberries began in Europe in mid-sixteenth century and they were used fresh or as jams and juices (Toshima *et al.*, 2021). Organic agricultural production of berries (excluding strawberries) in Europe increased from 29,933 tons to 69,506 tons between 2012 and 2018 (Eurostat, 2020). Argentina, for example, also exports 40% of its berry production. The export markets are in Canada, the United States of America and the countries of the European Union (Molina *et al.*, 2007), especially during the low season for berries in these countries (Van de Velde *et al.*, 2016). The fruits contain a rich source of bioactive substances with antioxidant potential such as retinol (vitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E), carotenoids, flavonoids and other phenolic compounds (Verma *et al.*, 2009; Hangun-Balkir & Mc Kenney, 2012). One of the most important and widely used plant antioxidants are polyphenols, including plant pigments, e.g. anthocyanins (Gupta & Sharma, 2006; Castaneda-Ovando *et al.*, 2012; Toshima *et al.*, 2021). These water-soluble pigments give the fruits a typical red, purple, blue or violet color (Andersen & Jordheim, 2006). Many types of berry fruits contain a rich mixture of the five most important anthocyanin aglycones (delphinidin-, petunidin-, cyanidin-, peonidin- and malvidin-), which are bound to monosaccharides (Poracova *et al.*, 2016; Huang *et al.*, 2022). Anthocyanins are the most important flavonoids in blackberry and raspberry fruits and contribute significantly to their antioxidant activity (Milivojević *et al.*, 2011; Pérez-Gregorio *et al.*, 2011; Dujmović Purgar *et al.*, 2012). Natural substances, especially phenols, contained in fruits play an important role in the prevention of many diseases (Kondakova *et al.*, 2009; Park *et al.*, 2019; Serino *et al.*, 2020; Dou *et al.*, 2021; Toshima *et al.*, 2021).

Antioxidants from natural products play many beneficial roles inprotecting cells from the harmful effects of free radicals and also help to eliminate oxidative damage to biomolecules (Toshima *et al.*, 2021). Interest in antioxidants from natural sources (e.g. plants and fruits) is increasing (Molyneux, 2004; Poracova *et al.*, 2011). These substances can scavenge free radicals and act as antioxidants (Guardado Yordi *et al.*, 2012; Ignat *et al.*, 2013). The high content of polyphenols is an indicator of presumed antioxidant activity (Abdel-Hameed, 2009; Sedlak *et al.*, 2016).

Reactive oxygen species play a role in the damage of various tissues, can limit or impair their function (Verma *et al.*, 2009) and have a negative effect on biomolecules (e.g. DNA, lipids and proteins) (Shukla *et al.*, 2009). The increased accumulation of free radicals leads to oxidative stress in the organism. This is involved in the development of human diseases (e.g. cancer, neurodegenerative and cardiovascular diseases, diabetes, arteriosclerosis, arthritis, inflammatory processes). The destructive effects of free radicals can be eliminated by taking antioxidants (Diaconeasa *et al.*, 2015). The antiradical activity of natural antioxidants represents the defense mechanisms against harmful processes in an organism (Jirovský, 2007).

Plants are also an unlimited source of bioactive compounds. Studies have confirmed the bioactive properties of polyphenols: antioxidant, anti-inflammatory, antiplatelet, hypocholesterolemic, vasorelaxant, antimicrobial, antiviral and gastroprotective effects (Subedi *et al.*, 2012; Ignat *et al.*, 2013; Pinho *et al.*, 2014; Nesello *et al.*, 2017; Goodman *et al.*, 2021; Lee *et al.*, 2024). Attention has been focused on the protective effects of polyphenols in cardiovascular diseases, cancer, stimulation of the immune system, modulation of detoxification enzymes, lowering of blood pressure and improvement of endothelial vascular functions (Domínguez-Ávila *et al.*, 2017). Based on numerous studies on antioxidants, dark-colored fruits are a potentially rich source of natural antioxidants and are widely used as medicinal, dietary and nutritional supplements (Gogaľová *et al.*, 2017, Huang *et al.*, 2022, Grabek-Lejko *et al.*, 2022; Varzaru *et al.*, 2024).

Although berries from different plants that can easily adapt to different ecological conditions, it is known that the growing environment can influence phenolic concentrations and profiles as well as the health-related effects of the berries (Grace *et al.*, 2014). In addition, there is increasing evidence that genotype can have a profound effect on the content of bioactive compounds and on the antioxidant and anti-inflammatory properties of berries (Anttonen & Karjalainen, 2005; Cuevas-Rodriguez *et al.*, 2010).

Most studies focus on comparing different parameters using different methods in berries harvested locally or comparing different cultivars (Van de Velde *et al.*, 2014; Mitic *et al.*, 2014; Wajs-Bonikowska *et al.*, 2017; Mihailović *et al.*, 2019; Anjos *et al.*, 2020). A simple comparison of biological activity between berries from different sources for their practical application is lacking.

The aim of the present study was to compare the antioxidant activity and total phenolic content of two fruit species, blackberries and raspberries, harvested in eastern Slovakia as a local source with those from an imported fruits from organic store.

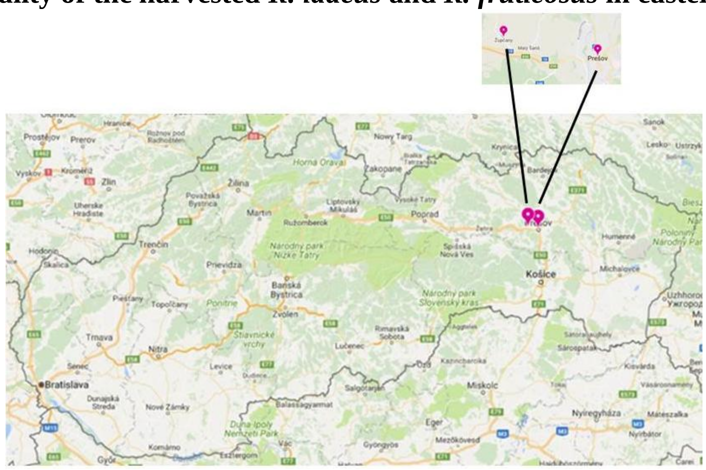
MATERIAL AND METHODS

Plant material

The plant material was raspberry (*Rubus idaeus* L.) and blackberry (*Rubus fruticosus* L.) fruits from two sources - locally harvested in eastern Slovakia private gardens and imported frozen berries from a health food store. Local ripe fruits of *R. idaeus* were harvested on July 16, 2017 and *R. fruticosus* on August 6, 2017 at Župčany (altitude 311m, a.s.l. N 49°01'00", E 21°16'00") near the town of Prešov in eastern Slovakia (Figure 1). After harvesting, all fruit samples were stored in a freezer at -20°C.

The imported frozen berries of *R. idaeus* came from the company Natural-Cool (Belgium) and *R. fruticosus* from the company ANO (Czech Republic). Both berries were packaged in 300 g and 350 g packs respectively and stored in the freezer at -18°C.

Figure No. 1
Map of locality of the harvested *R. idaeus* and *R. fruticosus* in eastern Slovakia



Chemicals and equipment

Chemicals and equipment used for the analyses were as follows: 96% ethylalcohol p.a. (Centralchem, Slovakia); distilled water; DPPH• (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany); $NaH_2PO_4.2H_2O$ (Merck, Germany); $Na_2HPO_4.12H_2O$ (Merck, Germany); EDTA (ethylenediamine-tetraacetic acid) (Sigma Aldrich, Germany); hypoxantin (Sigma Aldrich, Germany); xantinoxidase (X4376-5UN) (Sigma Aldrich, Germany); NTB (N6876, nitrotetrazolium blue chloride) (Sigma Aldrich, Germany); HOC_6H_4COONa (sodium salicylate) (Centralchem, Slovakia); Folin-Ciocalteau phenol reagent, (Merck, Germany); Na_2CO_3 (sodium carbonate) (Centralchem, Slovakia); L-ascorbic acid (Merck, Germany); $C_7H_6O_5$ (Gallic acid) (Merck, Germany); filter paper (KA-1-M, diameter: 150 mm) (Papírna Perštejn s.r.o., Czech Republic); water bath (Heidolph, Germany), hotair stabilizer (Memmert, Germany); ultrasound stirrer (Bandelin, SONOREX DIGITEC DT 100 H, Germany); UV spectrophotometer (SHIMADZU UV-1800, Japan); Soxhlet apparatus.

Extract preparation

The fruit extracts were prepared by the following percolation extraction procedure with ethanol according to a previous study (Cardona *et al.*, 2017). 5 g of frozen fruits were homogenized in a mortar with 100 mL of 70% ehanol. The percolation time for the extraction took 72 hours in a cool and dark place. Afterwards, the mixtures were filtered three times through the filter paper and stored in a freezer at -20°C until the next analysis. The extraction was performed in triplicate for each plant material.

Evaluation of dry mass of prepared extracts

The dry mass (DM) was determined gravimetrically after evaporation of the liquid portion of the extract in a drying oven at a temperature of 105°C to a constant weight. Determination was done in triplicate for each extract. Content of dry mass in extracts was expressed as mg/mL.

Total phenolic content

The total phenolic content in the ethanol extracts was determined using the Folin-Ciocalteu reagent (FCR) according to the previously described procedure (Singleton *et al.,* 1999) with slight modifications (Fejér *et al.,* 2019). The working solution was prepared by mixing one volume of FCR with nine volumes of 5% Na_2CO_3 solution. The solutions were pipetted in duplicate into the test tubes. The solutions were mixed well and after 20 minutes of incubation in a water bath (40°C), the absorbance was determined at 765 nm by spectrophotometer. Gallic acid (GA) was used as a standard and the total phenol content in extract was expressed as GA equivalents (mg GAE/L and mg GAE/g DM). All determinations of total polyphenols in the samples were performed at least four times.

DPPH• scavenging activity

The antioxidant activity of the fruit extracts was measured spectrophotometrically using the modified DPPH• method (Brand-Williams *et al.,* 1995; Sanchéz-Moreno *et al.,* 1998). The DPPH• stock solution (0.06 mM) was prepared by dissolving the powdered synthetic DPPH radical in ethanol and stored in a cool and dark place. The absorbance of the samples was detected at the wavelength of 517 nm. Decrease in absorbance due to scavenging the DPPH• by antioxidants was measured at 0 min and then every 5 min until the value stabilised. The percentage of inhibition of DPPH• activity (%) was calculated according Cipak *et al.* (2006), using the formula:

$$\text{\% of DPPH• inhibition} = \frac{AC(0) - AA(t)}{AC(0)} \times 100$$

Where AC(0) is the absorbance of the control (without antioxidant) at t=0 min and AA(t) is the absorbance of the sample (with antioxidant) in the final measurement time. The IC_{50} value, i.e., the concentration of the antioxidant that could scavenge 50% of DPPH radical, was calculated using the graph, in which % of inhibition was plotted against concentration (μg DM/mL). L-ascorbic acid was used as a reference antioxidant. A five-point calibration curve (5 - 102 μg/mL) was constructed to express the antioxidant activity of the fruit extracts in ascorbic acid equivalents. All determinations were performed in triplicate.

Superoxide anion radical scavenging activity

The assay was based on the work of Fridovich (1970), with slight modifications according to the previously published work (Fejér *et al.,* 2020). Phosphate buffer (PB) was prepared by dissolving 0.93 g/mL $NaH_2PO_4.2H_2O$; 6.81 g/mL $Na_2HPO_4.12H_2O$ and 0.0186 g/mL EDTA in 500 mL distilled water. The pH of the buffer was corrected to pH 7.4. Hypoxanthine (HX 0.0056 g) was dissolved in 100 mL phosphate buffer. Then 0.01 g xanthine oxidase (XO) was dissolved in 20 mL phosphate buffer and stored in a refrigerator at 4°C. 0.05 g nitroblue tetrazolium chloride (NBT) was dissolved in 12.2 mL distilled water. Finally, a control sample of 0.1604 g HOC_6H_4COONa (SO) was prepared in 100 mL PB. The antioxidant activity of the tested samples against superoxide radicals was compared with the antioxidant activity of sodium salicylate in phosphate buffer. The solutions were pipetted into test tubes (in duplicate) according to Table No. 1.

The solutions were mixed well and incubated in a water bath at 38°C for 40 minutes. After incubation and cooling, the absorbance of the solutions was determined in a 1 cm cuvette at 560 nm using a spectrophotometer. The antioxidant activity of each sample, expressed as percentage of inhibition (POI), was calculated according to the following formula:

Table No. 1
Reaction mixture composition [µL] in the superoxide anion radical scavenging activity determination

Test tube	PB	HX	XO	NBT	SO
0	2550	200	-	50	-
X0	2350	200	200	50	-
0-SO	2525	200	-	50	25
SO	2325	200	200	50	25
0-S_x	2525	200	-	50	25
S_x	2325	200	200	50	25

0=blank; PB=phosphate buffer; HX=hypoxanthine; XO=xanthine oxidase; NBT=nitro blue tetrazolium chloride; SO=sodium salicylate; Sx=sample of extract

$$\mathrm{POI} = \frac{[\mathrm{A(X0)} - \mathrm{A(0)}] - [\mathrm{A(Sx)} - \mathrm{A(0-Sx)}]}{\mathrm{A(X0)} - \mathrm{A(0)}} \times 100$$

All determinations of antioxidant activity against superoxide radicals in the samples were performed at least four times.

Statistical analysis

A multifactorial analysis of variance (MANOVA) was carried out using statistics and the Tukey test was applied post-hoc in the case of significant differences between the mean values. A confidence interval of 95% was used for the statistical analysis.

RESULTS

The dry matter content of the extracts of *R. idaeus* and *R. fruticosus* was evaluated. Two sources of the two species were examined and compared. *R. idaeus* from local harvest (RIL) and imported (RII) and *R. fruticosus* from local harvest (RFL) and imported (RFI). Significant differences were found between the dry matter of the local berries and the imported berries. The final DM represents the average of the triplicate measurements. While the DM content of the local raspberries (RIL) was 4.1 ± 0.04 mg/mL, the DM content of the imported raspberries was 10 percent lower (RII 3.7 ± 0.05 mg/mL). The same situation was found when comparing the samples of blackberries. The local sample reached a 30 percent higher DM content (4.2 ± 0.08 mg/mL) compared to the imported sample RFI (2.94 ± 0.03 mg/mL) (Table No. 2).

Table No. 2
Comparison of dry matter and total phenolic content in extracts

	Sample extracts			
	RIL	RII	RFL	RFI
DM ± SD [mg/mL]	$4.1^{b} \pm 0.04$	$3.7^{b} \pm 0.05$	$4.2^{b} \pm 0.08$	$2.9^{a} \pm 0.03$
Phenols ± SD [mg GAE/L]	$38.7^{a} \pm 3.6$	$37.2^{a} \pm 1.7$	$39.3^{a} \pm 4.2$	$40.1^{a} \pm 4.7$
Phenols ± SD [mg GAE/DM]	$9.4^{ab} \pm 0.9$	$10.1^{b} \pm 0.5$	$9.4^{ab} \pm 1.0$	$13.6^{c} \pm 1.6$

DM–dry matter, SD–standard deviation; RIL–*R. idaeus*, local; RII–*R. idaeus*, imported; RFL–*R. fruticosus*, local; RFI–*R. fruticosus*, imported; GAE–gallic acid equivalent, differences significantly in *p*<0.05 according to Multiple Range Test LSD (Least Significant Difference, ANOVA), a-c values express the evidence of differences between sample extracts

The inhibition potential of the tested samples was between 48.5% and 53.1%. No significant differences were found between the samples of *R. idaeus* (local 52.7% and harvest 51.5%). The lowest potential was found in *R. fructicosus* from local harvest and the highest potential in *R. fruticosus* from imported sample Table No. 3.

The IC_{50} value of *R. idaeus* was almost the same for both samples 42.8 µg/mL for the local sample and 42.1 µg/mL for the imported sample. Some significant differences were found between the samples of *R. fruticosus*. The IC_{50} for the local sample was 40.0 µg/mL and for the imported sample 43.1 µg/mL.

Table No. 3
Comparison of antioxidant potential of sample extracts and IC_{50}

Sample	DPPH• Inhib. [%]	SOR Inhib. [%]	SOR Inhib. [%/DM]	IC_{50} [µg/mL]	AA [mg /mL]
RIL	52.7^{c} ±1,35	47.5^{a} ±2.65	11.57^{ab} ±0.65	42.8^{c} ±4,18	0.045
RII	51.5^{b} ±4,70	46.0^{a} ±1.35	12.44^{b} ±0.37	42.1^{b} ±3,59	0.043
RFL	48.5^{a} ±4,86	46.0^{a} ±4.70	11.03^{a} ±1.13	40.0^{a} ±2,91	0.039
RFI	53.1^{c} ±2,65	45.4^{a} ±4.86	15.46^{c} ±1.65	43.1^{c} ±4,71	0.046

SOR – superoxide radical; RIL – *R. idaeus*, local; RII – *R. idaeus*, imported; RFL – *R. fruticosus*, local; RFI – *R. fruticosus*, imported; AA – L-ascorbic acid equivalent, differences significantly in p<0.05 according to Multiple Range Test LSD (Least Significant Difference, ANOVA), a-c values express the evidence of differences between sample extracts within individual methods

Superoxide is the starting material for the formation of other reactive oxygen species (hydroxyl radical, hydrogen peroxide, hydroperoxide radical). It appears to be less dangerous than the hydroxyl radical. This method utilizes the ability of the superoxide to reduce the tetrazolium salt. Under normal conditions, NTB has a yellow color, but in the presence of antioxidants with a reducing effect, its color changes to blue. The tested samples showed similar inhibition in the superoxide radical assay ranging from 45.4% to 47.5% (Table No. 3). No significant differences were found when comparing the two species. Sodium salicylate was used as a standard, which showed a prooxidant activity with a value of -9.77%.

DISCUSSION

The total value of antioxidant activity cannot be fully assessed. The reason for this is the influence of various factors that affect the change in the content of antioxidants in plant parts (e.g. the influence of climatic conditions on the growth site of the examined plants, the extraction itself, the choice of reagents, the temperature and extraction time or the storage of the fresh material) (Wang & Zheng, 2011). Polyphenol compounds from red and dark-colored fruits are generally extracted with methanol, ethanol, acetone or aqueous solvents (Hohnová *et al*, 2008). The maximum effective solutions with antiradical activity were observed in the extractions with ethanol. Ethanol extracts appear to contain a higher concentration of active partial substances that contribute significantly to the antioxidant properties (Molyneux, 2004; Peschel *et al.*, 2007; Angela & Meireles, 2008; Xia *et al.*, 2010; Bidchol *et al.*, 2011). The obtained values of total phenolic content and total antioxidant activity seem to be more objective to compare the analyzed samples by conversion to DM (Fejér *et al.*, 2020).

Researchers (Gülçin *et al.*, 2011) determined the phenolic content of wild raspberries in Turkey, which ranged from 5.83 to 26.66 mg GA/mL extract. Our samples showed differences in the content of total polyphenols. We found a significantly higher content of phenols in raspberries. Another study (Milivojević, *et al.*, 2011) examined the fruits of raspberries and blackberries. They reported the total phenolic content in the dry matter of the blackberry variety in the range of 1.74-1.97 mg/g and in the wild raspberry 3.20 mg/g. The polyphenols in wild blackberries were 4.95 mg/g. Study from Japan (Toshima *et al.*, 2021) of different varieties of raspberries and blueberries evaluated total phenolic content as an equivalent to gallic acid of fresh weight. The highest content was found as 309.4 mg gallic acid/100 g FW and the lowest was 43.2 mg gallic acid/100 g FW). Differences were found in the higher synthesis and concentration of antioxidants between cultivated varieties and wild raspberries. Wild raspberries are exposed to extreme temperatures, pests and diseases and the synthesis of phenolic compounds normally serves as a defense mechanism. Flavonoids play a major role in antioxidant capacity and are also related to vitamins and other substances (Huang *et al.*, 2022).

The total phenol content of various blackberry varieties determined in recent studies is associated with their strong antioxidant activity. In the study, the content of phenolics was determined from 1.74 mg GA/g to 1.97 mg GA/g, they found 95.37% antioxidant activity by DPPH• method and the IC_{50} value of 0.44 mg/mL, which is comparable to our results (Huang *et al.*, 2012). It was reported that the total phenolic content of the two blackberry varieties studied was 2.35 and 2.70 mg GA/g, and the antioxidant activity was expressed by IC_{50} values of

0.0616 and 0.0646 mg/mL (Stajčić *et al.*, 2012). The results of researchers (Zia-Ul-Haq *et al.*, 2014) reported polyphenol content values of 192.8 mg/100 g to 351.7 mg/100 g in blackberry fruits. These values were higher compared to our results.

In the study, the antioxidant activity of ethanol extracts from raspberries (*Rubus idaeus* L.) was tested in Lithuania using the DPPH• method (Dvaranauskait *at al.*, 2006). All tested extracts had an antioxidant activity of 52.9% to 92.6%. The total amount of phenolic compounds was determined in the range of 5.6 to 13.7 mg GA/g DM. We came to similar results in our work. The tested ethanolic extracts of raspberry showed an antioxidant activity of 52.71% and 51.53% in the DPPH• test. Their phenolic content was determined in the range of 9.44 to 10.06 mg GA/g dry matter of the extracts.

Slovakian researchers investigated the antioxidant activity of selected indigenous plants, which were expected to have a high anthocyanin content (Habánová & Habán, 2008). In the DPPH• tests, the forest blackberry achieved 75.26% antioxidant activity, the garden blackberry 62.80% and the mulberry 82.39%. Our tested samples showed a lower antioxidant activity against DPPH•. It amounted to 48.47% for the cultivated blackberries and 53.14% for the imported blackberries.

The study (Ryu *et al.*, 2016) investigated the properties of blackberry fruits (*Rubus fruticosus* L.) and their functional compounds with antioxidant capacity. They determined the total phenolic content in the values of 23.6-84.4 mg/g. Our analyzed blackberry samples contained total polyphenols in the amount of 9.42-13.64 g/kg. One blackberry sample purchased in the market had the highest phenolic content (13.64 g/kg) and the lowest dry matter content (2.94 mg/mL) of all extracts tested.

In another study an IC_{50} value of 13.74 μg/mL was determined in the fruits of *Rubus niveus* Thunb. using the DPPH method. The IC_{50} values of the raspberry extracts were 42.84 μg/mL for locally grown raspberries and 42.09 μg/mL for imported raspberries.

The antioxidant activity of raspberries, blackberries, blueberries and acai fruit was tested using the DPPH• method (Hangun-Balkir & Kenney, 2012). The authors found that blackberries (IC_{50}=1.40 mg/mL) and raspberries (IC_{50}=0.80 mg/mL) had the highest antioxidant activity. In study from China (Huang *et al*., 2023) was reported that the correlation between antioxidant capacity and metabolites was different in the different blackberry cultivars. The total antioxidant capacity of specified cultivar 'Boysen' was significantly positively correlated with the flavonoid while the total antioxidant capacity of another variety 'Hull' was positively correlated with the phenol content.

In vitro comparisons of the antioxidant activity of fruit extracts and synthetic antioxidants showed that the natural antioxidants are significantly more effective and underlined their importance (Farghali *et al.*, 2013). The antioxidant effect of polyphenols can be influenced by interactions with other compounds or antioxidants in the diet. Antioxidant mechanisms may also involve the mutual synergistic effects of polyphenols (De Kok *et al.*, 2008).

Superoxide radicals are extremely reactive oxygen species. They are formed in the cells during aerobic metabolism. They are dangerous and trigger the formation of other reactive oxygen species. They have mutagenic potential and are involved in the development of cancer (Prasad & Srivastava, 2020). In *R. ideaus* and *R. fruticosus*, they represent a significant benefit to human health, also in terms of their high nutritional value (Buczyǹski *et al.*, 2024; Chwil *et al.*, 2023). The antiradical activity against the superoxide radical of leaf extracts (Varazu *et al.*, 2024) and fruit extracts (Martins *et al.*, 2024) or juice (Wang & Jiao, 2000) has been reported. In a study by Morosan *et al.* (2022), the antioxidant activity was evaluated using the superoxide dismutase method. The hydroalcoholic extract from the fruits of *R. idaeus* showed an antioxidant activity of 83.8%. The extract from *R. fruticosus* fruits showed a significantly lower activity of 51.0%. In our studies, the activity against superoxide radicals of *R. idaeus* extracts was significantly lower (46.0-47.5%). Extracts of *R. fruticosus* showed almost comparable activity (45.4-46.0%), as reported by Morosan *et al.* (2022).

Antioxidant activity may vary as the content of total phenols and other bioactive compounds is influenced by specific natural and environmental site and cultivation conditions (soil conditions, temperature, humidity, precipitation, climate, etc.) (Krížová *et al.*, 2010). The content of bioactive substances in the fruit also depends on many factors (e.g. location, year and genotype). Climatic conditions can influence the content of phenolic compounds, which is reflected in antioxidant activity (Dragović-Uzelac *et al.*, 2010; Vagiri *et al.*, 2013). Antioxidant activity can also be increased by purification of the fruit extracts. The changes in antioxidant activity and antioxidant capacity during fruit development between blackberry and blackberry–raspberry hybrids fruits, providing some suggestions and data support for the production, cultivation and utilization of blackberry fruits (Huang *et al.*, 2023). As we have already reported, purified extracts are more concentrated in active substances due to the removal of inert components that do not contribute to antioxidant activity (Sedlak *et al.*, 2016).

CONCLUSION

The fruits of blackberries and raspberries are a rich source of biologically active substances with great potential for the pharmaceutical and food industries. Fruit extracts are a rich source of natural antioxidants and can counteract the negative effects of free radicals and contribute to the protection of human health. In the present study, known and unknown samples of two berry species were compared for their antioxidant activity that could be beneficial for human health. From the results, we can conclude that the antioxidant potential measured by two different methods DPPH• and superoxide radical - and the total phenolic content were comparable in all samples. Regardless of whether the dry matter content is high or low, even without specifying the individual polyphenols, we can say that the expected benefit was comparable in two samples of the species *Rubus idaeus*, comparing a locally harvested known sample with an imported sample from an unknown environment. The same is true for the species *Rubus fruticosus* and its local and imported samples. Both species, *R. idaeus* and *R. fruticosus*, which are very popular in different countries around the world, are characterized by their specific taste as well as their benefits for the human body.

Natural antioxidants continue to be a popular means of prevention and treatment. Natural phytochemical compounds found in local sources of red and dark-colored berry fruits provide beneficial effects in the prevention of diseases caused by high production of free radicals. The active phytochemicals can be used in the production of new alternative medicines and in the prevention of diseases related to the influence of free radicals in the human organism.

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