

Phenolics content and antioxidant activity of three *Sorbus* species

Tahirović, A.^a, Mehić, E.^b, Kjosevski, N.^c, Bašić, N.^a

^aUniversity of Sarajevo, Faculty of Forestry, Department of Forest Ecology, Zagrebačka 20, Sarajevo, B&H

^bDžemal Bijedić University of Mostar, Faculty of Education, Department of Chemistry, University campus,
Mostar, B&H

^cINSPEKT RGH doo, Hamdije Kreševljakovića 18, Sarajevo, B&H

Article info

Received: 17/04/2019

Accepted: 07/10/2019

Keywords:

Sorbus species

Phenolics

Antioxidant activity

DPPH

ABTS

FRAP

*Corresponding author:

Azra Tahirović

E-mail: a.tahirovic@sfsa.unsa.ba

Phone: 00-387-33-812-490

Abstract: The phenolic content and antioxidant activity of *Sorbus aucuparia* L., *Sorbus aria* (L.) Crantz and *Sorbus austriaca* (Beck) Hedlund leaves and fruit were investigated. The quantification of total phenolics, flavonoids and phenolic acids was performed using the Folin-Ciocalteu, Dowd and Arnow methods, respectively. The antioxidant activity of the extracts was evaluated using DPPH, TEAC and FRAP methods with Trolox as a standard. Leaves had a higher content of phenolic compounds and antioxidant activity than the fruits for all species. The highest content of phenolics (76.11 mg gallic acid equivalents (GAE)/g plant), flavonoids (15.86 mg rutin equivalents (RE)/g plant) and phenolic acids (44.54 mg caffeic acid equivalents (CAE)/g plant) was determined for *S. austriaca* leaves. *Sorbus austriaca* fruit had the highest content of phenolics (13.21 mg GAE/g plant), flavonoids (1.82 mg RE/g plant) and *S. aucuparia* fruit had the highest content of phenolic acids (9.05 mg CAE/g plant). The antioxidant activity was in the range: DPPH=38.42–274.52 μ mol Trolox equivalents (TE)/g plant; TEAC=43.23–403.02 μ mol TE/g plant; FRAP=47.13–706.96 μ mol TE/g plant. The highest values of antioxidant activity were found for *S. austriaca* leaf and fruit extracts while the lowest values were determined for *S. aucuparia* leaves and *S. aria* fruit. The antioxidant activity was highly correlated with total phenolics and phenolic acids.

INTRODUCTION

Sorbus L. genus includes about 250 species of deciduous trees and shrubs mainly widespread in the northern hemisphere (Olszewska and Michel, 2009). It is estimated that about one-third of *Sorbus* diversity in Europe is located in the Balkan Peninsula, including 18 species and 12 subspecies of the genus (Hajrudinović, Siljak-Yakovlev, Brown, *et al.*, 2015b and references therein). So far, eight *Sorbus* species have been recognized in Bosnia and Herzegovina including *Sorbus aria* (L.) Crantz, *Sorbus aucuparia* L., and *Sorbus austriaca* (Beck) Hedl. (Beck-Mannagetta, 1927; Hajrudinović, *et al.*, 2015b). Fruit of *S. aucuparia*, *S. aria*, *S. domestica* and *S. torminalis* have been used for medicinal purposes and as food ingredients in the production of jams and juices. (Mikulić-Petkovsek, Krška, Kiprovska *et al.*, 2017 and references therein). Extracts of leaves, inflorescences,

fruit and bark of various *Sorbus* species are used for their hypoglycaemic, diuretic, vasoprotective, anti-inflammatory and antidiarrhoeal properties (Raudonė, Raudonis, Gaivelyte, *et al.*, 2015 and references therein). In particular, inflorescences of *S. aucuparia* are used in traditional and European medicine as a diuretic and anti-inflammatory agent. The fruit of *S. aucuparia* and *S. aria* is used as a vitamin and antioxidant agent and for the treatment of diarrhoea (Olszewska and Michel, 2009 and references therein). Some species such as *S. aucuparia*, *S. aria* and *S. domestica* have been identified as possible rich sources of phenolics (Olszewska and Michel, 2009). The pharmacological properties of *Sorbus* species have been related to the presence of different phenolic compounds as the main antioxidants (Hukkanen, Pölönen, Kärenlampi, *et al.*, 2006). The presence of polyphenols,

flavonoids (quercetin derivatives rutin, hyperoside, isoquercitrin), phenolic acids (chlorogenic, neochlorogenic, caffeic acids), proanthocyanidins in leaves and fruit has already been mentioned by several authors (Olszewska, Nowak, Michel, *et al.*, 2010; Gaivelyte, Jakstas, Razukus, *et al.*, 2014; Raudoné, *et al.*, 2015). The fruit is also rich in tocopherols, ascorbic acid, carotenoids and anthocyanins (Mrkonjić, Nadpal, Beara, *et al.*, 2017; Šavikin, Zdunić, Krstić-Milošević, *et al.*, 2017, Mikulić-Petkovsek, *et al.*, 2017 and references therein). Most research on the antioxidant activity of various plant materials has been associated with their application in the food and pharmaceutical industries as a possible source of new natural additives and antioxidants that could replace synthetic ones (Finley, Kong, Hintze, *et al.*, 2011; Surwesvaran, Cai, Corke, *et al.*, 2007). Therefore, the aim of this study was to estimate the phenolic content and antioxidant activity of leaves and fruits of *S. aucuparia*, *S. aria* and *S. austriaca* from Bosnia and Herzegovina. Antioxidant activity was estimated using three different methods: DPPH, TEAC and FRAP with Trolox as a standard. The determination of total phenolics, total flavonoids, and total phenolic acids was conducted using the Folin–Ciocalteu, Dowd, and Arnov methods, respectively. Relationships between phenolic compounds and antioxidant activity were investigated. To our knowledge, this is the first report on phenolic content and antioxidant activity of selected *Sorbus* species from Bosnia and Herzegovina.

EXPERIMENTAL

All chemicals were of analytical grade. Caffeic acid was purchased from Merck Chemical Suppliers (Germany), and potassium peroxydisulfate from Fluka (Germany). Sodium acetate, sodium nitrite and sodium hydroxide were purchased from Kemika, (Croatia), and sodium molybdate from Acros Organics (USA). All other chemicals were obtained from Sigma-Aldrich (Germany).

Plant material

Leaf and fruit samples of *S. aucuparia*, *S. austriaca* and *S. aria* were collected in October 2016, in the area of Sarajevo, Bosnia and Herzegovina. Samples were identified by a plant taxonomist (one of the authors), and voucher specimens were stored in the Herbarium of Forest Ecology at the Faculty of Forestry. The samples were dried in a ventilated room for 15–20 days, and they were stored in paper bags in a dry and dark place until analysis.

Extraction procedure

Dried fruits and leaves were pulverised in an electric mill (Gorenje, Slovenia), and then extracted with 80% (*v/v*) aqueous methanol by ultrasound extraction (Ultrasound bath, Elmasonic, Italy). A modified extraction method previously described by Memon, Memon and Luthria (2010) and Raudonis, *et al.* (2014) was used. Briefly, plant material (0.5 g) was extracted twice with 25 mL of methanol (80%, *v/v*) for 10 minutes at 30°C. After the

centrifugation (3000 rpm, 10 min), the resulting supernatants were combined, filtered (Millipore nylon filters, 0.45 µm) in a 50 mL volumetric flask and supplemented with the extraction medium to the mark. The extracts were stored at -20°C until use. Yields were determined by evaporation of extracts (5 mL) to dryness.

Determination of total phenolics

Total phenolics were determined by the Folin–Ciocalteu method as modified and described by Luthria, Mukhopadhyay and Krizek (2004). Sample solution (0.1 mL) was mixed with distilled water (7.9 mL) and Folin–Ciocalteu reagent (500 µL) was added. After 5 minutes, Na₂CO₃ (20%, 1.5 mL) was added, and the total volume was adjusted with distilled water up to 10 mL. Samples were left for 30 minutes at 40°C in a water bath (INKO 1935, Zagreb). Absorbance was read at 765 nm against a blank. Gallic acid was used to prepare the standard curve, and the results were expressed as milligrams of gallic acid equivalents per gram of dry plant (mg GAE/g plant) and per gram of extract (mg GAE/g extract). A Shimadzu UV-mini 1240 spectrophotometer was used for all spectrophotometric determinations.

Determination of total flavonoids

A modified Dowd method was used to determine total flavonoids as described by Quettier-Deleu, Gressier, Vasseur (2000). Sample solution (1 mL) and AlCl₃ solution (2% in absolute methanol, 1 mL) were mixed. The mixture was incubated at room temperature for 1 h, and absorbance was measured at 415 nm against a blank. The standard curve was prepared with rutin, and the results were expressed as milligrams of rutin equivalents per gram of dry plant (mg RE/g plant) and per gram of extract (mg RE/g extract).

Determination of total phenolic acids

For the determination of total phenolic acids, the modified Arnov method given by Gawlic-Dziki (2012) was used. Sample solution (1 mL) was mixed with water (5 mL), HCl (0.5 M, 1 mL), Arnov reagent (1 mL) and NaOH (1 M, 1 mL), followed by the addition of distilled water to a total volume of 10 mL. The samples were incubated for 20 min at room temperature. Absorbance was measured at 490 nm against a blank. Total phenolic acids were calculated from the standard curve, and the results expressed as milligrams of caffeic acid equivalents per gram of dry plant (mg CAE/g plant) and per gram of extract (mg CAE/g extract).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Determination of the antioxidant activity with DPPH reagent (2,2-diphenyl-1-picrylhydrazyl radical) was carried out according to Thaipong, Boonprakob, Crosby (2006). The working solution of DPPH was prepared immediately before measurements by diluting the stock solution (3 mM) to the absorbance of A=1.1±0.02 at 515 nm. Sample solution (0.1 mL) and methanolic DPPH (1.9

mL) were mixed and left in the dark for 30 min. The decrease in absorbance was measured at 515 nm against methanol as a blank. The results were expressed as μmol of Trolox equivalents per gram of dry plant material ($\mu\text{mol TE/g plant}$) and per gram of extract ($\mu\text{mol TE/g extract}$) using the standard curve of Trolox.

Trolox equivalent antioxidant capacity (TEAC) assay

Determination of the antioxidant activity with ABTS⁺⁺ reagent (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was carried out following the method of Thaipong, *et al.* (2006). The ABTS⁺⁺ reagent was freshly prepared by mixing equal parts of the ABTS (7 mM) stock solution and K₂S₂O₈ (2.45 mM) solution. The mixture was left for 12 h in the dark before use. Immediately before use, the ABTS⁺⁺ solution was diluted to the absorbance of $A=1.1\pm 0.02$ at 734 nm. Sample solution (0.1 mL) was mixed with the ABTS⁺⁺ radical solution (1.9 mL) and left to stand for 10 minutes (Olszewska and Michel, 2009). The decrease in absorbance was measured at 734 nm against methanol as a blank. The results were expressed as μmol of TE per gram of dry plant material ($\mu\text{mol TE/g plant}$) and per gram of extract ($\mu\text{mol TE/g extract}$) using the standard curve of Trolox.

Ferric reducing antioxidant power (FRAP) assay

A modified FRAP method was used to determine antioxidant activity as described by Thaipong, *et al.* (2006). FRAP reagent was prepared immediately before use by mixing acetate buffer (300 mM, pH=3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and FeCl₃ (20 mM) in a volume ratio of 10:1:1. Sample solution (0.1 mL) was added to FRAP reagent (1.9 mL) in a test tube. The samples were incubated for 2 h at 37°C on a water bath (Olszewska and Michel, 2009) before measurements. Absorption of the blue-coloured complex was measured at 593 nm against a blank. The results were expressed as μmol of TE per gram of dry plant material ($\mu\text{mol TE/g plant}$) and per gram of extract ($\mu\text{mol TE/g extract}$) using the standard curve of Trolox.

Statistical analysis

All measurements were made in triplicate, and the results were expressed as the mean \pm standard deviation (SD). Data were subjected to one-way analysis of variance

followed by Duncan's multiple range test to separate the mean values. Statistical analysis was performed using IBM SPSS Statistics, version 20 (IBM Corp., Armonk, NY). The differences were considered statistically significant at $p<0.05$. The correlations between the contents of tested compounds and the antioxidant activity were determined by a linear regression method (Excel, Windows 10).

RESULTS AND DISCUSSION

Leaf and fruit extracts of the investigated *Sorbus* species were prepared by ultrasound extraction, and extraction yields were determined and presented in Table 1.

Table 1: Yields of extracts for *Sorbus* leaf and fruit samples

Plant species	Plant part	Mass of the sample (g)	Mass of the extract (g)	Yield (%)
<i>S. aria</i>	L	0.5002	0.0182	36.40
	F	0.5000	0.0352	70.40
<i>S. austriaca</i>	L	0.5001	0.0163	32.60
	F	0.5000	0.0242	48.40
<i>S. aucuparia</i>	L	0.5000	0.0176	35.20
	F	0.5002	0.0369	73.80

*L-leaves; F-fruit

The yields ranged from 48.40 to 73.80% for fruit samples, and from 32.60 to 36.40% for leaf samples. The highest yield was from *S. aucuparia* fruit, while the lowest yield was from *S. austriaca* leaves. It can be concluded that the extraction efficiency is higher for the fruit than the leaves. Similar observations were obtained in the study on the effects of extraction of *Prunus laurocerasus* leaves and fruit. Differences in extraction yields could be attributed to the presence of different compounds in each part of the plant as well as the solvent extraction activity (Karabegović, Stojičević, Veličković, *et al.*, 2014 and references therein).

The total phenolics, flavonoids and phenolic acids contents expressed as mg of standard equivalents per gram of dry plant/extract are given in Table 2. In leaf samples, the total phenolics ranged from 30.40 to 76.11 mg GAE/g plant, flavonoids from 10.94 to 15.86 mg RE/g plant, phenolic acids from 18.82 to 44.54 mg CAE/g plant. In fruit samples, determined values varied from 7.02 to 13.21 mg GAE/g plant for phenolics, from 0.87 to 1.82 mg RE/g plant for flavonoids and from 4.21 to 9.05 mg CAE/g plant for phenolic acids.

Table 2: Total phenolics, total flavonoids and total phenolic acids in *Sorbus* leaf and fruit samples.

Species	Sample	Total phenolics		Total flavonoids		Total phenolic acids	
		mg GAE/g plant	mg GAE/g extract	mg RE/g plant	mg RE/g extract	mg CAE/g plant	mg CAE/g extract
<i>S. aria</i>	Leaves	55.93 \pm 0.00 ^e	153.71 \pm 0.00 ^d	10.94 \pm 0.43 ^c	30.08 \pm 1.20 ^c	33.80 \pm 0.18 ^d	92.90 \pm 0.50 ^e
	Fruit	7.02 \pm 0.02 ^a	9.97 \pm 0.03 ^a	0.92 \pm 0.03 ^a	1.31 \pm 0.05 ^a	4.21 \pm 0.04 ^a	5.98 \pm 0.06 ^a
<i>S. austriaca</i>	Leaves	76.11 \pm 2.70 ^f	233.54 \pm 8.30 ^e	15.86 \pm 0.32 ^e	48.66 \pm 0.97 ^e	44.54 \pm 0.65 ^e	136.70 \pm 2.01 ^f
	Fruit	13.21 \pm 0.26 ^c	27.29 \pm 0.54 ^b	1.82 \pm 0.01 ^b	3.76 \pm 0.02 ^b	8.94 \pm 0.05 ^b	18.47 \pm 0.10 ^c
<i>S. aucuparia</i>	Leaves	30.40 \pm 0.04 ^d	86.33 \pm 0.11 ^c	14.01 \pm 0.15 ^d	39.81 \pm 0.43 ^d	18.82 \pm 0.10 ^c	53.48 \pm 0.28 ^d
	Fruit	10.13 \pm 0.05 ^b	13.74 \pm 0.07 ^a	0.87 \pm 0.01 ^a	1.18 \pm 0.02 ^a	9.05 \pm 0.06 ^b	12.27 \pm 0.08 ^b

Values with different upper-case letters in the same column are significantly different at $p<0.05$

Sorbus austriaca leaves had significantly higher ($p<0.05$) contents of all bioactive compounds. In general, the reported values of Olszewska and Michel (2009), Olszewska (2010, 2011) and Gaivelyte, *et al.* (2014), for total phenolics in *S. aucuparia* leaves (7.07-9.09% dry weight(DW)), are higher than in this work, and for flavonoids (0.038–1.58% DW) and phenolic acids (0.97-3.90% DW) they are comparable to the results of this study. Olszewska and Michel (2009) reported a higher content of phenols (6.06% DW) and flavonoids (1.30% DW) but lower content of phenolic acids (1.73% DW) in *S. aria* leaves than those given in this work. *Sorbus austriaca* fruit had the highest contents of phenolics and flavonoids. However, no significant difference was determined in the values of phenolic acids content between *S. austriaca* and *S. aucuparia* fruit.

In general, data for *S. austriaca* are quite limited. Raudoné, *et al.* (2015) reported similar values for total phenolic acids content between *S. aucuparia* (0.283% DW) and *S. austriaca* fruit (0.286% DW). Values for total flavonoids in *S. austriaca* fruit (0.0143% DW) and phenolic acids (0.286% DW) given by Raudonis, *et al.* (2014) were lower than the results in this study. In addition, there was no significant difference ($p<0.05$) in the values of total flavonoids content between the fruit of *S. aucuparia* and *S. aria*. According to Olszewska and Michel (2009) and Šavikin, *et al.* (2017), contents of phenolics, flavonoids and phenolic acids in *S. aucuparia* fruit yielded up to 2.68%, 0.104% and 1.52%, while for *S. aria* fruit they varied up to 2.98%, 0.093% and 0.63%, respectively. In general, the leaves have significantly higher ($p<0.05$) contents of all bioactive compounds in relation to the fruit which is in agreement with the findings of Olszewska and Michel (2009), Gaivelyte, *et al.* (2014) and references therein). Differences found between our results and previous investigations could be due to genetic factors, environmental conditions, maturity and time of harvest (Olszewska, 2011; Gaivelyte *et al.*, 2014 and references therein). It is important to emphasize that the extraction method and extraction medium could influence the differences as reported by Olszewska, Presler, Michel, *et al.* (2012), Aladedunye and Matthäus (2014). In addition, the phenolics content for *S. austriaca* and *S. aria* leaves are close to that of *Crataegus monogyna* (61.98 mg GAE/g DW), *Crataegus x macrocarpa* (82.44 mg GAE/g DW) (Tahirović and Bašić, 2015) and *Aloe littoralis* (62.00 mg GAE/g DW) (Surweswaran, *et al.*, 2007). The values are higher than those reported for *Artemisia abrotanum*

(4.9 mg GAE/g DW), *Euphorbia lathyrus* (11.50 mg GAE/g DW) and *Ocimum basilicum* (26.30 mg GAE/g DW) by Surweswaran, *et al.* (2007). On the other hand, the content of phenolics in fruit is much lower than in *Prunus spinosa* (25.14 mg GAE/g DW), *Crataegus monogyna* (28.19 mg GAE/g DW), *Rosa canina* (51.19 mg GAE/g DW) extracted with the same extraction medium (Tahirović, Bašić, Čopra-Janićijević, *et al.*, 2018; Tahirović and Bašić, 2015, 2017). In general, phenolics content in leaves of *S. austriaca* and *S. aria* were higher than 5%, so we can conclude on the basis of data and references given by Olszewska and Michel (2009) that leaves of these species represent a rich source of phenolic compounds. The results expressed per gram of extracts for leaves ranged from 86.33 to 233.54 mg GAE/g extract for phenolics, from 30.08 to 48.66 mg RE/g extract for flavonoids and from 53.48 to 136.70 mg CAE/g extract for total phenolic acids. For fruit samples, obtained values were in the range of 9.97-27.29 mg GAE/g extract for phenolics, 1.18-3.76 mg RE/g extract for flavonoids and 5.98-18.47 mg CAE/g extract for phenolic acids (Table 2). *Sorbus austriaca* is the species with the highest content of investigated bioactive compounds in leaves and fruit. In addition, there are no significant differences ($p<0.05$) in the contents of total phenolics and flavonoids for *S. aria* and *S. aucuparia* fruit samples. Mrkonjić *et al.* (2017) reported for *S. aucuparia* fruit values of 0.187 mg/g extract for total flavonoids and 0.0154 mg/g extract for phenolic acids, which is lower than the data in this study. Results of total phenolics for butanol and ethyl acetate extracts of *S. aucuparia* fruit (42-103 mg/g extract) given by Aladedunye and Matthäus (2014) were generally higher. The content of phenolics in fruit was lower than values reported for cherry laurel (42.2 mgGAE/g extract; Karabegović, *et al.*, 2014), rosehips (149.35 mg GAE/g extract; Barros, Carvalho, Ferreira, *et al.*, 2011) and hawthorn (274.27 mg GAE/g extract; Barros, Carvalho, Ferreira, *et al.*, 2011).

Antioxidant activity

The DPPH, TEAC and FRAP assays were performed to assess the antioxidant activity of leaf and fruit extracts. The obtained results are expressed as $\mu\text{mol TE/g plant}$ and $\mu\text{mol TE/g extract}$ and shown in Table 3. Antioxidant activity of *Sorbus* leaf samples ranged from 121.80 to 274.52 $\mu\text{mol TE/g plant}$ for DPPH, from 142.52 to 403.02 $\mu\text{mol TE/g plant}$ for TEAC, and from 287.03 to 706.96 $\mu\text{mol TE/g plant}$ for FRAP method.

Table 3: Antioxidant activity of *Sorbus* species leaf and fruit samples determined by DPPH, ABTS and FRAP methods

Species	Sample	DPPH		TEAC		FRAP	
		$\mu\text{mol(TE)/g plant}$	$\mu\text{mol(TE)/g extract}$	$\mu\text{mol(TE)/g plant}$	$\mu\text{mol(TE)/g extract}$	$\mu\text{mol(TE)/g plant}$	$\mu\text{mol(TE)/g extract}$
<i>S. aria</i>	Leaves	234.92±0.90 ^e	645.70±2.41 ^e	364.63±2.42 ^e	1002.13±6.64 ^e	551.94±0.33 ^e	1516.93±0.90 ^e
	Fruit	17.90±0.09 ^a	101.63±0.51 ^a	43.23±0.53 ^a	61.40±0.75 ^a	47.13±0.08 ^a	66.95±0.11 ^a
<i>S. austriaca</i>	Leaves	274.52±5.30 ^f	842.24±16.14 ^f	403.02±6.81 ^f	1236.51±20.90 ^f	706.96±29.82 ^f	2169.03±91.50 ^f
	Fruit	61.26±1.40 ^c	506.30±11.30 ^d	82.90±1.81 ^c	171.18±3.75 ^c	138.06±0.10 ^c	285.24±0.21 ^c
<i>S. aucuparia</i>	Leaves	121.80±0.80 ^d	346.02±2.30 ^c	142.52±2.34 ^d	404.90±6.64 ^d	287.03±0.32 ^d	815.41±0.92 ^d
	Fruit	38.42±0.72 ^b	208.32±3.90 ^b	67.64±1.70 ^b	91.70±2.24 ^b	101.10±0.70 ^b	137.04±1.30 ^b

Values with different upper-case letters in the same column are significantly different at $p<0.05$

Sorbus fruit samples gave results ranging from 17.90 to 61.26 $\mu\text{mol TE/g}$ for DPPH, from 43.23 to 82.90 $\mu\text{mol TE/g}$ plant for TEAC and from 47.13 to 138.06 $\mu\text{mol TE/g}$ plant for FRAP. The leaves revealed significantly higher ($p < 0.05$) antioxidant properties than those of fruit samples. The results agree with those observed by Cyboran, Bonarska-Kujawa, Pruchnik (2014) and Olszewska and Michel (2009). *Sorbus austriaca* leaf and fruit samples showed significantly higher ($p < 0.05$) antioxidant activity in the DPPH, TEAC, and FRAP assays.

The values of antioxidant activity decreased in the order: *S. austriaca* > *S. aria* > *S. aucuparia* leaves, and in the order: *S. austriaca* > *S. aucuparia* > *S. aria* fruit. Olszewska and Michel (2009) demonstrated that leaves of *S. aucuparia* have higher antioxidant activity than *S. aria*, but fruit of *S. aria* have higher antioxidant activity than *S. aucuparia*. These results could be associated with the quantitative contents of investigated compounds in the sample. The results in this study for *S. austriaca* leaves and fruit were higher than that of Raudoné, *et al.* (2015) for *S. austriaca* leaves (FRAP=78.29 $\mu\text{mol TE/g DW}$) and of Raudonis, *et al.* (2014) for *S. austriaca* fruit (TEAC=9.70 $\mu\text{mol TE/g DW}$; FRAP=9.58 $\mu\text{mol TE/g DW}$). The results of antioxidant activity given by Raudoné, *et al.*, (2015), Olszewska and Michel (2009), Olszewska *et al.* (2011) for *S. aucuparia* leaf (FRAP=144.31-1650 $\mu\text{mol TE/g DW}$) and fruit (ABTS=11.19-91.6; FRAP=11.83-347 $\mu\text{mol TE/g DW}$) and *S. aria* leaf (ABTS=265.5 $\mu\text{mol TE/g DW}$; FRAP=136.53-861.6 $\mu\text{mol TE/g DW}$) samples were in agreement with the results in this study. However, values of DPPH=400-628 $\mu\text{mol TE/g DW}$ for *S. aucuparia* leaves, collected in summer, were also reported (Olszewska and Michel, 2009, Olszewska, 2011). In general, the antioxidant activity of *S. aria* fruit was lower than the values given by Olszewska and Michel (2009). The differences can be explained in the same way as described for the phenolic content. In addition, antioxidant activity of investigated *Sorbus* fruit samples had lower values than blackthorn fruit (118.37-193.19 $\mu\text{mol TE/g DW}$), rosehip fruit (278.34-422.16 $\mu\text{mol TE/g DW}$), hawthorn species (0.11-0.51 mmol TE/g DW) determined by DPPH, TEAC and FRAP methods in 80% methanol extracts (Tahirović, *et al.*, 2018; Tahirović and Bašić, 2015, 2017). According to Surweswaran *et al.* (2007), antioxidant activity with TEAC values between 50.10 and 1000 $\mu\text{g TE/g DW}$ is considered as high. We can conclude that leaves of *Sorbus* species have high antioxidant activity, while the fruit have a medium antioxidant activity.

The antioxidant activity of *Sorbus* leaves, expressed as $\mu\text{mol TE/g}$ of the extracts, ranged from 346.02 to 842.24 $\mu\text{mol TE/g}$ extract for DPPH, from 404.90 to 1236.15 $\mu\text{mol TE/g}$ extract for TEAC and from 815.41 to 2169.03 $\mu\text{mol TE/g}$ extract for FRAP assay (Table 3). The results for *Sorbus* fruit samples varied from 101.63 to 506.30 $\mu\text{mol TE/g}$ extract for DPPH, from 61.40 to 171.18 $\mu\text{mol TE/g}$ extract for TEAC and from 66.95 to 285.24 $\mu\text{mol TE/g}$ extract for FRAP assay. It should be mentioned that FRAP values of leaves are higher than those of DPPH and TEAC assay (Table 3). Vitamin C contributes significantly to the reduction properties of

extracts, and its quantity in extracts of some *Sorbus* species was determined by several researchers (Egea, *et al.*, 2010 and references therein). The higher reduction capability could be attributed to the higher levels of strong reductants capable of donating electrons (Sasikumar, Patharaj, Adithya, *et al.*, 2012).

Correlation coefficients were investigated among the total phenolics, flavonoids, phenolic acids, and antioxidant activity with a linear regression method. The obtained results are presented in Table 4. The total phenols ($r^2=0.7761-0.9981$) and total phenolic acids ($r^2=0.7672-0.9969$) are highly correlated with DPPH, TEAC and FRAP activity. Correlations between total flavonoids ($r^2=0.3984-0.7974$) and antioxidant activity were lower. Higher correlation coefficients are probably associated with higher total phenolics and phenolic acids content of the samples (Table 2). Several studies suggest that phenolic compounds, including phenolic acids, can contribute to the antioxidant activity. The results obtained here were consistent with the results of other researchers confirming the correlations between different phenolic compounds and antioxidant activity (Olszewska and Michel, 2009; Raudonis *et al.*, 2014; Mikulić-Petkovsek, *et al.*, 2017; Šavikin *et al.*, 2017).

Table 4: Correlation coefficients between phenolics, flavonoids, phenolic acids and antioxidant activity

Bioactive compounds	Correlations for data expressed in (mg/g plant)		
	DPPH	TEAC	FRAP
Phenolics	0.985	0.9678	0.9965
Flavonoids	0.7529	0.6470	0.7488
Phenolic acids	0.9856	0.9703	0.9969
	Correlations for data expressed in (mg/g extract)		
	DPPH	TEAC	FRAP
Phenolics	0.7761	0.9753	0.9981
Flavonoids	0.3984	0.716	0.7974
Phenolic acids	0.7672	0.9775	0.9990

CONCLUSIONS

The most abundant phenolic compounds in the investigated *Sorbus* species are total phenolics and phenolic acids, while the content of total flavonoids was lower. *Sorbus austriaca* leaves and fruit had a significantly higher ($p < 0.05$) content of active compounds than other species, except the total phenolic acids in fruit. However, no significant difference ($p < 0.05$) was found in the phenolic acids content between *S. austriaca* and *S. aucuparia* fruit. In addition, *S. austriaca* leaf and fruit extracts had significantly higher antioxidant activity than extracts of other species. In general, the leaf samples have a significantly higher content of bioactive compounds and antioxidant activity than the fruit samples. Based on the results obtained in this study, we can conclude that the investigated *Sorbus* species, in particular, *S. austriaca*, represent a valuable source of natural antioxidants with the possibility of their application in medicine, pharmacy and the food industry. In this regard, more detailed investigations on the quantitative composition of individual phenolic compounds and their antioxidant properties are necessary.

REFERENCES

- Aladedunye, F., Matthäus, B. (2014). Phenolic extracts from *Sorbus aucuparia* (L.) and *Malus baccata* (L.) berries: antioxidant activity and performance in rapeseed oil during frying and storage. *Food Chemistry*, 159, 273-281.
- Barros, L., Carvalho, A.M., Ferreira, I.C. F.R. (2011). Exotic fruit as a source of important phytochemicals. Improving the traditional use of *Rosa canina* fruits in Portugal. *Food Research International*, 44, (7), 2233-2236.
- Barros, I., Carvalho, A.M., Ferreira, I.C. (2011). Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine. *Phytochemical Analysis*, 22, 181-188.
- Beck-Mannagetta, G. (1927). Flora Bosne, Hercegovine i oblasti Novog Pazara, III. Horipetale. Državna štamparija u Sarajevu, Beograd-Sarajevo, 184.
- Cyboran, S., Bonarska-Kujawa, D., Pruchnik, H., Zylka, R., Oszmianski, J., Kleszczynska, H. (2014). Phenolic content and biological activity of extracts of blackcurrant fruit and leaves. *Food Research International*, 65, Part A, 47-58.
- Egea, I., Sánchez-Bel, P., Romojaro, F., Pretel, M.T. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods for Human Nutrition*, 65, (2), 121-129
- Finley, J.W., Kong, A-N., Hintze, K.J., Jeffery, E.H., Ji, L.L., Lei, X.G. (2011). Antioxidants in foods: state of the science important to the food industry. *Journal of Agricultural and Food Chemistry*, 59, (13), 6837-6846.
- Gaivelyte, K., Jakstas, V., Razukas, A., Janulis, V. (2014). Variation of quantitative composition of phenolic compounds in rowan (*Sorbus aucuparia* L.) leaves during the growth season. *Natural Product Research*, 28(13), 1018-1020.
- Gawlic-Dziki, U. (2012). Changes in the antioxidant activities of vegetables as a consequence of interactions between active compounds. *Journal of Functional Foods*, 4 (4), 872-882
- Hajrudinović, A., Siljak-Yakovlev, S., Brown, S.C., Pustahija, F., Bourge, M., Ballian, D., Bogunić, F. (2015b). When sexual meets apomict: genome size, ploidy level and reproductive mode variation of *Sorbus aria* s.l. and *S. austriaca* (Rosaceae) in Bosnia and Herzegovina. *Annals of Botany*, 116 (2): 301-312.
- Hukkanen, A. T., Pölönen, S. S., Kärenlampi, S. O., Kokko, H. I. (2006). Antioxidant capacity and phenolic content of sweet rowanberries. *Journal of Agricultural Food Chemistry*, 54, (1), 112-119.
- Karabegović, I.T., Stojičević, S.S., Veličković, D.T., Todorović, Z.B., Nikolić, N.Č., Lazić, M.L. (2014). The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts. *Industrial Crops and Products*, 54, 142-148.
- Luthria, D.L., Mukhopadhyay S., Krizek, D.T. (2006). Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis*, 19, 771-777.
- Memon, A., Memon, N., Luthria, D.L., Bhanger, M. I., Pitafi, A. A. (2010). Phenolic acids profiling and antioxidant potential of mulberry (*Morus laevigata* W., *Morus nigra* L., *Morus alba* L.). Leaves and fruits grown in Pakistan. *Polish Journal of Food and Nutrition Sciences*, (1), 25-32
- Mikulić-Petkovsek, M., Krska, B., Kiproviski, B., Veberic, R. (2017). Bioactive components and antioxidant capacity of fruits from nine *Sorbus* genotypes. *Journal of Food Science*, 82(3), 647-658.
- Mrkonjić, Z., Nađpal, J., Beara, I., Sabo, V. A., Četojević-Simin, D., Mimica-Dukić, N., Lesjak, M. (2017). Phenolic profiling and bioactivities of fresh fruits and jam of *Sorbus* species. *Journal of Serbian Chemical Society*, 82(6), 651-664.
- Olszewska, M.A., Michel, P. (2009). Antioxidant activity of inflorescences leaves and fruits of three *Sorbus* species in relation to their polyphenolic composition. *Natural Product Research*, 23, (16), 1507-1521.
- Olszewska, M.A., Nowak, S., Michel, P., Banaszczak, P., Kicel, A. (2010). Assessment of the content of phenolics and antioxidant action of inflorescences and leaves of selected species from the genus *Sorbus sensu stricto*. *Molecules*, 15, (12), 8769-8783.
- Olszewska, M.A. (2011). Variation in the phenolic content and *in vitro* antioxidant activity of *Sorbus aucuparia* leaf extracts during vegetation. *Acta Poloniae Pharmaceutica*, 68, (6), 937-944.
- Olszewska, M. A., Presler, A., Michel, P. (2012). Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected *Sorbus* species. *Molecules*, 17(3), 3093-3113.
- Quettier-Deleu, C., Gressier, B., Vasseur J., Dine T., Brunet C., Luyckx M.C., Cazin M., Cazin, J.C., Bailleul F., Trotin F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal Ethnopharmacology*, 72, (1-2), 35-42.
- Raudonė, L., Raudonis, R., Gaivelyte, K., Pukalskas, A., Viškelis, P., Venskutonis, P. R., Janulisa, V. (2015). Phytochemical and antioxidant profiles of leaves from different *Sorbus* L. Species. *Natural Product Research*, 29 (3), 281-285
- Raudonis, R., Raudonė L., Gaivelyte, K., Višelis, P., Janulis, V. (2014). Phenolic and antioxidant profiles of rowan (*Sorbus* L.) fruits. *Natural Product Research*, 28 (16), 1231-1240.

- Sasikumar, J. M., Patharaj, J., Adithya, E. S., Christabel, P. H., Shamna, R. (2012). Antioxidant capacity and phenolic content of *Elaeagnus kologa* schlecht. An underexploited fruit from India. *Free Radicals and Antioxidants*, 2(3), 28–35.
- Surveswaran, S., Cai, Y-Z., Corke, H. Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry*, 102, (3), 938-953.
- Šavikin, K. P., Zdunić, G. M., Krstić-Milošević, D. B., Šircelj, H. J., Stešević, D. D., Pljevljakušić, D. S. (2017). *Sorbus aucuparia* and *Sorbus aria* as a Source of Antioxidant Phenolics, Tocopherols, and Pigments. *Chemistry and Biodiversity*, 14(12).
- Tahirović, A., Bašić, N. (2015). Phenolic content and antioxidant activity of *Crataegus monogyna* Jacq. and *Crataegus macrocarpa* Hegetschw. leaves and fruits extracts. *Works of the Faculty of Forestry University of Sarajevo*, 45(2) 37-51.
- Tahirović, A., Bašić, N. (2017). Determination of phenolic content and antioxidant activity of *Rosa canina* L. fruits in different extraction systems. *Works of the Faculty of Forestry University of Sarajevo*, 47 (1) 47-59.
- Tahirović, A., Bašić, N., Čopra-Janićijević, A. (2018). Effect of solvents on phenolic compounds extraction and antioxidant activity of *Prunus spinosa* L. fruits. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, 50, 19-24.
- Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevalos, L., Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Compostion and Analysis*, 19, 669-675.

Summary/Sažetak

Ispitivan je sadržaj fenola i antioksidacijska aktivnost lišća i plodova vrsta *Sorbus aucuparia* L., *Sorbus aria* (L.) Crantz i *Sorbus austriaca* (Beck) Hedlund. Kvantifikacija ukupnih fenola, flavonoida i fenolnih kiselina provedena je Folin-Ciocalteu, Dowd-om i Arnou-om metodom. Antioksidacijska aktivnost ekstrakata je procijenjena korištenjem DPPH, ABTS i FRAP metode s Troloxom kao standardom. Lišće je imalo veći sadržaj fenolnih spojeva i veću antioksidacijsku aktivnost od plodova za sve vrste. Najveći sadržaj fenola (76,11 mg ekvivalenata galne kiseline (GAE)/g biljke), flavonoida (15,86 mg ekvivalenata rutina (RE)/g biljke) i fenolnih kiselina (44,54 mg ekvivalenata kafene kiseline (CAE)/g biljke) utvrđen je za listove *S. austriaca*. Plodovi *S. austriaca* imali su najveći sadržaj fenola (13,21 mg GAE/g biljke) i flavonoida (1,82 mg RE/g biljke), a plodovi *S. aucuparia* imali su najveći sadržaj fenolnih kiselina (9,05 mg CAE/g biljke). Antioksidacijska aktivnost kretala se u području: DPPH=38,42-274,52 $\mu\text{mol TE/g}$ biljke; ABTS=43,23-403,02 $\mu\text{mol TE/g}$ biljke; FRAP=47,13-706,96 $\mu\text{mol TE/g}$ biljke. Najveće vrijednosti antioksidacijske aktivnosti utvrđene su za ekstrakte lista i ploda *S. austriaca*, dok su najniže vrijednosti utvrđene za listove *S. aucuparia* i plodove *S. aria*. Antioksidacijska aktivnost je bila u visokoj korelaciji sa ukupnim fenolima i fenolnim kiselinama.