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Fresh fruits and jam of *Sorbus domestica* L. and *Sorbus intermedia* (Ehrh.) Pers.: phenolic profiles, antioxidant action and antimicrobial activity

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ABSTRACT: A detailed examination of the phenolic profile, ascorbic acid content and antioxidant and antimicrobial capacities of extracts and jams of the fruits of two traditionally used *Sorbus* species, viz., *S. domestica* and *S. intermedia*, was carried out in the present study. Use of the LC-MS/MS technique revealed the presence and content of 44 phenolics, the most dominant compounds being amentoflavone in *S. domestica* and chlorogenic acid in *S. intermedia* extracts. Jam of both species showed the highest content of ascorbic acid. In comparison with BHT (butylated hydroxytoluene) and PG (propyl gallate) antioxidant standards, both *Sorbus* species exhibited moderate antioxidant action. Some extracts of *S. domestica* and *S. intermedia* inhibited the growth of two clinically relevant bacterial strains. The presented results support the belief that *Sorbus* fruits are food with health-promoting properties.

Keywords: *Sorbus*, phenolics, ascorbic acid, antioxidant action, antimicrobial activity

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INTRODUCTION

Nowadays consumer interest in foods or their particular compounds that have health-promoting effects is increasing enormously worldwide. Accordingly, the term functional food, meaning food that ensures additional physiological benefits beyond just providing basic nutritional values, has been introduced in food science (Siró *et al.* 2008). Numerous scientific results have confirmed that functional foods and their nutraceuticals promote health and mitigate certain diseases, such as cancer, cardiovascular disorders and diabetes, in addition to reducing healthcare costs, which further contributes to their recognition (Shahidi 2009). Since a vast number of naturally occurring health-promoting agents are plant-derived, greater attention should be given to plant-derived foods and confirmation of their beneficial role in health.

Fruits of *Sorbus* species (Rosaceae, Maloideae) are sour berry-like fruits with a unique flavour. They are consumed after the first autumn frost as overripe fruits having a soft texture and sweet flavour. These fruits have been traditionally used for the production of preserves such as jam, jelly, syrup, compote, liquor, wine or tea. In addition, *S. domestica* is used in production of alcohol drinks like schnapps, liquor and cider called „*Sorbette*" in France and „*Sperbel*" in Germany (Barbieri *et al.* 2011; Lim 2012). *Sorbus* fruits are also well known worldwide as a traditional remedy for treating respiratory tract infections, fever, cold, flu, rheumatism, gout, anaemia, oedema, dyspepsia and various digestive dis-

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orders. In addition, recent pharmacological studies suggested that fruits of *Sorbus* species possess antioxidant, antidiabetic, anti-inflammatory, antimicrobial and antiproliferative activities (Hukkanen *et al.* 2006; Ganhão *et al.* 2010; Vogl *et al.* 2013; Uddin *et al.* 2014).

The best-known and most utilised *Sorbus* species, *Sorbus aucuparia* L., has been extensively investigated in studies of its phytochemical profile and biological potential in which its beneficial effects on health were confirmed. On the other hand, there are limited data on other edible *Sorbus* species, such as *S. domestica* L. (service tree) and *S. intermedia* (Ehrh.) Pers. (Swedish whitebeam), even though their traditional use is recognised. To be specific, fruits of *S. domestica* have been traditionally used against chronic diarrhea, against dysentery and in the treatment of pyelonephritis (Tuzlaci & Aymaz 2002; Kültür 2007; Termentzi *et al.* 2008a; Miletić & Paunović 2012; Polat & Satil 2012). In Europe it is traditionally believed that consumption of *S. domestica* fruits reduces complications caused by diabetes, such as peripheral neuropathy, nephropathy, retinopathy and cataract (Termentzi *et al.* 2008a). It has been suggested that the high aldose reductase inhibitory activity exhibited by fruits of *S. domestica* greatly contributes to its antidiabetic properties (Termentzi *et al.* 2008b). Nevertheless, there are only a few studies on the content of phenolics (Ölschläger *et al.* 2004; Termentzi *et al.* 2008a, b; Piagnanini *et al.* 2012; Forino *et al.* 2015), carotenoids (Egea *et al.* 2010), vitamins (A, B_2 and C) (Brindza *et al.* 2009; Egea *et al.* 2010; Altuntas *et al.* 2015) and micro- and macroelements (Majić *et al.* 2015) in *S. domestica* fruits. Also, their notable antioxidant potential was reported previously (Ölschläger *et al.* 2004; Termentzi *et al.* 2006; Egea *et al.* 2010; Piagnanini *et al.* 2012; Forino *et al.* 2015). A more detailed report on the antioxidant action of *S. domestica* fruits, together with data indicating significant differences in the profile and content of phenolics at different stages of fruit maturity, was given by Termentzi *et al.* (2006).

On the other hand, to the best of our knowledge, only one study showed that fruits of *S. intermedia* are rich in flavonols (Olszewska 2008), whereas there are no data on biological activity, except for poorly argued evidence of an antioxidant potential (Olszewska & Michel 2009).

Bearing in mind that fruits of *S. domestica* and *S. intermedia* are commonly present in the human diet and traditional medicine, we decided to make them the focus of the present research. To be specific, the aim of this study was to conduct a comprehensive investigation of the phenolic profile and ascorbic acid content of fruits of the insufficiently investigated *S. domestica* and completely unexplored *S. intermedia*. In addition, a detailed evaluation of their antioxidant action and antimicrobial potential was conducted using six *in vitro* assays. For this purpose, water and methanol extracts were made of fresh fruits, and jams were prepared according to a traditional Serbian recipe.

MATERIALS AND METHODS

Chemicals and reagents. All phenolic compound standards and all other chemicals were acquired from Sigma–Aldrich Chem (Steinheim, Germany), Fluka Chemie GmbH (Buchs, Switzerland) or ChromaDex (Santa Ana, USA). All reagents used in this study were of analytical grade.

Collection of plant material and preparation of extracts. Plant material (fruits) of *S. domestica* L. 1753 was collected in October of 2013 in the village of Vranjak on Mt. Trebava in the Republic of Bosnia and Herzegovina, while *S. intermedia* (Ehrh.) Pers. 1806 was collected in November of 2013 on the outskirts of the city of Novi Sad in the Republic of Serbia. The specimen vouchers (*S. domestica* No. 2-1566 and *S. intermedia* No. 2-1572) were prepared and identified by Dr. Goran Anačkov and deposited in the herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Sciences University of Novi Sad, Republic of Serbia.

Three types of extracts of each species were prepared according to a previously published procedure (Mrkonjić *et al.* 2017): methanol extracts of fresh fruits with seeds (M), water extracts of fresh fruits with seeds (W) and jam extracts (J). All extracts were prepared in triplicate, making 18 extracts in total.

Briefly, for preparation of the water and methanol extracts, 30 g of fresh fruits was ground and immediately extracted using a method involving maceration with boiling distilled water (for extracts) or 80% aqueous methanol (for methanol extracts) (both, 1 mL of solvent/0.1 g of plant material) and constant shaking at 120 rpm/min for 1 h (for water extracts) or 72 h (for methanol extracts) at room temperature. Plant material leftovers were removed by filtration and solvents (water or 80% aqueous methanol) were evaporated *in vacuum* at 40°C. Crude residues were dissolved in hot distilled water. Non-polar compounds were removed using extraction with petroleum ether (fraction 40–60°C) and concentrated to dryness under a vacuum. The obtained extract yields were: 15.33 and 12.07% for M and W extracts of *S. domestica* fruits, respectively; and 9.84 and 6.84% for M and W extracts of *S. intermedia* fruits, respectively.

Jam was prepared according to a traditional Serbian recipe previously described by Mrkonjić *et al.* (2017). For preparation of jam extracts, 10 g of each jam sample of *Sorbus* species was weighed out and concentrated to dryness *in vacuum* at 40°C. Crude residues of jam were dissolved in hot distilled water (10 mL of water/1g of crude residue). Extracts were filtered and concentrated to dryness *in vacuum* at 40°C. The obtained yields of jam

extracts were 27.27 and 16.19% for *S. domestica* and *S. intermedia*, respectively.

Dried residues of methanol, water and jam extracts were dissolved in distilled water to obtain 300 mg/mL stock solutions and used for evaluation of ascorbic acid content and determination of antioxidant action and antimicrobial activity. Additionally, dried extracts were dissolved in distilled water to obtain 20 mg/mL stock solutions for LC-MS/MS analysis. All extracts were stored in a freezer until analysis.

LC-MS/MS analysis of single phenolic compounds. Quantification of 44 selected phenolics in extracts of *S. domestica* and *S. intermedia* was carried out according to a previously published procedure (Orčić *et al.* 2014). In brief, samples and standards (prepared in serial dilutions ranging from 1.53 to $25.0/10^{-3}$ ng/mL) dissolved in a mixture of 0.5% formic acid and methanol (in a 1:1 ratio) were analysed using an Agilent Technologies 1200 Series HPLC instrument coupled with an Agilent Technologies 6410A QqQ mass spectrometer with an electrospray ion source and controlled by the Agilent Technologies MassHunter Workstation software (ver. B.03.01). Injection volume was 5 µL. The column used in this study was a Zorbax Eclipse XDB–C18 column from Agilent Technologies – a rapid resolution column with length of 50 mm, radius of 4.6 mm and particle size of 1.8 µm. This column is commonly used in our laboratory for chemical characterisation of phenolic compounds and has shown itself to possess exceptional separation properties. Consisting of 0.05% aqueous formic acid (A) and methanol (B), the mobile phase was delivered at a flow rate of 1 mL/min in gradient mode (0 min 30% B, 6 min 70% B, 9 min 100% B, 12 min 100% B, post time 3 min). Ion source parameters were as follows: nebulisation gas pressure 40 psi, drying gas flow rate 9 L/min, temperature 350°C and capillary voltage 4000 V. All compounds were detected in negative mode, using dynamic selected reaction monitoring with optimised compound-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage). Calibration curves were plotted and concentrations of phenolics in extracts were calculated using OriginLabs Origin Pro (ver. 8.0).

For quantification of the compounds specified in this paper, a previously published procedure of Orčić *et al.* (2014) was used. The cited paper indicates that solvent calibration standards were analysed in five replicates each, with relative standard deviation of repeatability being plotted against concentration. The value of Loq was then estimated as the lowest concentration resulting in acceptable repeatability (<20%). That of Lod was estimated as the lowest concentration resulting in a well-defined peak. The compounds analysed were as follows: *p*-hydroxybenzoic, protocatechuic, ferulic, chlorogenic, 2,5-dihydroxybenzoic, vanillic, gallic, cinnamic, caffeic, syringic, *o*-coumaric, *p*-coumaric, 3,4-dimethoxycinnamic, sinapic and quinic acids, in addition to amentoflavone, quercetin-3-*O*-glucoside, hyperoside, epicatechin, catechin, apigenin, apigenin-7-*O*-glucoside, baicalin, baicalein, apiin, daidzein, naringenin, vitexin, genistein, isorhamnetin, luteolin, luteolin-7-*O*-glucoside, myricetin, kaempferol, kaempferol-3-*O*-glucoside, epigallocatechingallate, chrysoeriol, quercetin, quercitrin, rutin, umbelliferone, scopoletin, aesculetin, matairesinol and secoisolariciresinol.

Ascorbic acid content. The content of ascorbic acid was examined by a previously described method (Mrkonjić *et al.* 2017). In brief, prepared extracts in *meta*-phosphoric acid (30 µL) were mixed with 270 µL of 2,6-dichlorophenolindophenol (72 mg/mL) and absorbance was measured within 5 min at 515 nm. Ascorbic acid content was determined using the standard calibration curve of ascorbic acid (in a range of from 0 to 320 µg/mL) and results are presented as the mean value of three measurements.

Antioxidant activity. The reason for examining the antioxidant potency of *S. domestica and S. intermedia* derives from the fact that antioxidants can be effective in the prevention and treatment of numerous chronic diseases, such as metabolic syndrome, cardiovascular disease and neurodegenerative disorders. In order properly to evaluate their antioxidant potential, several *in vitro* assays showing neutralisation of different reactive species, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH), super oxide anion ($O_2^{\bullet-}$), nitric oxide ($^{\bullet}NO$) and hydroxyl ($HO^{\bullet}$) radical scavenger capacity tests, reducing power (FRAP) assay and test of lipid peroxidation (LP) inhibition were performed according to previously described procedures (Beara *et al.* 2014). The results obtained were compared to the synthetic antioxidants PG and BHT. The percentage of inhibition achieved by different concentrations of extracts in the antioxidant assays was calculated by the following equation: $I\ (\%) = (A_0 - A)/A_0 \times 100$, where A_0 is absorbance of the control reaction and A is absorbance of the examined samples, corrected for the value of the control. The corresponding inhibition–concentration curves, as well as the calibration curves, were drawn using OriginLabs Origin Pro (ver. 8.0), and IC_{50} values (concentration of extract that inhibited $DPPH^{\bullet}$, $^{\bullet}NO$, $O_2^{\bullet-}$, $HO^{\bullet}$ and malondialdehyde production by 50%) were determined.

Antimicrobial activity. The antimicrobial activity of extracts was tested using the microdilution technique against reference bacterial strains from the American Type Culture Collection (ATCC), viz., *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). The method used herein is not described in detail since it is a globally applied assay which was published previously (Mrkonjić *et al.* 2017). To sum up, the method is

based on reduction of TTC (2,3,5-triphenyltetrazolium chloride) to formazane. The lowest concentration of extracts that inhibited bacterial growth, which was identified by the absence of red formazane, was considered as the MIC (minimal inhibitory concentration). Determination of MIC values was carried out in three replicates and three independent experiments. Amikacin was used as a positive control.

Statistical analysis. The final results were expressed as the mean ± standard deviation (SD) of the measurements of three separate extracts, while measurements for each extract were done in three different trials. Comparison of group means and significance of the difference between groups were verified by Student's t-test. Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phenolic profile. Phenolic compounds are widely distributed in plant tissues and have a protective role and numerous beneficial health effects on the human organism. Their health-promoting effects are based on their antioxidant, antiproliferative, anticancer and anti-inflammatory activities, as well as their antibacterial, antiviral and antifungal potential (Halliwell 2006). Accordingly, we felt that it would be worthwhile to examine the detailed phenolic profiles of *S. domestica* and *S. intermedia* fruits using the LC-MS/MS technique and mark which phenolics could be carriers of their biological activity.

The conducted analysis yielded determination of 15 phenolic acids, 25 flavonoids, three coumarins and two lignans, and it resulted in quantification of 10 phenolics (Table 1).

Regarding phenolic acids, protocatechuic acid was the most abundant acid among extracts of *S. domestica* fruits, particularly in the methanol extract, while chlorogenic acid was dominant in *S. intermedia* extracts. Examination of selected flavonoids showed that amentoflavone and quercetin-3-*O*-glucoside were prevalent in both species. While considerable amounts of hyperoside, epicatechin and catechin were determined among *S. domestica* extracts, they were not present in *S. intermedia*. Moreover, coumarins and lignans were not detected in the examined extracts. Altogether, the most abundant compound was quinic acid in both species.

Generally, it could be assumed that methanol extracts, as expected, were the richest in phenolics because methanol is a more suitable solvent for isolation of polar components. In this they were followed by jam and water extracts, respectively.

Previously published data (Termentzi *et al.* 2008a, b) on phenolic content in *S. domestica* fruits indicate the presence of ferulic and protocateic acids in amounts several times greater than those obtained in the present study. Also, the mentioned authors confirmed the presence of quercetin-3-*O*-glucoside, which is in accordance with our results. On the other hand, no previous study reported the presence of amentoflavone in *S. domestica* fruits, as was found in the present study. Furthermore, the work of other investigators indicates *S. domestica* fruits to be a valuable source of chlorogenic acid (Forino *et al.* 2015), whereas it was not identified in the present study. Similarly, gallic acid was previously detected in fruits of *S. domestica* (Piagnanini *et al.* 2012), but it was not quantified in the present study. The listed differences between authors in regard to phenolic profiles could be affected by various factors apart from experimental conditions, such as the stage of fruit maturity, genetic factors, environmental considerations and stress (Pandey & Rizvi 2009). However, all of the mentioned authors point out that *S. domestica* fruits possess simple phenolic acids and have a flavonoid profile that is in a good accordance with our data.

A previous limited study of their phenolic profile cited *S. intermedia* fruits as a valuable source of compounds not identified in our research, viz., quercetin, kaempferol and isorhamnetin (Olszewska 2008). To the best of our knowledge, the present study represents the first comprehensive analysis of phenolic compounds in *S. intermedia* fruits and can serve as a valuable basis for further approval of their utilisation as functional food with health benefits.

Ascorbic acid content. It is known that ascorbic acid has numerous biological functions, including a role in prevention of various diseases due to its ability to efficiently scavenge free radicals (Phillips *et al.* 2016). Hence, everyday consumption of functional food with high ascorbic acid levels contributes to well-being and reduces the risk of chronic illnesses. In the present study, the highest level of ascorbic acid (Table 2) was found in the jam extract of *S. domestica* fruits (290 μg g^{-1}/dw), followed by the methanol extract (140 μg g^{-1}/dw), while the water extract showed the lowest level (50 μg g^{-1}/dw). The low but still significant level of ascorbic acid in jam extracts indicates that the jam can serve as part of a seasonal diet rich in vitamins. In spite of destructive processes involved in preparation of jam (cooking), a certain amount of ascorbic acid still remains present. Moreover, a short time of cooking the jam and storing it in a refrigerator will surely contribute to ascorbic acid stability. In this connection, it has been confirmed that long-term cooking of jam and keeping it at room temperature leads to clear reduction of ascorbic acid content (Nojavan *et al.* 2008).

Generally, there is a lack of published data regarding ascorbic acid content in *S. domestica* (Brindza *et al.* 2009; Egea *et al.* 2010). Moreover, it is difficult to make comparisons between this and previous studies due to different experimental conditions and applied

Table 1. Content[a] of detected phenolics in *S. domestica* and *S. intermedia* fruit extracts

Compound	Content of selected phenolics (μg/g of dw[b])					
	S. domestica			*S. intermedia*		
	W	M	J	W	M	J
Phenolic acids						
p–Hydroxybenzoic acid	< loq [c]	< loq[c]	< loq[c]	13.52±0.13 a	< loq[c]	< loq[c]
Protocatechuic acid	7.76±0.07 d	51.79±3.04 a	16.08±0.80 b	< loq[c]	8.09±0.2 d	10.92±0.77 c
Ferulic acid	< loq[c]	16.48±1.34 b	27.62±1.57 a	4.92±0.12 e	7.79±0.47 d	12.04±1.07 c
Chlorogenic acid	< loq[c]	< loq[c]	< loq[c]	< loq[c]	785.51±7.13 a	336.73±8.24 b
Flavonoids						
Amentoflavone	39.79±4.24 b	52.39±3.31 a	35.66±2.40 b	6.30±0.47 c	6.23±0.14 c	6.13±0.17 c
Quercetin-3-*O*-glucoside	3.47±0.02 e	63.56±0.04 a	11.20±0.01 c	< loq[c]	34.92±0.01 b	8.00±0.04 d
Hyperoside	< loq[c]	63.10±0.5 a	9.89±0.04 b	< loq[c]	< loq[c]	< loq[c]
Epicatechin	< loq[c]	< loq[c]	43.95±3.34 a	< loq[c]	< loq[c]	< loq[c]
Catechin	< loq[c]	< loq[c]	10.74±0.96 a	< loq[c]	< loq[c]	< loq[c]
Organic acid						
Quinic acid	(3.25±0.02) /10^{-3}d	(7.45±0.04) /10^{-3}b	(1.18±0.00) /10^{-3}e	(6.48±0.04) /10^{-3}c	(9.75±0.04) /10^{-3}a	(1.30±0.00) /10^{-3}e

[a]Values are means ± SD of measurements of three separate extracts. Measurement for each extract was done in triplicate. Means within each row with different letters (a–e) differ significantly ($p \leq 0.05$).
[b]dw – dry weight.
[c] Reference: Orčić *et al.* 2014.
W – water extract, M – methanol extract, J – jam extract.

Table 2. Total ascorbic acid content[a] in *S. domestica* and *S. intermedia* fruit extracts

	S. domestica			*S. intermedia*		
	W	M	J	W	M	J
Ascorbic acid content (μ/g of dw[b])	0.05± 0.09c	0.14± 0.07b	0.29 ± 0.05a	n.d.[c]	n.d.	0.16 ± 0.05b

[a]Values are means ± SD of measurements of three separate extracts. Measurement for each extract was done in triplicate. Means within each column with different letters (a–c) differ significantly ($p \leq 0.05$).
[b]dw – dry weight.
[c]n.d. – not detected.
W – water extract, M – methanol extract, J – jam extract.

techniques. Apart from that, ascorbic acid content can be influenced by the period of fruit ripening and environmental considerations such as geographic origin and habitat (Mlcek *et al.* 2014). On the other hand, to the best of our knowledge, there has been no prior study of ascorbic acid content in *S. intermedia* fruits, a fact which

Table 3. Antioxidant action and antimicrobial activity[a] of *S. domestica* and *S. intermedia* fruit extracts and standard antioxidants

		S. domestica			*S. intermedia*			*Standard*	
		W	M	J	W	M	J	PG	BHT
Antioxidant action									
IC_{50} values	DPPH•(mg/mL)	1.72 ± 0.10 f	0.20 ± 0.01 c cccfg	0.32 ± 0.02 d	0.43 ± 0.02 e	0.20 ± 0.01 c	0.23 ± 0.02 c	(0.38 ± 0.00)/10^3 a	(9.32 ± 0.05)/10^3 b
	•NO (mg/mL)	n.a.[b]	1.15 ± 0.12 b cd	1.86 ± 0.01 c ddcd d	n.a.	1.09 ± 0.11 b 0.35 c	3.11 ± 0.18 d	(6.58 ± 0.43)/10^3 a	n.a.
	$O_2^{\bullet -}$ (µg/mL)	21.4 ± 0.29 b	30.5 ± 0.98 c	21.5 ± 0.02 b	107 ± 4.19 e	55.7 ± 2.00 d	190 ± 1.77 f	9.73 ± 0.25 a	n.a.
	HO• (mg/mL)	0.27 ± 0.02 d fg	0.21 ± 0.02 c	0.51 ± 0.11 e	0.82 ± 0.07f fg	0.18 ± 0.01 bc	0.88 ±0.00 g	(30.0 ± 0.00)/10^3 a	(160 ± 1.00)/10^3 b
	LP (mg/mL)	n.a.	n.a.	1.86 ± 0.35 b	n.a.	n.a.	n.a.	n.a.	(14.0 ± 3.00)/10^3 a
FRAP (mg of AAE[c]/g dw)		1.63 ± 0.63 d g	4.29 ± 0.17 b	2.63 ± 0.14 c	2.37 ± 0.13 c df	4.47 ± 0.39 b	2.73 ± 0.07 c	n.a.	124 ± 12.4 a
Antimicrobial activity									
MIC (mg/mL)								Amikacin	
	E. coli	n.a.	64	n.a.	64	n.a.	n.a.	4	
	S. aureus	n.a.	64	n.a.	64	n.a.	n.a.	4	

[a]Values are means ± SD of measurements of three separate extracts. Measurement for each extract was done in triplicate.
Means within each column with different letters (a–f) differ significantly ($p \leq 0.05$).
[b]n.a. –inhibition level of 50 % not achieved.
[c]AAE– ascorbic acid equivalents.
W – water extract, M – methanol extract, J – jam extract.

additionally highlights the significance of results obtained in thepresent research.

Antioxidant activity. Exogenous antioxidants can have a wide range of beneficial effects, such as inhibition of oxidising enzymes, chelation of transitional metals, transfer of hydrogen or single electrons to radicals or deactivation of singlet oxygen, all leading to lowering of oxidative stress and protection against degenerative diseases such as cancer, inflammatory diseases, diabetes, etc. Polyphenols are known to be potent antioxidants and radical scavengers due to their multiple phenolic hydroxyl groups (Leopoldini 2011). Some phytochemicals of *Sorbus* species can be regarded as good antioxidants, especially flavonoids and phenolic acids such as amentoflavone, quercetin-3-*O*-glucoside and protocateic and chlorogenic acids.

The antioxidant potential of *Sorbus* extracts was evaluated using several assays based on single-proton/electron transfer (the DPPH• FRAP assay), neutralisation of free radicals ($O_2^{\bullet-}$, •NO, HO•) and measuring the potential to inhibit LP (Table 3).

Sorbus domestica extracts showed themselves to be the more active in all of the conducted assays, except in the case of neutralisation of DPPH• and FRAP, where *S. intermedia* extracts were slightly better. Interestingly, only the jam extract of *S. domestica* was active in inhibition of LP. In general, methanol extracts exhibited better action compared to water extracts. However, water extracts still demonstrated some antioxidant activity, while jam was shown to be a moderate source of antioxidants in the diet. Also, it should be emphasised that jam extracts exhibited a considerable potential for neutralisation of •NO, while water extracts were inactive. This fact additionally supports the usefulness of consumption of jams as functional food with nutritional values. In comparison with the antioxidant potential of the well-known synthetic antioxidants BHT and PG, the examined extracts manifested moderate antioxidant action.

The limited published data regarding the antioxidant potential of *S. domestica* fruits (Termentzi *et al.* 2006; Egea *et al.* 2010; Piagnanini *et al.* 2012) indicate that they possess a moderate antiradical capacity toward DPPH• and •NO, which is in reasonable agreement with the results obtained herein. Also, Termentzi *et al.* (2006) demonstrated higher antioxidant activity of methanol extracts compared to water extracts, as we also confirmed. Moreover, the mentioned authors reported that the pulp of *S. domestica* fruits is more active toward the examined assays compared to other extracts. This fact is particularly important and suggests that considerable antioxidant activity of pulp is retained in jam regardless of cooking.

The only study of the antioxidant potential of *S. intermedia* fruits (Olszewska & Michel 2009) is to some extent in accordance with our results. Still, it should be emphasised that this is the first detailed research regarding the antioxidant action of *S. intermedia*.

Antimicrobial activity. Resistance to antibiotics is one of the greatest threats to global health and food security. Thus, research on natural antimicrobial agents such as plants is highly recommended (Shahidi Bonjar 2004). It was previously reported that berry-like fruits, including *Sorbus* fruits, have a positive effect on the gastrointestinal tract and are introduced as antimicrobial agents (Nohynek *et al.* 2006). For this reason, the antimicrobial activity of *Sorbus* fruit extracts was evaluated against Gram-negative and Gram-positive bacterial strains, as shown in Table 3. It can be concluded from the presented results that both species expressed moderate activity against growth of clinically relevant bacterial strains, *E. coli* and *S. aureus*. To be precise, only the methanol extract of *S. domestica* and water extract of *S. intermedia* showed reasonably high antimicrobial activity, reaching the same MIC. Jam extracts were inactive in the mentioned assays. Neither *S. domestica* nor *S. intermedia* extracts were found to be more active than the antibiotic amikacin. A previously published study (Hwang *et al.* 2013) showed a significant antibacterial potential of amentoflavone, supporting the expediency of using it as an antimicrobial agent with potential therapeutic effects. Although moderate inhibition against bacterial strains was confirmed, the present research strongly supports the need for additional investigation in order to find which compounds are the most responsible for this activity. However, as far as we know, there are no prior data on the antibacterial potential of fruits of the two investigated species. Thus, the results obtained in the present study contribute significantly to expanding our overall knowledge about the biological activity of *Sorbus* species.

CONCLUSION

Nowadays the increasing importance of fruits as functional foods creates the need to analyse and define the best species for nutrition. In the present research, we critically assess two *Sorbus* species, comparing their chemical composition with respect to content of health-promoting compounds naturally occurring in thefruits, along with their antioxidant action and antimicrobial activity. The LC–MS/MS analysis of selected plant phenolics revealed that amentoflavone was dominant in *S. domestica* extracts, while chlorogenic acid was the most abundant in *S. intermedia* extracts. The jam showed itself to be the richest source of ascorbic acid. Regarding antioxidant action, extracts of *S. domestica* manifested a slightly better potential in comparison with *S. intermedia* extracts. A moderate antimicrobial potential was revealed for both of the investigated species. In conclusion, the results obtained in this study justify the traditional

use of *Sorbus* fresh fruits and jam as food with potential health and nutritional benefits.

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Botanica SERBICA

REZIME

Sorbus domestica L. i *Sorbus intermedia* (Ehrh.) Pers. sveži plodovi i pekmez: fenolni profil, antioksidantna i antimikrobna aktivnost

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Cilj rada predstavljao je detaljno ispitivanje fenolnog profila, sadržaja vitamina C, antioksidantne i antimikrobne aktivnosti ekstrakata i pekmeza plodova dve tradicionalno korišćene vrste roda *Sorbus*: *S. domestica* i *S. intermedia*. Pristustvo i sadržaj 44 fenolna jedinjenja urađena je pomoću LC-MS/MS, gde je dominantno jedinjenje bio amentoflavon u ekstraktima *S. domestica*, odnosno hlorogenska kiselina u ekstraktima *S. intermedia*. Najveći sadržaj vitamina C detektovan je u pekmezima. U poređenju sa standardnim antioksidantima BHT i PG, obe vrste roda *Sorbus* ispoljile su umerenu antioksidantnu aktivnost. Pojedini ekstrakti *S. domestica* i *S. intermedia* pokazali su aktivnost u inhibiranju rasta dva klinički releventna bakterijska patogena. Prezentovani rezultati podržavaju potencijal voća vrsta roda *Sorbus* kao hrane sa pozitivnim dejstvom na zdravlje.

Ključne reči: *Sorbus*, fenoli, askorbinska kiselina, antioksidantna aktivnost, antimikrobna aktivnost