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Phenolic profiling and bioactivities of fresh fruits and jam of *Sorbus* species

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Abstract: The purpose of this study was a comprehensive examination of the phenolic profile, the vitamin C content, and the antioxidant, anti-acetylcholinesterase, cytotoxic and antimicrobial activities of extracts and jams of fruits of two edible *Sorbus* species: well characterized *S. aucuparia* and two insufficiently explored forms of *S. torminalis* (*torminalis* and *semitorminalis*). Characterisation of 44 phenolics was realized using LC–MS/MS and 15 compounds were confirmed, with chlorogenic acid being the most dominant in *S. aucuparia* and ferulic acid in both *S. torminalis* forms. *S. aucuparia* demonstrated potent antioxidant activity, while that of both *S. torminalis* forms was moderate. Jam extracts had the highest content of vitamin C. *S. aucuparia* exhibited some anti-acetylcholinesterase activity, while *S. torminalis* f. *torminalis* showed the best antimicrobial activity against *Staphylococcus aureus* and both forms (*torminalis* and *semitorminalis*) possessed the highest activity against *Escherichia. coli*. The results obtained herein are a great base for further research of edible *Sorbus* species with the aim of promoting their better usage as nutraceuticals.

Keywords: phenolics; antioxidant; anti-acetylcholinesterase activity; cytotoxic activity; antimicrobial activity.

INTRODUCTION

In spite of their widespread use in diet, preservatives preparation, beverage manufacture and traditional medicine (the details are presented in the Supple-

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mentary material to this paper), there are still very few reports concerning the detailed chemical composition and biological activities of fruits of different *Sorbus* species. Bearing this in mind, in this paper, the fruits of the well-known *S. aucuparia* and the unexplored *S. torminalis* f. *torminalis* and *S. torminalis* f. *semitorminalis* were the subjects of extensive study.

Namely, within the *Sorbus* genus, the most renowned species is *S. aucuparia* (rowan), which is edible for humans and domestic animals. It was confirmed that rowan fruits contain a high content of natural products, such as phenolics,[1–6] vitamins C, B_1, B_2, E and K,[5,7] carotenoids,[8] carboxylic acids[9] and sugars,[7] which surely contribute to their salubrity. *S. aucuparia* is a very variable species and occurs in nature as five subspecies: *S. aucuparia* subsp. *aucuparia*, *S. aucuparia* subsp. *fenenkiana*, *S. aucuparia* subsp. *glabrata*, *S. aucuparia* subsp. *praemorsa* and *S. aucuparia* subsp. *sibirica*. In this study, *S. aucuparia* subsp. *aucuparia* var. *aucuparia* fruits were the subject of in-depth investigation whereby their detailed phenolic profile was elucidated for the first time. Herein, fresh fruit extracts and jam were investigated, as those are the forms in which the fruits of *Sorbus* species are usually consumed.

On the other hand, other species from *Sorbus* genus, such as *S. torminalis* (wild service or chequer tree), have been poorly studied to date. As the fruits of *S. aucuparia*, the fruits of *S. torminalis* are commonly present in human and animal diet. This species occur in wide variety of forms, mostly differing in leaf shape. In this paper, two forms of *S. torminalis*, f. *torminalis* and f. *semitorminalis*, were studied. The morphological differences between leaves of these two forms are barely noticeable, while their colour and fruits are identical. The only variations between leaves are hairs on underside, which are hard to observe. Specifically, leaves of f. *torminalis* are hairy only during a short period when young, while those of f. *semitorminalis* are permanently covered with hairs.[10] Thus, due to their strong morphological similarities, these two forms could be easily mistaken for each other if they were not harvested by professional plant collectors. Consequently, it was considered worthwhile to determine whether their fruits have a similar chemical composition and biological potency, and conclude if substituting one of these species with the other in functional foods could result in quality differences. While the antioxidant and anti-acetylcholinesterase (AChE) activities, as well as total phenolic and flavonoid contents of *S. torminalis* fruits have only been poorly investigated,[11,12] no literature data related to the detailed phenolic profile, vitamin C content and other biological activities of these fruits has hitherto been reported. For this reason, the present research was focused on the examination and comparison of the phenolic profile, vitamin C content, and antioxidant, anti-AChE, cytotoxic and antimicrobial activities of water and methanol extracts of the edible fresh fruits, and traditionally prepared

jams of *S. aucuparia* and of the uninvestigated *S. torminalis* f. *torminalis* and *S. torminalis* f. *semitorminalis* plant species.

EXPERIMENTAL

Experimental details related to the employed chemicals and reagents, plant material collection and extracts preparation, are given in the Supplementary material to this paper.

LC–MS/MS analysis of single phenolic compounds

Determination of selected phenolic compounds in the extracts of *S. aucuparia* and both *S. torminalis* forms, *torminalis* and *semitorminalis*, was performed according to a previously reported procedure.[13] Additional brief details are given in the Supplementary material.

Vitamin C content

The vitamin C content was determined by a method given in the literature[14] adapted for 96-well microplates.[15] Briefly, each sample was evaporated *in vacuo* at 40 °C and mixed with metaphosphoric acid (0.1 g mL^{-1}) to obtain final concentrations of 60, 90 and 120 mg mL^{-1} for all extracts, except for the extracts of jam that were prepared in concentrations of 40, 60 and 80 mg mL^{-1}. The mixtures were stirred for 45 min at room temperature. The prepared extracts in metaphosphoric acid (30 µL) were mixed with 270 µL of 2,6-dichlorophenolindophenol (72 mg mL^{-1}) and the absorbance at 515 nm was measured within 5 min. The vitamin C content was determined using a standard calibration curve of vitamin C (ranging 0–320 µg mL^{-1}) and the results are presented as the mean value of three measurements.

Antioxidant and anti-AChE activities

The scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide anion ($O_2^{\bullet-}$), nitric oxide ($^{\bullet}NO$) and hydroxyl ($HO^{\bullet}$) radical, reducing power (FRAP assay) and inhibition of lipid peroxidation (LP) were tested according to previously published methods.[16] To evaluate the inhibitory activity of the extracts, the AChE Ellman method[17] with numerous modifications was used. Details of these experiments are given in the Supplementary material.

Cell growth activity

Preparation of the samples and standards, experimental conditions for maintenance of the cell lines, and the sulforhodamine B (SRB) assay procedure were performed according to previously published procedures.[16] Each experimental method is briefly explained in the text below.

Preparation of samples and standards. For the evaluation of the cell growth activity, aqueous stock solutions of the extracts and podophyllotoxin (10 mg mL^{-1} in dimethyl sulfoxide (DMSO)) were diluted in NaCl (9 mg mL^{-1}) to obtain ranges of concentrations 0.1–1000 and 0.00001–1 µg mL^{-1}, respectively.

Maintenance of cell lines. Cell growth activity was evaluated *in vitro* using human cell lines: HeLa (cervix epitheloid carcinoma, European Collection of Authenticated Cell Cultures (ECACC), No. 93021013), MCF7 (breast adenocarcinoma, ECACC No. 86012803), HT-29 (colon adenocarcinoma, ECACC No. 91072201) and MRC-5 (human foetal lung, ECACC 84101801). The cell lines were grown in Dulbecco-modified Eagle's medium with 4.5 % glucose, supplemented with 10 % heat-inactivated foetal calf serum (FCS), 100 IU mL^{-1} penicillin and 100 µg mL^{-1} streptomycin. The cells were cultured in 25-cm^2 flasks at 37 °C, in a 5 % CO_2 atmosphere of high humidity, and sub-cultured twice a week. A single cell suspension was obtained using 0.1 % trypsin with 0.04 % EDTA.

SRB assay. The cell lines were harvested and plated into 96-well microtitre plates at seeding density of (3–5)×10^3 cells $well^{-1}$ in a volume of 199 or 180 μL, and pre-incubated in complete medium supplemented with 5 % FCS at 37 °C for 24 h. Serial dilutions of the extracts (1 μL) were added into 199 μL of medium, while serial dilutions of podophyllotoxin (20 μL) were added into 180 μL of medium, in order to achieve the required final concentrations. All samples were filtered through 0.22 μm microfilters to obtain sterility. Equal volumes of water and DMSO were added in the control wells. The concentration of DMSO in the cell cultures was ≤ 5 μL mL^{-1}. After adding the dilutions, the microplates were incubated at 37 °C for 48 h. The cell growth was evaluated by the colorimetric SRB assay.[18] Colour development was measured using photometer at 540 nm against 620 nm as the background.

Antimicrobial activity

To evaluate minimum inhibitory concentration (*MIC*) of the extracts, the method for the determination of the antimicrobial activity of antimicrobial agents was used.[19] First, two-fold dilutions of the tested plant extracts were prepared in microtitre plates. The final concentration of each extract ranged 1–128 mg mL^{-1}. Subsequently, a bacterial suspension, previously adjusted to match a turbidity of a 0.5 McFarland nephelometer standard, was diluted in double-strength Mueller–Hinton medium (1:100 volume ratio). Into each well, inoculated double-strength medium were added to the same volume as each extract (1:1 volume ratio). The number of the bacteria in the assay was about 1×10^6 colony forming units (CFU) mL^{-1}. Antimicrobial activity of the extracts was tested against Gram negative bacteria, *Escherichia coli* American Type Culture Collection (ATCC) 25922 and Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923. The microtitre plates were incubated overnight at 37 °C, without shaking, amended with a 10 μL of a 1 % solution of 2,3,5-triphenyltetrazolium chloride and incubated additionally for 2 h until development of the red colour. Controls for plate sterility and bacterial growth without extracts were also included. The lowest concentration of extracts that inhibited bacterial growth, which was identified by the absence of red formazane, was considered as the *MIC*. The MIC determination was performed in three replicates and three independent experiments.

RESULTS AND DISCUSSION

Phenolic profile

Qualitative and quantitative analyses of 44 phenolics in all extracts were performed using the LC–MS/MS technique. The content of the 12 determined phenolics are presented in Table I and differences between the species and two *S. torminalis* forms are evident. Concerning phenolic acids, chlorogenic acid was the most abundant compound in *S. aucuparia* extracts, while ferulic acid was the most dominant in extracts of both *S. torminalis* forms. Analyses of the selected flavonoids showed that all extracts contain amentoflavone, which was dominant flavonoid in both *S. torminalis* forms, especially in *semitorminalis* form. Additionally, rutin, quercetin-3-*O*-glucoside and hyperoside were characteristic for extracts of *S. aucuparia*. Furthermore, coumarins and lignans were not detected, with the exception of aesculetin, which was present only in *S. torminalis* f. *semitorminalis*.

It is possible to see a common trend among the extracts concerning determined phenolic amounts was recognizable. Namely, aqueous methanol was more efficient than the extraction in water, while jam extracts contained significantly lower amounts of most phenolics, than did the fresh fruits.

TABLE I. Contents of detected phenolics (μg g^{-1} dw) in *S. aucuparia* and *S. torminalis* (forms *torminalis* and *semitorminalis*) fruit extracts; means within each row with different letters (a–i) differ significantly ($p \leq 0.05$); compounds analyzed, but the peak was below the limit of quantification (*LOQ*):[13] *p*-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, vanillic acid, cinnamic acid, caffeic acid, syringic acid, *o*-coumaric acid, *p*-coumaric acid, 3,4-dimethoxycinnamic acid, sinapic acid (phenolic acids); apigenin, apigenin-7-*O*-glucoside, baicalin, baicalein, apiin, daidzein, naringenin, vitexin, genistein, isorhamnetin, luteolin, luteolin-7-*O*-glucoside, myricetin, kaempferol, epigallocatechin gallate, chrysoeriol, quercetin, epicatechin (flavonoids); umbelliferone, scopoletin (coumarins); matairesinol, secoisolariciresinol (lignans) W – water extract, M – methanol extract, J – jam

Compound	Species								
	S. aucuparia			*S. torminalis* f. *torminalis*			*S. torminalis* f. *semitorminalis*		
	W	M	J	W	M	J	W	M	J
Phenolic acids									
Gallic acid	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	5.69± 0.51^{a}	*<LOQ*	*<LOQ*
Protocatechuic acid	*<LOQ*	*<LOQ*	12.5± 0.01^{a}	13.7± 0.24^{b}	23.2± 0.13^{c}	5.92± 0.41^{d}	4.61± 0.04^{e}	3.44± 0.03^{f}	2.11± 0.19^{g}
Chlorogenic acid	(5.69± 0.30)×10^{3c}	(5.80± 0.30)×10^{3c}	(2.60± 0.10)×10^{3b}	*< LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Ferulic acid	7.80± 0.46^{h}	9.59± 0.80^{i}	11.4± 0.93^{g}	27.8± 0.97^{d}	62.6± 3.18^{c}	13.3± 0.67^{f}	43.3± 1.44^{b}	38.3± 1.46^{a}	18.4± 0.55^{e}
Flavonoids									
Amentoflavone	10.7± 0.91^{h}	11.9± 0.33^{g}	8.40± 0.56^{i}	15.8± 0.98^{f}	19.3± 1.17^{d}	16.8± 1.31^{e}	362± 8.67^{b}	974± 13.2^{c}	195± 3.76^{a}
Kaempferol-3-*O*-glucoside	9.00± 0.45^{c}	8.56± 0.33^{c}	3.99± 0.27^{b}	*<LOQ*	*<LOQ*	*<LOQ*	2.34± 0.02^{a}	2.43± 0.01^{a}	*<LOQ*
Quercitrin	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	6.53± 0.51^{b}	11.0± 0.97^{a}	3.76± 0.27^{c}
Quercetin-3-*O*-glucoside	49.3± 1.34^{b}	55.8± 2.16^{c}	17.9± 1.24^{a}	*<LOQ*	13.6± 0.64^{d}	2.53± 0.01^{f}	3.33± 0.02^{e}	2.06± 0.02^{f}	1.60± 0.01^{g}
Hyperoside	36.6± 1.47^{c}	39.6± 1.57^{c}	9.68± 0.33^{b}	*<LOQ*	10.4± 1.02^{b}	1.61± 0.21^{c}	*<LOQ*	*<LOQ*	*<LOQ*
Rutin	82.3± 1.25^{c}	80.4± 2.76^{c}	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Catechin	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	10.6± 1.24^{c}	*<LOQ*	*<LOQ*
Coumarin									
Aesculetin	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	3.07± 0.14^{c}	*<LOQ*

Furthermore, previously published data also highlighted fruits of *Sorbus* species as a rich source of phenolic compounds. Specifically, chlorogenic and neochlorogenic acid, as well as quercetin glycosides (rutin, hyperoside and isoquercitrin), were identified in fruit samples of nineteen different *Sorbus* species, hybrids and varieties, including *S. aucuparia*.[20] Similarly, the same compounds were detected by others in ten fruit samples, including nine varieties of *S. aucuparia*.[21] When compared to the results of these authors,[21] the content of rutin was the same, while the content of chlorogenic acid was a few times higher than that found in the present study. On the other hand, others detected hyperoside in the fruits of *S. aucuparia*[20–22] in higher amounts than in the presented data. According to a few previous studies, hydroxycinnamic acids, mainly chlorogenic and neochlorogenic acids, were identified in *S. aucuparia* fruits.[1–3] These authors reported hydroxycinnamic acids to be the most abundant phenolics and obtained amounts of chlorogenic acid in *S. aucuparia* fruits that are in agreement with those found in the present research. Moreover, another study pointed out that fruits of *S. aucuparia* present a valuable source of ferulic acid, which was higher than in the present extracts.[23] Significant contents of both quercetin and kaempferol and their glucosides were previously confirmed in the fruits of *S. aucuparia*.[5,23] However, quercetin was absent in the present extracts, while the content of kaempferol reported previously was in agreement with data presented herein. Similarly, *S. aucuparia* fruits were shown previously to be a rich source of rutin, quercetin-3-*O*-glucoside and quercetin-3-*O*-galactoside,[22] whereby the determined contents of rutin and quercetin-3-*O*-glucoside were higher than in data obtained in the present study. In addition, another study indicates that the fruits of *S. torminalis* contain a high amount of quercetin as well as isorhamnetin.[24] Besides, amentoflavone was detected in the present *S. aucuparia* fruit extracts, while no prior study specifically mentioned this compound. Generally, the obtained results were in a good accordance with a few previously published studies regarding the phenolic profile of *S. aucuparia* fruits. However, to the best of our knowledge, this is the first detailed report concerning the phenolic composition of fruits of either *S. torminalis* forms (*torminalis* and *semitorminalis*), as well as some phenolics in *S. aucuparia* fruits.

Interestingly, the dominant compounds found in the investigated *Sorbus* species are proven to express valuable health benefits, which additionally support their usage as nutraceuticals. Namely, it was previously reported that the consumption of fruits rich in chlorogenic acid and its derivates decrease the risk of type 2 diabetes. In addition, most of chlorogenic acids convert to caffeic acid, which reduces glucose absorption and oxidative stress *in vitro* and inhibits glucose-6-phosphate translocase, thereby decreasing glucose output in the liver.[2] In addition, amentoflavone is recognized as a potent anti-inflammatory agent by

inhibiting prostaglandin E_2 biosynthesis *via* downregulation of cyclooxygenase-2 expression.[25]

Vitamin C content

As shown in Table II, the amount of vitamin C ranged from 40 to 420 μg g^{-1} dw in the extracts of fresh fruits and jam of the examined species. Interestingly, the jam extracts were shown to be the richest sources of vitamin C. This could be explained by the fact that the preparation of the jam (cooking) lasted a short time (a few minutes) and it was stored in the cold (4 °C) until analysed. Namely, a group of authors[26] showed that long-time cooking, as well as storage at room temperature, causes an evident reduction in the vitamin C content. In addition, a study indicated that the stability of vitamin C is higher in a fruit matrix than in extracts.[27] To the best of our knowledge, there are no prior investigations on vitamin C content in *S. torminalis* species. On comparing the two investigated forms of *S. torminalis*, it could be noted that the form *semitorminalis* was slightly richer in vitamin C than the form *torminalis*.

TABLE II. Total ascorbic acid contents (means within each column with different letters (a–f) differ significantly ($p \leq 0.05$)) in *S. aucuparia* and *S. torminalis* (forms *torminalis* and *semitorminalis*) fruit extracts; W – water extract, M – methanol extract, J – jam

Extract	vitamin C content, μg g^{-1} dw
S. aucuparia	
W	100±10.0[a]
M	40.0±1.10[b]
J	420±10.0[c]
S. torminalis f. *torminalis*	
W	40.0±1.20[ab]
M	220±20.3[d]
J	300±10.1[de]
S. torminalis f. *semitorminalis*	
W	80.0±7.90[a]
M	320±20.1[e]
J	360±10.7[f]

Generally, to date, there is a lack of information on the vitamin C content in *Sorbus* species.[5,28] One study indicated a higher amount of vitamin C in *S. aucuparia* fruits[5] compared with the results obtained in the present study. This disagreement is probably due to differences in the maturity stage of the investigated fruits. Namely, the others investigated unripe fruits, while in this study, the fruits where overripe.

Vitamin C is one of the most important antioxidants and everyday food supplement, which is purported to prevent respiratory tract infections and accelerate recovery from various conditions, such as fever, cold and flu.[29] Compared with

some other fruits that are valuable sources of vitamin C, the content of vitamin C in *Sorbus* fruits is about six times lower than in orange and eight times lower than in lemon and kiwi fruit.[30] However, fresh *Sorbus* fruits and jam are valuable sources of micronutritients and could serve in a healthy seasonable diet.

Antioxidant activity

The reason to examine the antioxidant potency of *S. aucuparia*, *S torminalis* f. *torminalis* and *S. torminalis* f. *semitorminalis* derives from the fact that the antioxidant supplements in food, particularly those rich in phenolic compounds, could markedly diminish amount of free radicals, leading to the prevention of some severe diseases, such as metabolic syndrome, and cardiovascular and neurodegenerative disorders.[31] In the light of this, antioxidant therapy by functional food is considered to be promising approach to postpone or retard such diseases.[32] In order to extensively characterize the antioxidant activity of the fruits and jam, of *S. aucuparia* and two *S. torminalis* forms, several *in vitro* assays were applied: the DPPH$^{\bullet}$, $^{\bullet}$NO, HO$^{\bullet}$ and $O_2^{\bullet-}$ scavenger capacity tests, FRAP assay and Fe^{2+}/ascorbate induced LP inhibition. The determined antioxidant activities are given in Table III. In comparison with well-known synthetic antioxidants butylated hydroxytoluene (BHT) and propyl gallate (PG), all extracts exhibited considerable antioxidant effects. Generally, *S. aucuparia* extracts were the most active in all the performed tests, except toward $O_2^{\bullet-}$ scavenging activity, where *S. torminalis* f. *torminalis* was the most powerful. Namely, its fruit extracts (water and methanol) showed better or the same activity as PG, respectively, toward the neutralisation of $O_2^{\bullet-}$. The water extract of *S. aucuparia* fruits demonstrated a significant HO$^{\bullet}$ scavenger activity similar to that of the standard antioxidant BHT. These facts are particularly important because $O_2^{\bullet-}$ reacts rapidly with other free radicals, such as NO$^{\bullet}$, and can cause the biological damage occurring in many human diseases.[33] Additionally, HO$^{\bullet}$ is the most potent radical in initiating LP in the cell and is capable of damaging almost every molecule found in living cells.[34] Overall, the extracts of the fruits of *S. aucuparia* had the highest antioxidant activity in the present study. By comparing two forms of *S. torminalis*, it could generally be noted that they express similar antioxidant potential, because the extracts of the form *torminalis* had a slightly better antiradical capacity towards $^{\bullet}$NO, $O_2^{\bullet-}$ and HO$^{\bullet}$, while the extracts of the form *semitorminalis* were more effective in scavenging of DPPH$^{\bullet}$ and inhibition of LP and had a better reducing power. According to the results presented, it could be noted that the fresh fruits exhibited some antioxidant activity while the jam was shown to be a moderate source of antioxidants in the diet.

Anti-AChE activity

It has been suggested that AChE may participate in the development of neurodegenerative disorders, such as Alzheimer's disease and tumorigenesis.[35] Thus, inhibition of AChE serves as a promising strategy for the treatment of neurodegenerative disorders and the search for new sources of effective anti--AChE compounds, such as natural products, is worthwile.[36]

TABLE III. Antioxidant activities of *S. aucuparia* and *S. torminalis* (forms *torminalis* and *semitorminalis*) fruit extracts; means within each column with different letters (a–h) differ significantly ($p \leq 0.05$); AAE – ascorbic acid equivalents; NA – 50 % inhibition not achieved; PG – propyl gallate; BHT – butylated hydroxytoluene; W – water extract, M – methanol extract, J – jam

Extract	IC_{50} / mg mL^{-1}					FRAP
	DPPH·	·NO	$O_2{\cdot}^-$ (×10^3)	HO·	LP	mg of AAE g^{-1} dw
			S. aucuparia			
W	0.07± 0.00^{a}	1.43± 0.03^{a}	20.16± 0.90^{b}	0.16± 0.00^{c}	6.40± 0.14^{c}	10.6± 0.20^{c}
M	0.08± 0.01^{a}	0.43± 0.03^{c}	20.5± 0.50^{b}	0.24± 0.00^{a}	7.38± 0.08^{d}	11.2± 0.43^{c}
J	0.13± 0.01^{b}	2.26± 0.04^{d}	67.8± 2.06^{e}	0.61± 0.02^{e}	4.08± 0.05^{a}	4.22± 0.08^{b}
			S. torminalis f. *torminalis*			
W	1.38± 0.01^{f}	NA^{a}	7.09± 0.50^{a}	0.30± 0.00^{d}	NA	1.11± 0.13^{f}
M	0.57± 0.02^{e}	2.82± 0.19^{g}	12.2± 1.21^{ca}	0.26± 0.01^{ab}	NA	2.12± 0.15^{e}
J	0.44± 0.02^{f}	1.64± 0.03^{b}	36.9± 2.19^{d}	1.11± 0.01^{f}	NA	3.10± 0.18^{g}
			S. torminalis f. *semitorminalis*			
W	1.27± 0.02^{f}	NA	12.8± 0.53^{a}	0.43± 0.01^{g}	NA	2.12± 0.27^{e}
M	0.42± 0.01^{g}	3.12± 0.06^{f}	12.5± 1.29^{cc}	0.27± 0.01^{b}	NA	3.81± 0.04^{d}
J	0.18± 0.00^{d}	2.45± 0.01^{dg}	50.3± 0.36^{g}	0.29± 0.02^{bd}	3.02± 0.02^{c}	6.41± 0.11^{a}
			References			
PG	(0.38± 0.00)×10^{-3h}	(6.58± 0.43)×10^{-3h}	9.68± 0.29^{c}	(20.0± 0.40)×10^{-3h}	NA	NA
BHT	(9.36± 0.05)×10^{-3c}	NA	NA	(160± 5.00)×10^{-3c}	(14.0± 3.00)×10^{-3h}	124± 12.4^{h}

In this study, as shown in Table IV, only the extracts of *S. aucuparia* possessed inhibitory activity toward AChE. Interestingly, the methanol extracts of fresh fruits as well as traditionally made jam exhibited the highest anti-AChE activity, but were not as good as galanthamine. Inhibitory potential against AChE

of *S. aucuparia* was also reported previously, but in the present study, the activity was weaker.[37] Even though some authors[12] reported anti-AChE activity of *S. torminalis* water extract, in the present study, neither form *torminalis* nor form *semitorminalis* were found to be active. The obtained results suggest that *S. aucuparia* fruits could potentially be used as sources of natural anti-AChE agents.

TABLE IV. Cytotoxic and anti-AChE activities (IC_{50} / μg mL^{-1}) of *S. aucuparia* fruit extracts; means within each column with different letters (a–c) differ significantly ($p \leq 0.05$); NA – 50 % inhibition not achieved; na – non-applicable; W – water extract, M – methanol extract, J – jam

Extract	Cytotoxic activity				Anti-AChE activity
	MRC-5	HeLa	MCF7	HT-29	
W	532±7.79^{a}	NA	526±7.21^{a}	592±70.6^{a}	(3.81±0.21)×10^{3a}
M	517±28.5^{b}	965±27.9^{b}	414±12.5^{b}	432±4.15^{b}	(2.02±0.02)×10^{3b}
J	NA	NA	NA	NA	(2.08±0.04)×10^{3b}
			Standard		
Podophyllotoxin	(4.70±0.80)×10^{-3c}	(4.10±0.30)×10^{-3c}	(1.30±0.20)×10^{-3a}	(3.00±0.50)×10^{-3c}	na
Galanthamine	na	na	na	na	0.39±0.01^{c}

Cytotoxic activity

The cytotoxity of the *Sorbus* extracts was evaluated *in vitro* using tumour HeLa, MCF7 and HT-29 and healthy MRC-5 cell lines by the SRB assay. In addition, the activity of podophyllotoxin, a potent cytotoxin, was investigated (Table IV). Overall, only extracts of *S. aucuparia* demonstrated cytotoxic activity, while the *S. torminalis* forms expressed no activity in the applied concentration range. Unfortunately, *S. aucuparia* was not selective towards tumour cell lines, as it was also cytotoxic to the healthy MRC-5 cell line. Furthermore, the growth of examined cell lines was not affected by jam. In comparison to podophyllotoxin, the extracts exhibited much lower activity. Nevertheless, since there is only one literature reference on the antiproliferative activity of *S. aucuparia*,[38] the obtained results could contribute to the overall knowledge of the bioactivity of *Sorbus* species.

Antimicrobial activity

The antimicrobial activity of extracts was evaluated against clinically relevant Gram-positive and Gram-negative bacterial strains (Table V).

One of the most important human and animal pathogens, *E. coli*, is responsible for a broad spectrum of diseases, including urinary tract infections and other clinical infections. On the other hand, *S. aureus* are causative agents of food poisoning in humans.[39] It was previously reported that multiple mechanisms of organic and phenolic acids, tannins and anthocyanins are responsible for the antimicrobial activity of berries.[39,40] In presented study all investigated extracts were

more effective in growth inhibition of *S. aureus* compared to *E. coli*. Extracts of *S. torminalis* f. *torminalis* possessed the best activity against *S. aureus*, while *E. coli* was more sensitive to both *S. torminalis* forms compared with *S. aucuparia*. It is noteworthy that among the jam extracts only the jam from *S. torminalis* f. *semitorminalis* exhibited inhibition of bacterial growth. The antibacterial activity of *S. aucuparia* was previously poorly argued,[4,39] while there are no previous reports on either *S. torminalis* forms. Taking into account that the investigated extracts showed moderate effects against bacterial growth, further studies are recommended in order to verify which constituents and mechanisms are responsible for the observable activity.

TABLE V. Antimicrobial activities (*MIC* / mg mL^{-1}) of *S. aucuparia* and *S. torminalis* (forms *torminalis* and *semitorminalis*) fruit extracts; NA – inhibition not achieved; W – water extract, M – methanol extract, J – jam

Extract	Test microorganism	
	E. coli	*S. aureus*
S. aucuparia		
W	64	32
M	64	32
J	NA	NA
S. torminalis f. *torminalis*		
W	32	16
M	32	16
J	NA	NA
S. torminalis f. *semitorminalis*		
W	32	32
M	32	32
J	64	32
Standard		
Amikacin	4	4

Overall, no clear correlation between the content of the examined phenolic compounds and results of antioxidant assays and analyzed activities were found. This indicates a strong synergistic effect of the phenolics, flavonoids and vitamin C, as well as other uninvestigated bioactive compounds, such as tannins and anthocyanins, which could be responsible for the determined bioactivities.

CONCLUSIONS

In the present study, the edible fruits of *S. aucuparia*, and two forms of *S. torminalis* (*torminalis* and *semitorminalis*) were extensively investigated for their phenolic profile, vitamin C content and their antioxidant, anti-AChE, cytotoxic and antimicrobial activities. The contents of 44 phenolics were investigated in the fresh fruits and jam using LC–MS/MS and 12 compounds were detected, with the most dominant being chlorogenic acid (*S. aucuparia*) and amentoflavone (*S.*

torminalis f. *semitorminalis*). Regarding the vitamin C content, the fruit jam was the richest source. Although the fruits of both *S. torminalis* forms showed moderate antioxidant potency, those of *S. aucuparia* were the most powerful antioxidant agents. Only *S. aucuparia* fruits were effective in the inhibition of AChE and the cytotoxic assay, but regrettably, they showed no selectivity towards tumour cell lines. Regarding antimicrobial activity, the fruits of *S. torminalis* f. *torminalis* were the most active in the inhibition of the growth of *S. aureus*, while both forms (*torminalis* and *semitorminalis*) exerted the best activity on *E. coli*. To sum up, the results obtained in this study indicated that the fruits of *S. aucuparia* had higher biopotential than those of the *S. torminalis* forms. Furthermore, the fruits of the examined *S. torminalis* forms have somewhat different secondary metabolites profile and bioactivities and thus should be clearly distinguished from one another. The fruits of *Sorbus* species should be consumed mainly fresh, even though the jams should not be disregarded from the diet, as they showed greater biopotential. The presented results firmly support further studies with the aim of promoting everyday consumption of edible *Sorbus* fruits as functional food with valuable health benefits.

SUPPLEMENTARY MATERIAL

More details about studied species and additional experimental details are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ФЕНОЛНИ ПРОФИЛ И БИОАКТИВНОСТ СВЕЖИХ ПЛОДОВА И ПЕКМЕЗА ВРСТА РОДА *Sorbus*

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Циљ приказаног рада представљао је детаљно испитивање фенолног профила, садржаја витамина це, антиоксидантне, анти-ацетилхолинестеразне, цитотоксичне и антимикробне активности екстраката и пекмеза плодова две јестиве врсте рода *Sorbus*: добро окарактерисане врсте *S. aucuparia* и две недовољно проучене форме врсте *S. torminalis* (*torminalis* и *semitorminalis*). Карактеризација 44 фенолна једињења урађена је помоћу LC–MS/MS технике и потврђено је присуство 15 једињења. У екстрактима плодова *S. aucuparia* доминантно једињење била је хлорогенска киселина, док је у обе форме врсте *S. torminalis* доминантна била ферулна киселина. *S. aucuparia* је испољила снажну антиоксидантну активност, док су обе форме врсте *S. torminalis* показале умерен антиоксидантни потенцијал. Највећи садржај витамина це детектован је у пекмезима. *S. aucuparia*

је показала извесну анти-ацетилхолинестеразну активност, док је *S. torminalis* f. *torminalis* испољила најбољи антимикробни потенцијал. Резултати овог рада представљају добру базу за даља испитивања плодова јестивих врста рода *Sorbus* у циљу њихове боље искоришћености као нутрацеутика.

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