TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2017) 41: 154-164

doi:10.3906/tar-1702-26

Phenolic compounds in floral infusions of various *Sambucus* species and their interspecific hybrids

Biljana TODOROVIC[1], Maja MIKULIC-PETKOVSEK[1,*], Franci STAMPAR[1], Anton IVANCIC[2]

[1]Chair for Fruit, Wine and Vegetable Growing, Biotechnical Faculty, University of Ljubljana, Department of Agronomy, Ljubljana, Slovenia
[2]Chair for Genetics, Faculty of Agriculture and Life Sciences, University of Maribor, Hoce, Slovenia

Received: 07.02.2017 • **Accepted/Published Online:** 13.04.2017 • **Final Version:** 03.05.2017

Abstract: The main purpose of the present study was to evaluate the diversity and content of various phenolic compounds in floral infusions of 14 elderberry genotypes (3 species and 9 interspecific hybrids). Detailed phenolic analysis was performed using HPLC-MS. Hydroxycinnamic acids (HCAs) were the major phenolic group identified and 3-, 4-, and 5-caffeoylquinic acids were detected in infusions prepared from all analyzed species and interspecific hybrids. Several flavonols were identified in elderberry floral infusions: 8 isorhamnetin derivatives, 6 quercetin derivatives, 2 flavanones, and 6 kaempferol derivatives. The main flavonols detected in all samples were isorhamnetin-3-rutinoside, isorhamnetin dihexoside, quercetin-3-rutinoside, kaempferol-3-rutinoside, and kaempferol dihexoside. The highest levels of flavonols and hydroxycinnamic acids were measured in interspecific hybrid infusions of *Sambucus javanica* × *S. cerulea*, (*S. javanica* × *S. nigra*) × *S. nigra* cv. Black Beauty, (*S. javanica* × *S. nigra*) × *S. nigra*, and *S. javanica* × *S. racemosa*.

Key words: Elderberry, *Sambucus* spp., interspecific hybrids, inflorescence, tea extract, flavonoids, phenolic acids

1. Introduction

Horticulture plants are deemed important in many ways besides being a source of food and are central to the healthy diets of modern urban populations and these plants benefit us economically, mentally, environmentally, and educationally (Ercisli, 2009; Erturk et al., 2010; Hricova et al., 2016; Yazıcı and Şahin, 2016; Zorenc et al., 2016).

Black elderberry fruits (*Sambucus nigra*) are one of the richest sources of bioactive compounds compared to other fruits (Bermúdez-Soto and Tomás-Barberán, 2004; Duymuz et al., 2014; Mikulic-Petkovsek et al., 2015a). *S. nigra* is a traditionally cultivated and harvested wild-growing species, which was mainly appreciated in the past for its medicinal properties (Akbulut et al., 2009). Fruits and inflorescences have been used to heal various diseases, such as cough, influenza, and bronchitis (Kaack and Austed, 1998). Elderberry inflorescences have been regarded as less important than fruits; however, the former may represent a valuable source of phenolic acids and flavonoids (Christensen et al., 2008).

The wide range of elderberry use requires a broad range of genotypes (cultivars) suitable for the production of inflorescences, fruits, or both. Genotypic and phenotypic variations within *Sambucus nigra* appear to be insufficient to satisfy all specific demands, especially those associated with yield (number and size of inflorescences), chemical composition of inflorescences, growth characteristics, uniformity of flowering, and resistance to pests and diseases. Our studies indicate that interspecific hybridization efficiently increases the variation in the chemical composition of elderberry fruits (Mikulic-Petkovsek et al., 2014, 2016).

Polyphenolic compounds are regarded as important chemical compounds due to their high antioxidant efficacy (Ozgen et al., 2010), which has a positive impact on people's health, and may impede the development of cancer and inhibit oxidative damage (Akbulut et al., 2009). Phenolic compounds possess various pharmacological effects: antiviral, antibacterial, antiinflammatory, and anticancer functions. Positive effects of flavonoids and phenolic acids have also been reported in connection with cardiovascular diseases (Olthof et al., 2001; Netzel et al., 2002; Murkovic et al., 2004). It has been reported that the concentration of phenolics is closely connected with the antioxidant activity of plant tissue and thus an assessment of the phenolic profile is important for the determination of antioxidant capacity (Choi and Kwak, 2014). The antioxidant activity of elderberry inflorescence extracts ranged from 44.8 to 118.2 mM TE/kg DW (Mikulic-Petkovsek et al., 2016).

* Correspondence: maja.mikulic-petkovsek@bf.uni-lj.si

Elderberry inflorescences can be used as infusions, juices, syrups, liqueurs, sabesa (a traditional, nonalcoholic sweet beverage), and yoghurts. The content of phenolic acids and flavonoids in dried elderberry inflorescences has been studied by Serkedjieva (1996) and Zakay-Rones et al. (2004). All authors reported relatively high levels of these compounds in infusions prepared from elderberry inflorescences. Bioactive compounds in elderberry inflorescences and elderflower beverages were also analyzed by Christensen et al. (2008) and Mikulic-Petkovsek et al. (2015b).

Elderberry inflorescences have often been used in traditional folk medicine (Zakay-Rones et al., 2004; Ozgen et al., 2010). Elderflower extracts are recognized to have anticatarrhal, diuretic, and antiinflammatory properties (Cejpek et al., 2009). Bhattacharya et al. (2013) reported that elderflowers also have antidiabetic characteristics.

Only a few published studies have focused on elucidation of the phenolic profile of elderberry inflorescence infusions. Kaack and Christensen (2010) identified quercetin-3-rutinoside, kaempferol-3-rutinoside, and isorhamnetin-3-rutinoside as the most frequently detected flavonol derivatives in elderberry infusions. The main phenolic acids in elderflower infusions were 5-caffeoylquinic and 1,5-dicaffeoylquinic acid. The authors also concluded that the content and stability of individual phenolic compounds largely depended on the genotype. The importance of genotype has also been highlighted by other authors (e.g., Akbulut et al. 2009; Mikulic-Petkovsek et al., 2014, 2016).

Traditional elderflower beverages (sabesa, syrups) are characterized by high levels of total phenolic compounds. The content depends on the type of solvent and time of the extraction (Mikulic-Petkovsek et al., 2015b). The authors also suggest that traditional elderflower beverages could be incorporated into people's diets, since they are a good source of phenolics.

The aim of the present study was to analyze variations associated with the levels of various phenolic compounds in elderberry floral infusions. Significant differences in the contents of phenolics have already been confirmed among species, their botanical varieties, and interspecific hybrids (Mikulic-Petkovsek et al., 2014, 2016). Interspecific hybridization can thus potentially increase the diversity and contents of phenolic compounds. Floral infusions prepared from interspecific hybrids may contain a wide diversity of phenolic compounds, combining traits of the parental species.

Since this is the first published study on the chemical composition of floral infusions prepared from interspecific elderberry hybrids, the results will be very useful for reviewing the potential of interspecific hybridization aimed at improving the chemical composition of elderberry inflorescences. Data analysis could single out *Sambucus* species best suited for genetic recombination and selection of interspecific hybrids for direct use in agricultural production and/or for further genetic breeding.

2. Materials and methods

2.1. Plant material

The investigation included 14 genotypes: 3 species (*S. cerulea*, *S. javanica*, and *S. nigra* (*S. nigra* subsp. *nigra* (the common genotype), *S. nigra* var. *laciniata*, *S. nigra* var. *viridis*), and 9 interspecific hybrids JA/RAC (*S. javanica* × *S. racemosa*), JA/CER (*S. javanica* × *S. cerulea*), (JA/NI)/CER ((*S. javanica* × *S. nigra*) × *S. cerulea*), JA/NI (*S. javanica* × *S. nigra*), JANI/NI ((*S. javanica* × *S. nigra*) × *S. nigra*), JA/JANI (*S. javanica* × (*S. javanica* × *S. nigra*)), JANI/SIB ((*S. javanica* × *S. nigra*) × *S. sibirica*), (JA/NI) × cv. Black Beauty ((*S. javanica* × *S. nigra*) × *S. nigra* cv. Black Beauty), and JANI/NI ((*S. javanica* × *S. nigra*) × *S. nigra* cv. Black Beauty). According to Bolli (1994), *S. sibirica* Nakai belongs to *S. racemosa* L.

The samples were collected at Maribor University Gene Bank. The sampling took place from the end of May 2013 (some genotypes of *S. nigra*) to the beginning of August 2013 (*S. cerulea* and the hybrid *S. javanica* × (*S. javanica* × *S. nigra*)). From each sampled plant (n = 14), 7–10 fully developed inflorescences were collected and dried in a dry room, following traditional recipes used for preparation of flowers of various plant species for floral infusions. When drying was completed, individual samples were packed into brown paper bags, labelled, and stored until further analyses.

2.2. Extraction of phenolic compounds

Dried inflorescences were mixed and crushed in a mortar into fine powder. For the extraction, 0.5 g of elderflower samples (n = 14) was infused in 80 mL of boiling water (100 °C) and left for 5 min. After the extraction, the inflorescence extracts were centrifuged for 10 min at 10000 rpm. Each supernatant was filtered through a Chromafil AO-20/25 cellulose ester filter produced by Macherey-Nagel (Düren, Germany) and transferred into a vial prior to injection into the HPLC system.

2.3. Determination of individual phenolic compounds using HPLC-DAD-MS^n analysis

Phenolic compounds were analyzed on a Thermo Finnigan Accela HPLC system (Thermo Scientific, San Jose, CA, USA) with a diode array detector at 280 nm (hydroxycinnamic acid derivatives, flavanones) and 350 nm (flavonols). The spectra of the compounds were recorded between 200 and 600 nm. The column was a Gemini C_{18} (150 × 4.6 mm 3 µm; Phenomenex, Torrance, CA, USA) operated at 25 °C. The elution solvents were aqueous 0.1% formic acid in double distilled water (A) and 0.1% formic acid in acetonitrile (B).

The samples were eluted according to a linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, and then anisocratic mixture for 15 min before returning to the initial condition (Wang et al., 2002). The injection amount was 20 µL and the flow rate was 0.6 mL min^{-1}.

All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with electrospray ionization (ESI) in negative ion mode. The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 115 to 1500. The injection volume was 10 µL and the flow rate maintained at 0.6 mL min^{-1}. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, the source voltage was 3 kV, and normalized collision energy was 20%–35%. Spectral data were elaborated using the software Excalibur (Thermo Scientific). All phenolic compounds were identified by retention time (Rt), UV-visible absorbance (λ_{max}), mass of parent ions in negative mode ($[M-H]^-$), and mass of aglycone ions after fragmentation ($[A-H]^-$).

Contents of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg per 100 g of dried elderflowers. Phenolic compounds were calculated on the same phenolic standards and for compounds lacking standard quantification was carried out using similar compounds as standards. Thus, ferulic acid derivatives were quantified in equivalents of ferulic acid, *p*-coumaric acid derivatives in equivalents of *p*-coumaric acid, naringenin derivatives in equivalent of naringenin, isorhamnetin derivatives in equivalents of isohamnetin-3-glucoside, kaempferol derivatives in equivalents of kaempferol-3-glucoside, and all quercetin derivatives in equivalents of quercetin-3-galactoside. The contents of all identified phenolic compounds were summarized within each phenolic group and the results are presented in Table 1.

2.4. Statistical analysis

The data were analyzed using Statgraphics Centurion XVI. I based on one-way analysis of variance (ANOVA). The differences in composition of elderflower infusions were assessed among species and interspecific hybrids and tested using Duncan's test at the 0.05 significance level. Means and standard errors of the means are reported (mean ± SE).

3. Results and discussion

HPLC-MS analysis of elderberry infusions included samples of 14 genotypes with specific phenolic profiles. The majority of phenolics in elderberry inflorescences have hydroxycinnamic acids (HCAs). They represented 35%–70% of total analyzed phenolics (TAPs). 3-Caffeoylquinic acid, 4-caffeoylquinic acid, and 5-caffeoylquinic acid were detected in infusions from all analyzed species and interspecific hybrids (Table 2), and 3-feruloylquinic acid, 5-feruloylquinic acid, 3-*p*-coumaroylquinic acid, 5-*p*-coumaroylquinic acid, and dicaffeoylquinic acid were additionally detected in infusions prepared from specific hybrids/species.

Infusions prepared from JANI/NI × cv. Black Beauty (1285.86 mg 100 g^{-1} DW) and JANI/NI (1251.83 mg 100 g^{-1} DW) interspecific hybrids were characterized by the highest levels of total HCAs (Table 1). Among the analyzed species, *S. nigra* var. *laciniata* (1035.79 mg 100 g^{-1} DW), *S. cerulea* (973.05 mg 100 g^{-1} DW), JA/CER (985.34 mg 100 g^{-1} g DW), and JANI/CER (1016.76 mg 100 g^{-1} g DW) infusions contained comparably high levels of total HCA. The lowest contents of HCA were determined in the infusion of *S. javanica* (327.57 mg 100 g^{-1} g DW) and *S. nigra* (414.53 mg 100 g^{-1} DW) (Table 1).

The highest levels of 4-caffeoylquinic acid (4CQA) were detected in JANI/NI × cv. Black Beauty and JANI/SIB infusions and 5-caffeoylquinic acid (5CQA, chlorogenic acid) prevailed in infusions of *S. nigra* var. *laciniata* and JANI/NI and JANI/NI × cv. Black Beauty hybrids. Compared to *S. javanica* infusion, the hybrid JANI/NI × cv. Black Beauty had a 9.9 times higher concentration of 4-caffeoylquinic acid and 8.2 times higher concentration of 5-caffeoylquinic acid (Table 2).

The highest content of 3-caffeoylquinic acid (neochlorogenic acid) was determined in infusions prepared from JA/CER interspecific hybrid (423.51 mg 100 g^{-1} DW) and the lowest in infusions of *S. nigra* var. *laciniata* (82.32 mg 100 g^{-1} DW). The former was thus characterized by 5.1-fold higher levels of 3-caffeoylquinic acid than *S. nigra* var. *laciniata* and a 2.9-fold higher content than in infusions prepared from the common *S. nigra* type.

Dicaffeoylquinic acid was typical of the botanical varieties *S. nigra* var. *viridis* and *S. nigra* var. *laciniata*. Among interspecific hybrids, it was detected only in infusions of JANI/NI × cv. Black Beauty. The content of this compound in *S. nigra* var. *laciniata* was 8.7 and 6.1 times higher than in *S. nigra* var. *viridis* and the interspecific hybrid JANI /NI × cv. Black Beauty, respectively (Table 2).

Infusions prepared from JANI/NI × cv. Black Beauty and JANI/NI interspecific hybrids contained from 1.2- to 3.9-fold higher average levels of total HCA than infusions of other hybrids or *Sambucus* species. The second most valuable group of interspecific hybrids consisted of JANI/CER (1016.75 mg 100 g^{-1} DW), JA/CER (985.34 mg 100 g^{-1} DW), JANI/SIB (926.93 mg 100 g^{-1} DW), and JA/JANI (920.62 mg 100 g^{-1} DW) hybrids (Table 1).

In terms of the content and diversity of all studied phenolics belonging to HCAs among the involved species

Table 1. Content of individual phenolic groups (mean ± SE in mg 100 g^{-1} DW) in infusions prepared from different elderberry species and interspecific hybrids.

Genotype	Total hydroxycinnamic acids		Total isorhamnetin glycosides		Total quercetin glycosides		Total kaempferol glycosides		Total flavanones	
S. nigra	414.53 ± 47.24	a	137.09 ± 13.45	e	670.23 ± 74.74	e	14.64 ± 0.87	a	38.79 ± 11.83	bc
S. cerulea	973.05 ± 88.60	d	117.6 ± 20.14	d	34.09 ± 7.16	a	32.27 ± 7.27	cde	5.79 ± 1.11	ab
S. javanica	327.57 ± 25.19	a	49.24 ± 9.93	ab	186.65 ± 37.48	b	11.12 ± 1.77	a	_	
S. nigra var. *viridis*	793.62 ± 90.43	bc	29.74 ± 4.84	a	237.25 ± 45.79	b	21.39 ± 5.43	abc	17.52 ± 8.53	ab
S. nigra var. *laciniata*	1035.79 ± 93.15	d	98.97 ± 17.33	cde	370.84 ± 54.04	c	22.01 ± 3.11	abc	6.59 ± 2.67	a
JA/RAC	783.36 ± 34.00	bc	197.45 ± 29.91	f	411.36 ± 50.58	c	49.87 ± 7.67	fg	112.74 ± 72.23	d
JA/CER	985.34 ± 60.24	d	294.00 ± 17.40	g	243.31 ± 14.02	b	92.97 ± 5.80	h	5.79 ± 1.11	a
JANI/CER	1016.75 ± 101.11	d	83.12 ± 12.21	bcd	483.08 ± 84.36	cd	42.39 ± 3.27	ef	4.02 ± 3.68	a
JA/NI	679.19 ± 53.32	b	128.80 ± 18.72	de	182.65 ± 27.90	b	29.46 ± 3.21	bcd	_	
JANI/NI	1251.83 ± 37.14	e	105.00 ± 8.02	cde	560.30 ± 31.90	de	58.36 ± 4.42	g	49.01 ± 8.04	c
JA/JANI	920.62 ± 38.66	cd	67.62 ± 8.88	abc	380.12 ± 27.82	c	35.92 ± 4.22	de	_	
JANI/SIB	926.93 ± 32.50	cd	61.33 ± 6.98	abc	417.95 ± 34.53	c	36.44 ± 4.04	de	9.69 ± 4.55	a
JANI/BB	768.90 ± 53.67	bc	39.04 ± 4.34	ab	224.15 ± 32.62	b	17.19 ± 2.00	ab	24.81 ± 14.67	abc
JANI/NI/BB	1285.86 ± 56.69	e	194.24 ± 36.97	f	480.86 ± 59.72	cd	23.73 ± 4.04	abcd	_	

Mean values marked with the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$

Table 2. Content of individual hydroxycinnamic acids (mean ± SE in mg 100 g^{-1} DW) in infusions prepared from different elderberry species and interspecific hybrids.

Genotype	3-caffeoylquinic acid		4-caffeoylquinic acid		5-caffeoylquinic acid		3-feruloylquinic acid		5-feruloylquinic acid		3-*p*-coumaroylquinic acid		5-*p*-coumaroylquinic acid		Dicaffeoylquinic acid	
S. nigra	145.12 ± 12.10	cd	50.22 ± 5.39	de	258.02 ± 257.83	ab										
S. cerulea	178.78 ± 21.62	defg	52.81 ± 7.68	e	630.72 ± 47.25	cde	76.80 ± 8.82	g	7.12 ± 1.61	ab	8.47 ± 1.15	b	5.99 ± 0.72	a		
S. javanica	158.99 ± 12.41	cde	7.33 ± 1.10	a	111.33 ± 8.37	a	4.45 ± 0.66	a	15.07 ± 0.96	cd	17.92 ± 1.07	f	8.77 ± 0.56	a		
S. nigra var. *viridis*	152.22 ± 18.02	cde	33.96 ± 4.39	b	536.49 ± 57.28	cd	19.10 ± 2.47	b	26.34 ± 5.37	fg	5.54 ± 0.79	a			3.91 ± 0.94	a
S. nigra var. *laciniata*	82.32 ± 7.58	a	46.8 ± 94.48	cde	820.14 ± 69.28	ef	3.40 ± 0.32	a	8.37 ± 1,25	ab	5.35 ± 0.76	a	24.11 ± 2.44	c	34.20 ± 9.50	b
JA/RAC	190.69 ± 7.68	efg	47.19 ± 2.16	cde	400.10 ± 14.90	bc			17.33 ± 2.54	de	14.78 ± 0.93	e	28.94 ± 2.21	cd		
JA/CER	423.51 ± 22.11	i	44.20 ± 2.25	bcde	458.97 ± 73.48	bcd					8.77 ± 0.28	bc				
JANI/CER	207.34 ± 20.13	fg	53.21 ± 3.38	e	642.06 ± 73.48	de	58.86 ± 3.74	f	17.64 ± 1.92	de	13.36 ± 1.01	de	18.23 ± 0.96	b		
JA/NI	126.88 ± 10.07	bc	41.95 ± 3.33	bcd			30.82 ± 2.45	cd	2.74 ± 0.77	a	12.50 ± 1.35	de	33.60 ± 4.91	d		
JANI/NI	97.56 ± 4.65	ab	49.81 ± 1.66	de	987.50 ± 21.14	f	27.56 ± 0.92	bcd	29.26 ± 1.81	g	9.36 ± 0.37	bc	40.31 ± 1.18	e		
JA/JANI	157.20 ± 9.66	cde	38.0 ± 1.94	bc	632.19 ± 22.91	cde	50.69 ± 2.59	ef	22.34 ± 1.83	f			15.45 ± 1.16	b		
JANI/SIB	326.30 ± 8.47	h	70.06 ± 2.92	f	449.31 ± 16.57	bcd	34.95 ± 1.46	d	5.63 ± 0.68	ab	17.98 ± 1.26	f	18.15 ± 1.22	b		
JANI/BB	171.10 ± 13.10	def	49.38 ± 2.29	de	462.57 ± 36.54	bcd	44.79 ± 2.08	e	8.95 ± 0.57	bc	11.28 ± 0.73	cd	14.94 ± 0.80	b		
JANI/NI/BB	214.61 ± 12.69	g	72.67 ± 5.42	f	910.28 ± 30.35	f	23.73 ± 1.77	bc	16.17 ± 2.11	de	9.26 ± 0.80	bc	26.73 ± 1.64	c	5.56 ± 2.89	a

Mean values marked with the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$

(Table 2), it can be concluded that first place in high content of HCAs belongs to inflorescences of *S. cerulea* and *S. nigra* var. *laciniata*. The inflorescences of *S. nigra* and *S. javanica* are poor in HCA content. Among the interspecific hybrids, the most valuable in HCA content are JANI/NI and JANI/NI × cv. Black Beauty.

Dadáková et al. (2010) studied the diversity of bioactive compounds in tea extracts of well-known medicinal plants: *Filipendula ulmaria*, *Melissa officinalis*, *Betula pendula*, *Sambucus nigra*, *Achillea millefolium*, *Agrimonia eupatoria*, and *Glechoma hederacea*. Chlorogenic acid was detected in three species: *Betula pendula*, *Sambucus nigra*, and *Achillea millefolium*. A 6.6-fold higher level of chlorogenic acid (5 CQA) was detected in *Sambucus nigra* (166 mg L^{-1}) infusions compared to *Betula pendula* (25.2 mg L^{-1}) leaf extracts. Raal et al. (2012) studied the content of polyphenolic compounds in German chamomile (*Chamomilla recutita*) infusions of samples originating from different countries. The total content of polyphenolics and phenolic acids did not depend on the origin of the sample. The content of phenolic acids varied between 7.7 and 91.4 mg/200 mL. The main phenolic compounds detected in German chamomile infusions were chlorogenic acid, ferulic acid glycosides, dicaffeoylquinic acids, and apigenin glycosides. Elderflower infusions analyzed in our study contained higher levels of phenolic acids than German chamomile extracts. Horzic et al. (2009) also reported different phenolic acids (chlorogenic, *p*-coumaric, and ferulic acid) in chamomile infusions.

In elderberry infusions, various glycosides of isorhamnetin, quercetin, and kaempferol were identified (Tables 3–5). Flavonols represented approximately 20%–60% of total analyzed phenolics in elderflower infusions. These phenolic compounds are most frequently present in foods of plant origin (Mikulic-Petkovsek et al., 2015a). In our study, we determined the following isorhamentin derivatives (Table 3): isorhamnetin-3-rutinoside, isorhamnetin dihexoside, isorhamnetin dihexoside rhamnoside, isorhamnetin rhamnoside hexoside rhamnoside, isorhamnetin dihexoside pentoside, isorhamnetin acetyl hexoside, isorhamnetin acetyl dihexoside, and isorhamnetin hexoside pentoside. Isorhamnetin glycosides represented 10%–65% of all analyzed flavonols in elderflower infusions.

JA/CER hybrid infusions contained the highest levels of total isorhamnetin glycosides (294.09 mg 100 g^{-1} DW), followed by JA/RAC (197.45 mg 100 g^{-1} DW) and JANI/NI × cv. Black Beauty (194.24 mg 100 g^{-1} DW) (Table 1). The content of isorhamnetin-3-rutinoside was also highest in infusions prepared from JA/CER, JA/RAC and JANI/NI × cv. Black Beauty interspecific hybrids (Table 3). Interestingly, the content was significantly higher in infusions of hybrids than in extracts prepared from their corresponding parental species. Among the studied species, *S. nigra* infusions were characterized by the highest levels of total isorhamnetin glycosides (137.09 mg 100 g^{-1} DW) (Table 1) and a particularly high content of isorhamentin-3-rutinoside (107.57 mg 100 g^{-1} DW). The lowest average value of total isorhamnetin glycosides was determined in *S. nigra* var. *viridis* (29.72 mg 100 g^{-1} DW) infusions (Table 1). Infusions prepared from this genotype were characterized by 9.9-fold lower average values of total isorhamnetin glycosides than JA/CER infusions.

From the group of quercetin glycosides, quercetin-3-rutinoside, quercetin-3-glucoside, quercetin acetyl hexoside, quercetin hexoside pentoside, quercetin dihexoside, and quercetin dihexoside rhamnoside were identified in elderberry infusions (Table 4). Quercetin-3 rutinoside and quercetin acetyl hexoside were detected in nearly all analyzed infusions prepared from *Sambucus* species and their interspecific hybrids. Quercetin-3-rutinoside has previously been reported as the main quercetin glycoside in elderberry inflorescences (Mikulic-Petkovsek et al., 2015b) and it is also accumulated in elderberry fruits (Mikulic-Petkovsek et al., 2015a). The highest content of quercetin-3-rutinoside was found in *S. nigra*, JANI/CER, JANI/NI, and JANI/NI × cv. Black Beauty infusions (Table 4). Similar values for rutin were reported by Veljkovic et al. (2013) in elderflower tea.

Quercetin-3-glucoside was found in the botanical variety *S. nigra* var. *viridis* and the interspecific hybrid JA/RAC. The difference in its content was not significant. Quercetin-3-glucoside was not identified in inflorescence extracts of other analyzed genotypes (Table 4). Analysis of the contents of quercetin hexoside pentoside showed that there were some significant differences between the studied interspecific hybrids. The highest values were obtained in the interspecific hybrid JA/CER and JANI/NI and the lowest in JA/JANI (the concentration in JANI/NI and JA/CER was 3.6 to 4.4 times higher than in JA/JANI). Infusions prepared from *S. nigra* and JANI/NI elderflowers were very rich in total quercetin derivatives. In contrast, infusions of *S. cerulea* (34.09 mg 100 g^{-1} DW) were characterized by 16.5- to 19.6-fold lower contents of quercetin derivatives compared to the previously mentioned samples. Comparatively high levels of quercetin derivatives have been quantified in chamomile tea (Nováková et al., 2010). In contrast, Raal et al. (2012) reported their share in a range from 0.29% to 1.21% TAPs in infusions from commercial German chamomile. As mentioned previously, flavonols represented from 20% to 60% TAPs in elderflower infusions analyzed in the present study.

Herbal infusions are considered a very important source of flavonoids and other biologically active compounds in human medicine and nutrition (Veljkovic

Table 3. Content of individual isorhamnetin glycosides (mean ± SE in mg 100 g^{-1} DW) in infusions prepared from different elderberry species and interspecific hybrids.

Genotype	Isorha-3-rut		Isorha-dihex		Isorha-dihex-rha		Isorha-rha-hex-rha		Isorha-dihex-pent		Isorha-acetyl-hex		Isorha-acetyl-dihex		Isorha-hex-pent	
S. nigra	107.57 ± 9.00	ef	1.39 ± 0.20	ab	1.02 ± 0.39	a	4.04 ± 0.94	c			31.56 ± 3.60	f				
S. cerulea	7.46 ± 1.62	a	31.82 ± 4.42	f	14.33 ± 1.38	a					7.03 ± 2.16	cd	11.05 ± 1.12	a	1.99 ± 0.20	b
S. javanica	46.53 ± 9.75	bcd	0.86 ± 0.07a	a	1.32 ± 0.08	a	0.52 ± 0.07	a								
S. nigra var. *viridis*	11.75 ± 0.68	ab	3.83 ± 0.83	abcd					6.56 ± 1.15	b	7.09 ± 2.36	cd			0.49 ± 0.11	a
S. nigra var. *laciniata*	81.87 ± 14.72	de	8.00 ± 1.45	d	4.30 ± 0.61	a	0.92 ± 0.30	a			3.86 ± 0.64	ab				
JA/RAC	174.49 ± 24.97	g	3.37 ± 0.90	abc	11.97 ± 2.04	a	4.20 ± 0.47	c			3.41 ± 0.90	ab				
JA/CER	231.23 ± 14.56	h	13.90 ± 1.19	e					3.20 ± 0.84	a	3.89 ± 0.67	ab	8.85 ± 0.52	a		
JANI/CER	74.49 ± 13.54	de	2.62 ± 0.61	abc	2.33 ± 0.64	a	3.67 ± 0.79	bc								
JA/NI	124.44 ± 18.41	f	2.70 ± 0.16	abc	0.24 ± 0.12	a	0.67 ± 0.07	a			0.72 ± 0.67	a				
JANI/NI	77.28 ± 5.56	de	13.48 ± 1.89	e	8.44 ± 8.4	b					9.79 ± 0.62	e				
JA/JANI	64.64 ± 8.41	cd	2.70 ± 0.46	abc	0.28 ± 0.07	a										
JANI/SIB	48.77 ± 5.27	bcd	6.41 ± 0.94	cd	3.86 ± 1.10a	a	2.28 ± 0.4	abc								
JANI/BB	28.94 ± 3.09	abc	5.18 ± 0.80	bcd	3.33 ± 0.32	a	1.58 ± 0.60	ab								
JANI/NI/BB	168.34 ± 33.35	g	5.98 ± 1.07	cd	7.79 ± 0.92	a	4.25 ± 0.53	c			4.28 ± 0.86	ab			3.58 ± 0.48	c

Mean values marked with the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$
Legend: Isorha…isorhamnetin; rut…rutinoside; dihex…dihexoside; rha…rhamnoside; hex…hexoside, pent…pentoside.

Table 4. Content of individual quercetin glycosides and two flavanones (mean ± SE in mg 100 g^{-1} DW) in infusions prepared from different elderberry species and interspecific hybrids.

Genotype	Querc-3-rut		Querc-3-glu		Querc-acetyl-hex		Querc-hex-pent		Querc-dihex		Querc-dihex-rha		Naring-hex 1		Naring-hex 2	
S. nigra	588.97 ± 48.77	f			90.38 ± 10.60	d	5.77 ± 0.72	b					16.77 ± 2.51	b	24.56 ± 3.69	a
S. cerulea	5.01 ± 0.86	a			11.07 ± 4.35	ab	5.16 ± 0.68	b	12.91 ± 1.58	b			13.27 ± 1.84	ab		b
S. javanica	186.19 ± 37.43	b			0.45 ± 0.09	a										
S. nigra var. *viridis*	185.18 ± 36.07	b	9.25 ± 2.74	a	21.16 ± 4.68	bc	5.91 ± 1.40	b	15.73 ± 2.31	bc			17.52 ± 3.81	b		a
S. nigra var. *laciniata*	333.95 ± 49.29	c			10.13 ± 2.03	a			19.51 ± 2.72	cd			6.59 ± 1.19	A		
JA/RAC	393.42 ± 45.36	cde	7.70 ± 1.95	a	10.23 ± 4.22	a							28.62 ± 7.63	c	84.11 ± 24.97	b
JA/CER	196.94 ± 12.24	b					9.89 ± 0.54	c	23.60 ± 1.22	d	3.52 ± 0.33	a	5.79 ± 0.49	a		
JANI/CER	479.08 ± 83.56	def			3.99 ± 0.94	a							4.02 ± 1.64	a		
JA/NI	177.37 ± 27.33	b			5.28 ± 0.96	a										
JANI/NI	487.48 ± 26.90	ef			29.58 ± 2.21	c	7.90 ± 1.18	bc	30.51 ± 2.32	e	4.82 ± 1.19	a	49.01 ± 3.60	c		
JA/JANI	368.08 ± 26.53	cd			4.24 ± 0.85	a	2.20 ± 0.48	a	5.58 ± 0.74	a						
JANI/SIB	406.90 ± 34.09	cde			5.29 ± 0.85	a			5.75 ± 1.47	a			9.69 ± 2.03	ab		
JANI/BB	185.86 ± 28.95	b			7.17 ± 1.20	a	6.72 ± 1.52	b	24.38 ± 2.58	d			12.98 ± 2.01	ab	11.83 ± 4.81	a
JANI/NI/BB	475.16 ± 59.09	def			5.70 ± 0.76	a										c

Mean values marked with the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$
Legend: Querc…quercetin; rut…rutinoside; glu…glucoside; hex…hexoside; pent…pentoside; dihex…dihexoside; rha…rhamnoside; naring…naringenin.

Table 5. Content of individual kaempferol glycosides (mean ± SE in mg 100 g^{-1} DW) in infusions prepared from different elderberry species and interspecific hybrids.

Genotype	Kaempf-3-rut		Kaempf-dihex		Kaempf-dihex-rha		Kaempf-rha-hex		Kaempf-acetyl-hex 1		Kaempf-acetyl-hex 1	
S. nigra	11.14 ± 1.13	bcd	5.23 ± 0.78	cd								
S. cerulea	2.46 ± 0.34	a	20.52 ± 2.85	f			0.71 ± 0.19	a	1.29 ± 0.37	ab		
S. javanica	5.03 ± 1.59	ab	2.25 ± 0.19	ab	3.83 ± 0.24	b						
S. nigra var. *viridis*	12.09 ± 2.88	cd	3.70 ± 0.80	abc							5.59 ± 1.90	b
S. nigra var. *laciniata*	11.75 ± 1.55	bcde	1.93 ± 0.35	a							1.35 ± 0.29	a
JA/RAC	24.89 ± 4.85	f	3.88 ± 1.03	abc							2.30 ± 0.82	ab
JA/CER	65.73 ± 4.30	g	13.15 ± 1.13	e	4.91 ± 0.43	bc	9.15 ± 1.15	b				
JANI/CER	16.60 ± 2.74	d	1.87 ± 0.44	a								
JA/NI	9.31 ± 1.31	abc	2.50 ± 0,15	abc	0.26 ± 0.13	a			0.79 ± 0.64	a		
JANI/NI	12.75 ± 1.30	cd	7.12 ± 1.00	d	5.08 ± 0.64	bc			2.25 ± 0.23	b		
JA/JANI	13.66 ± 1.79	cd	4.19 ± 0.70	abc	0.61 ± 0.16	a						
JANI/SIB	12.87 ± 1.23	cd	5.01 ± 0.73	bcd	4.72 ± 1.34	bc						
JANI/BB	7.69 ± 1.04	abc	3.19 ± 0.49	abc	6.30 ± 0.61	c						
JANI/NI/BB	18.94 ± 3.40	ef	1.44 ± 0.25	a	3.34 ± 0.39	b						

Mean values marked with the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$
Legend: Kaempf…kaempferol; rut…rutinoside; dihex…dihexoside; rha…rhamnoside; hex…hexoside.

et al., 2013). Among seven herbal infusions studied by Dadáková et al. (2010), the highest level of total quercetin derivatives was detected in infusions prepared from *Filipendula ulmaria* and *S. nigra* inflorescences (120 and 108 mg L^{-1}, respectively). Infusions of *S. nigra* analyzed in the present study were characterized by particularly high levels of rutin, which has only been detected in traces in previously studied infusions (Dadáková et al., 2010). *S. nigra* infusions were characterized by higher contents of total quercetin glycosides compared to other floral infusions and stood out as the richest samples analyzed in the present study (670.23 mg 100 g^{-1} DW) (Table 1). Infusions prepared from JANI/NI (560.38 mg 100 g^{-1} DW), JANI/CER (483.08 mg 100 g^{-1} DW), and JANI/NI × cv. Black Beauty (480.86 mg 100 g^{-1} DW) interspecific hybrids similarly contained high levels of total quercetin glycosides (Table 1).

From the group of flavanones, we found two naringenin hexosides in elderberry extracts (Table 4). The highest content of naringenin hexoside 1 was determined in JANI/NI and JA/RAC hybrid infusions. Naringenin hexoside 2 was only detected in three analyzed elderflower extracts (Table 4). Wilcox et al. (1999) state that naringenin is only moderately soluble in water but more soluble in organic solvents such as alcohol. Mikulic-Petkovsek et al. (2015b) similarly reported that water extract contained lower levels of naringenin in comparison with methanolic extracts.

Elderberry inflorescences also contained kaempferol glycosides, an important group of flavonoids. Kaempferol intake reduces the risk of various cancers and cardiovascular diseases (Calderon-Montaño et al., 2011). In elderflower infusions, kaempferol-3-rutinoside, kaempferol dihexoside, kaempferol dihexoside rhamnoside, kaempferol rhamnoside hexoside, and two kaemferol acetyl hexosides were identified (Table 5). Kaempferol-3-rutinoside and kaempferol dihexoside were recorded in inflorescence extracts of all 14 genotypes included in the analysis. The highest concentration of kaempferol-3-rutinoside was determined in the interspecific hybrid JA/CER and the lowest in *S. cerulea*. The hybrid JA/CER had a 27 times higher concentration than its male parent, *S. cerulea*. Kaempferol dihexoside rhamnoside was detected in infusions prepared from most interspecific hybrids but only from *S. javanica* species (Table 5). Linden blossoms are a good source of kaempferol derivatives, containing 565 mg kaempferol/100 g (Karakaya et al., 1999). High total kaempferol levels were characteristic of infusions prepared from JA/CER (92.97 mg 100 g^{-1} DW), JANI/NI (58.36 mg 100 g^{-1} DW), JA/RAC (49.87 mg 100 g^{-1} DW), and JANI/CER (42.39 mg 100 g^{-1} DW) interspecific hybrids (Table 1).

Taking into account the contents of all analyzed glycosides of kaempferol (Table 5), the most valuable interspecific hybrid is JA/CER, which also has the highest level of hybrid effect (16.55%, considering the parental mean value for the concentration of kaempferol-3-rutinoside). In terms of diversity, the most interesting are *S. cerulea*, JA/CER, JA/RAC, JANI, and JANI/NI infusions.

Generally, interspecific genetic recombination among *Sambucus* species positively affects phenolic compound diversity (the number of different phenolic compounds in elderflower infusions) (Tables 2–5). However, it is not possible to conclude that hybrids based on three diverse species always exhibit higher phenolic diversity than individual parental species or two-species hybrids. Some of the latter (e.g., hybrids involving *S. javanica* and *S. cerulea*, and *S. javanica* and *S. nigra*; Tables 3–5) were characterized by a much richer phenolic profile than three-species hybrids.

According to our survey, interspecific hybridization is a very useful tool for diversification of the phenolic composition in elderflower infusions. Among the involved species, *S. cerulea* was probably the most valuable because of exhibiting exceptionally high phenolic diversity. In order to improve further the levels and diversity of phenolic compounds in infusions, it would be advisable to recombine promising interspecific hybrids or to backcross these with one or more parents, or to include additional *Sambucus* species. It would also be advisable to produce and evaluate large progenies, because genotypic and phenotypic segregation is very common among *Sambucus* hybrids. Another (cheaper and simpler) method for improving the chemical composition of elderflower infusions is to combine elderberry inflorescences of various species and interspecific hybrids and/or flowers, leaves, and fruits of other medicinal plants.

Acknowledgments

This work is part of the programs No P1-0164 and No P4-0013-0481 funded by the Slovenian Research Agency (ARRS).

References

Akbulut M, Ercisli S, Tosun M (2009). Physico-chemical characteristics of some wild grown European elderberry (*Sambucus nigra* L.) genotypes. Pharmacog Mag 20: 320-323.

Bolli R (1994). Revision of the genus *Sambucus*. Diss Bot 223: 1-227.

Bermúdez-Soto MJ, Tomás-Barberán FA (2004). Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. Eur Food Res Technol 219: 133-141.

Bhattacharya S, Christensen KB, Olsen L CB, Christensen LP, Grevsen K, Faergeman NJ, Kristiansen K, Young JF, Oksbjerg N (2013). Bioactive components from flowers of *Sambucus nigra* L. increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in *Caenorhabditis elegans*. J Agric Food Chem 61: 11033-11040.

Calderon-Montaño JM, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M (2011). A review on the dietary flavonoid kaempferol. Mini Rev Med Chem 11: 298-344.

Cejpek K, Malouskova I, Konecny M, Velisek J (2009). Antioxidant activity in variously prepared elderberry foods and supplements. Czech J Food Sci 27: S45-S48.

Choi ILS, Kwak EJ (2014). Comparison of antioxidant activities and bioactive compounds between bokbunja (*Rubus coreanus* Miq.) and other berries. Food Sci Biotech 23: 1677-1682.

Christensen LP, Kaack K, Frette XC (2008). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of elderflower extracts rich in flavonoids and phenolic acids. Eur Food Res Technol 227: 293-305.

Dadáková E, Vrchotová N, Tříska J (2010). Content of selected biologically active compounds in tea infusions of widely used European medicinal plants. J Agrobiol 27: 27-34.

Duymus HG, Goger F, Baser KHC (2014). In vitro antioxidant properties and anthocyanin compositions of elderberry extracts. Food Chem 155: 112-119.

Ercisli S 2009. Apricot culture in Turkey. Sci Res Essays 4: 715-719.

Erturk Y, Ercisli S, Haznedar A, Cakmakci R 2010. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biological Research 43: 91-98.

Horzic D, Komes D, Belscak A, Ganic KK, Ivekovic D, Karlovic D (2009). The composition of polyphenols and methylxanthines in teas and herbal infusions. Food Chem 115: 441-448.

Hricova A, Fejer J, Libiakova G, Szabova M, Gazo J, Gajdosova A (2016). Characterization of phenotypic and nutritional properties of valuable *Amaranthus cruentus* L. mutants. Turk J Agric For 40: 761-771.

Kaack K, Austed, T (1998). Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. Plant Food Hum Nutr 52: 187-198.

Kaack K, Christensen LP (2010). Phenolic acids and flavonoids in tea processed from flowers of black elder (*Sambucus nigra* L.) stored in different packing materials. Eur J Hortic Sci 75: 214-220.

Karakaya S, El SN (1999). Quercetin, luteolin, apigenin and kaempferol contents of some foods. Food Chem 66: 289-292.

Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Todorovic B, Veberic R, Stampar F, Ivancic A (2014). Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. J Agric Food Chem 62: 5573-5580.

Mikulic-Petkovsek M, Ivancic A, Todorovic B, Veberic R, Stampar F (2015a). Fruit phenolic composition of different elderberry species and hybrids. J Food Sci. 80: C2180-C2190.

Mikulic-Petkovsek M, Samoticha J, Eler K, Stampar F, Veberic R (2015b). Traditional elderflower beverages: a rich source of phenolic compounds with high antioxidant activity. J Agric Food Chem 63: 1477-1487.

Mikulic-Petkovsek M, Ivancic A, Schmitzer V, Veberic R, Stampar F (2016). Comparison of major taste compounds and antioxidative properties of fruits and flowers of different *Sambucus* species and interspecific hybrids. Food Chem 200: 134-140.

Murkovic M, Abuja PM, Bergmann AR, Zirngast A, Adam U, Winklhofer-roob BM, Toplak H (2004). Effects of elderberry juice on fasting and postprandial serum lipids and low-density lipoprotein oxidation in healthy volunteers: a randomized, double-blind, placebo-controlled study. Eur J Clin Nutr 58: 244-249.

Netzel M, Strass G, Kaul C, Bitsch I, Dietrich H, Bitsch R (2002). In vivo antioxidative capacity of a composite berry juice. Food Res Int 35: 213-216.

Nováková L, Vildová A, Mateus JP, Gonçalves T, Solich P (2010) Development and application of UHPLC-MS/MS method for the determination of phenolic compounds in chamomile flowers and chamomile tea extracts. Talanta 82: 1271-1280.

Olthof MR, Hollman PCH, Kata MB (2001). Chlorogenic acid and caffeic acid are absorbed in humans. J Nutr 131: 66-71.

Ozgen M, Scheerens JC, Reese RN, Miller RA (2010). Total phenolic, anthocyanin contents and antioxidant capacity of selected elderberry (*Sambucus canadensis* L.) accessions. Pharmacogn Mag 6: 198-203.

Raal A, Orav A, Püssa T, Valner C, Malmiste B, Elmar Arak E (2012). Content of essential oil, terpenoids and polyphenols in commercial chamomile (*Chamomilla recutita* L. Rauschert) teas from different countries. Food Chem 131: 632-638.

Serkedjieva J (1996). A polyphenolic extract from *Geranium sanguineum* L. inhibits influenza virus protein expression. Phytother Res 10: 441-443.

Veljkovic JN, Pavlovic AN, Mitic SS, Tosic SB, Stojanovic GS, Kalicanin BM, Stankovic DM, Stojkovic MB, Mitic MN, Brcanovic JM (2013). Evaluation of individual phenolic compounds and antioxidant properties of black, green, herbal and fruit tea infusions consumed in Serbia: spectrophotometrical and electrochemical approaches. J Food Nutr Res 52: 12-24.

Wang SY, Zheng W, Galletta GJ (2002). Cultural system affects fruit quality and antioxidant capacity in strawberries. J Agric Food Chem 50: 6534-6542.

Wilcox LJ, Borradaile NM, Huff MW (1999). Antiatherogenic properties of naringenin, a citrus flavonoid. Cardiovasc Drug Rev 17: 160-178.

Yazıcı K, Şahin A 2016. Characterization of pomegranate (*Punica granatum* L.) hybrids and their potential use in further breeding. Turk J Agric For 40: 813-824.

Zakay-Rones Z, Thom E, Wollan T, Wadstein J (2004). Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. J Int Med Res 32: 132-140.

Zorenc Z, Veberic R, Stampar F, Koron D, Mikulic-Petkovsek M 2016. Changes in berry quality of northern highbush blueberry (*Vaccinium corymbosum* L.) during the harvest season. Turk J Agric For 40: 855-864.