European Food Research and Technology (2019) 245:469–478
https://doi.org/10.1007/s00217-018-3178-1

ORIGINAL PAPER

Nutraceutical properties and phytochemical characterization of wild Serbian fruits

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Received: 31 May 2018 / Revised: 5 October 2018 / Accepted: 14 October 2018 / Published online: 31 October 2018

Abstract

Wild fruits grown in Serbia, i.e., elderberry (*Sambucus nigra*), hawthorn (*Crataegus monogyna*), cornelian cherry (*Cornus mas*), and blackthorn (*Prunus spinosa*), are rich in secondary metabolites. In this study, the polyphenolic composition of wild fruit extracts and their antioxidant capacity were investigated by in vitro assays. Elderberry was characterized by the presence of arbutin (a skin protector), and cornelian cherry by syringic and gallic acids. In blackthorn, at least 11 different phenolic compounds were reported for the first time, including vanillic acid and naringin, the metabolite present in the highest amount. Blackthorn extracts were the richest in polyphenols (11.24–18.70 g GAE/kg FW) and had the highest activity in the DPPH radical test (180.93–267.11 mMTE/mL), while cornelian cherry extracts showed the most effective ferric ion chelating (81.37–90.66%) and antityrosinase inhibition capacities (21.75–74.23%). No sample was able to scavenge NO. Using the principal component analysis, wild fruit samples were classified into four separate clusters due to distinctive phenolic profiles and antioxidant capacity. Our investigation showed how every fruit could be considered unique in terms of its phytonutrient content. Thus, Serbian wild fruits may be a great source of bioactive natural compounds and could be therefore considered particularly useful in food supplement production. Particularly, as a source of natural antioxidants, these species could be used to extend the shelf life of food products and replace synthetic antioxidants, avoiding potential health risks and toxicity.

Keywords Wild Serbian fruits · UHPLC–DAD–HESI–MS/MS · Phenolic profile · Antioxidant potential · Functional foods

Introduction

Over the last decade, wild fruits have received increasing attention due to their medicinal properties and high nutritional and nutraceutical values, being a rich source of antioxidant compounds, vitamins, minerals, and polyphenols [1]. Serbia is considered to be a secondary gene center for some representatives of the *Rosaceae* family, as it has a very rich gene pool for indigenous fruits with more than 100 species classified into 15 different families. It is mostly used as rootstocks for fruit production, and as noble-wood production for making furniture in prevention of erosion, for landscape architecture, and in bee keeping [2]. The most widespread species belong to *Crataegus, Prunus, Cornus*, and *Sambucus* genera. Due to their abundance in proanthocyanidins, catechins, phenolic acids, essential oils, and terpenoids, *Crataegus monogyna* (hawthorn, HW) fruits possess strong antioxidant potential [3]. Furthermore, several studies have showed the positive influence of HW fruit extracts on the cardiovascular system, acting as antiarrhythmic, hypolipidemic, and

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00217-018-3178-1) contains supplementary material, which is available to authorized users.

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hypotensive agents [4, 5]. *Prunus spinosa* (blackthorn or sloe, BL) is mainly used to make jellies, jams, vinegar, wine, ice-cream, and distilled alcoholic and non-alcoholic beverages [6]. BL fruits are rich in polyphenols, vitamins, and minerals [7]. Significant antimicrobial activity was reported, suggesting the usefulness of this fruit in food and pharmaceutical industries [8]. *Cornus mas* L. (cornelian cherry, CC) is mainly used as food and possesses many pharmacological and therapeutic effects [9–11]. *Sambucus nigra* (elderberry, EL) fruits are generally eaten when fully ripe, after cooking, or can be used to make jams and jellies [12]. Literature reports the use of EL in treating several diseases such as diabetes, colic, diarrhea, fever, coughs, colds, congestion, bronchitis, influenza, allergies, rheumatism, swollen limbs, burns, and inflamed mucous membranes [13].

According to the International Federation of Organic Agriculture Movements (IFOAM) and Mediterranean Organic Agriculture Network (MOAN), wild fruits in Serbia are collected on around 450,000 ha, but just 1550 ha are certified for organic production [14]. Harvesting of wild berries is traditionally performed in rural economies and at least ten companies are exporting collected wild fruits worth several millions of euros. Wild fruits are processed in households as a part of tradition in the entire Serbia and are sold through specialized retail points such as green markets and specialty shops. Although the domestic market is growing, wild berries collection is export driven. Besides, fruits are used in folk medicine, and the plants as ornamental and honey plants. The aim of including wild berries in Serbian agriculture is the diversification of agriculture throughout the region, the development of new types of agricultural products and their offer in local markets, the introduction of new raw materials in the food industry and of new sources of income for local producers and family farms, as well as the preservation of biodiversity [2].

Chemical fingerprinting is a valuable method to find distinctive patterns or to identify a specific feature that could be potentially used to verify the identity of a certain plant or food material and is generally strictly related to the antioxidant capacity. Therefore, the aim of this study was to investigate the polyphenolic profile and nutraceutical properties of Serbian hawthorn, blackthorn, cornelian cherry, and elderberry fruits. The total phenolic content (TPC) was evaluated and the quantification of each phenolic compound was performed by ultrahigh performance liquid chromatography (UHPLC) coupled with a diode array detector (DAD) and connected to a triple-quadrupole mass spectrometer. The nutraceutical properties of the wild fruit extracts were evaluated using five assays: DPPH●-scavenging activity, ferric ion-reducing capacity (FRC), ferrous ion-chelating capacity (FCC), nitric oxide-scavenging activity (NO), and antityrosinase activity test (Tyr). The characterization of the phenols was reported, and the main quantitative differences in phenolic composition among the berries are emphasized and discussed comparing the results available in literature. Finally, principal component analysis (PCA) was employed to find chemical markers for discrimination among studied berries on the basis of the polyphenolic composition and different nutraceutical properties. The presented approach is in agreement with the most commonly used procedures in the analysis of plant material. Mass spectrometry coupled to liquid chromatography and statistical data analysis in food profiling enables finding key compounds responsible for discrimination and classification of samples. Such an investigation could contribute to the assessment of the possible usage of wild fruit extracts in the formulation of food supplements and/or cosmetic products.

Materials and methods

Wild fruit samples and extract preparation

The autochthonous *Crataegus monogyna* (single seeded hawthorn, HW), *Crataegus oxyacantha* (Northern Europian hawthorn, HW), *Prunus spinosa* (blackthorn, BL), *Sambucus nigra* (elderberry, EL), and *Cornus mas* (cornelian cherry, CC) fruits (identified according to the taxonomical criteria of [15]) were selected from the spontaneous flora and collected for experiments from different sites (Table 1). Each plant was authenticated and preserved at the Department of Botany and Systematic at the Faculty of Agriculture, University of Belgrade. The voucher numbers for the analyzed wild species are: *Cornus mas*—PF 2016/10211 (flowers, leaves, fruits) *Crataegus monogyna*—PF 2016/11367 (flowers, leaves, fruits) *Crataegus oxyacantha*—PF 2016/11370 (flowers, leaves, fruits) *Prunus spinosa*—PF 2106/12763 (flowers, leaves, fruits) *Sambucus nigra*—PF 2016/13287 (flowers, leaves, fruits).

Thirty mature fruits of each genotype were picked at the full maturity stage. Three different samples were collected from the same tree/bush, but picked from different sides; each sample was submitted to the extraction procedure and analyzed in triplicate. After harvesting, fruits were placed in AC/DC hand-refrigerator (− 20 °C) to prevent oxidation up to the extract preparation. Each sample of frozen fruits were ground up with a Mikro-Dismembrator S (Sartorius, Göttingen, Germany) and extracted under magnetic stirring for 10 min (500 mg/10 mL 70% methanol). The supernatant was collected after centrifugation at 4000 rpm for 5 min, filtered through a 0.45 µm membrane filter (Millex-HV hydrophilic polyvinylidene difluoride (PVDF), Millipore, Billerica, MA, USA) and kept at − 20 °C prior to analysis that were all performed in triplicate.

Table 1 Sample codification, location and main traditional and not traditional usage of different berries

Species	Code	Location	Usage
Sambucus nigra Elderberry	EL1	Deliblato Sands	Folk medicine (kidney stones, digestive ailments, dyspnea and cardiovascular disorders), anti-inflammatory, gastroprotective, antiproliferative activity against different tumor cell lines, hedge plant, jellies, jams, and syrups, used to make wine, or to add flavour to brandy [3, 16, 17]
	EL2	Deliblato Sands	
	EL3	Deliblato Sands	
	EL4	Deliblato Sands	
	EL5	Tara	
	EL6	Tara	
	EL7	Tara	
Cornus mas Cornelian cherry	CC1	Fruška Gora	Folk medicine (cardiotonic effect), antiarrhythmic and inducer of rhythmicity in quiescent cardiomyocytes, anti-inflammatory, inhibitor of PA-induced tumor transformation, inhibitor of tumor promotion, hedge plant, jellies, jams, and syrups, used to make wine, or to add flavor to brandy [18–20]
	CC2	Fruška Gora	
	CC3	Fruška Gora	
	CC4	Fruška Gora	
	CC5	Stara Planina, Radičevac	
	CC6	Stara Planina, Radičevac	
	CC7	Stara Planina, Radičevac	
	CC8	Stara Planina, Zlot	
	CC9	Stara Planina, Zlot	
	CC10	Stara Planina, Zlot	
Prunus spinosa Blackthorn	BL1	Radmilovac	Folk medicine, ornamental plant, syrups, juices, jams, compotes, spirits, pickled like olives, used to prepare wine and other traditional products Wood is used to make furniture, jewellery and traditional musical instruments [21]
	BL2	Radmilovac	
	BL3	Saranovo	
	BL4	Saranovo	
	BL5	Beograd	
Crataegus monogyna Hawthorn	HW1	Radmilovac	Jams, jellies, preserves, wine, vinegar, and distilled alcoholic beverages, digestive and laxative liqueurs, or ingredients for pastries. Flowers and petals used for tea, syrup, fresh juice Folk medicine, bee pasture, ornamental tree, often as hedges [8, 22, 23]
	HW2	Radmilovac	
	HW3	Gornji Milanovac	
Crataegus oxyacantha Hawthorn	HW4	Stara Planina, Radičevac	Folk medicine, jam, yoghurt, juice, wine, liqueurs, marmalade, juice, and pies [24]

Determination of the total phenolic content (TPC)

The total phenolic content was determined spectrophotometrically by Folin–Ciocalteu method [25] following the procedure reported in [26]. Gallic acid standard solutions (20–100 mg/L) were used for constructing a calibration curve and the results were expressed in grams of gallic acid equivalents (GAE) per kilogram of frozen sample (g GAE/kg FW).

Antioxidant activity assays

The antioxidant activity of the extracts was evaluated by different methods: DPPH [27], Fe^{2+}-chelating capacity (FCC) [28], and Fe^{3+}-reducing capacity (FRC) [29] assays. Nitric oxide (NO)-scavenging activity [30] and antityrosinase activity (Tyr) [31] were also evaluated. A more detailed description of the used methods is reported in Supplementary material.

Analysis of polyphenols

Polyphenols were separated, quantified and identified using a UHPLC system (Dionex Ultimate 3000, ThermoFisher Scientific, Bremen, Germany) equipped with a diode array detector (DAD) and triple-quadrupole mass spectrometer. Chromatographic separations and quantitative analysis of phenolic compounds were achieved following the optimized method previously reported [26]. More details can be found in Supplementary material.

Statistical analysis

Measurements done in triplicate are presented as the mean values ± standard deviation (SD). Tukey's test was performed using NCSS program. Principal component analysis (PCA) was done using the PLS_Tool Box software package for MATLAB (Version 7.12.0).

Results and discussion

Investigation of *Crataegus monogyna* started long ago, but in most of the studies carried out so far, the analysis of phenolic extracts from leaves and flowers has been done [4, 5, 32]. Nowadays, oil obtained from the seeds is examined for its antioxidant and antibacterial activities [33]. Even if Cornelian cherry (*Cornus mas*) has been an important plant from ancient time, its analysis has started recently. References [20 and 21] determined vitamin C, sugars, anthocyanins, organic acids and tannins in its fruits, and [9 and 11] continued those studies. Since the consumer awareness of high quality and healthy food has been increasing, blackthorn (*Prunus spinosa*) has become an interesting species for chemical analysis. Reference [32] proved that it contains substantial quantities of phenolic antioxidants. Besides, [34, 35] showed that extracts and liquors made from blackthorn can treat hypertension and gastrointestinal disturbances. In elderberry, flowers and fruits have been gaining a lot of attention. They have been present in folk medicine for centuries, but the pharmacokinetics of many of their constituents has not been completely understood. Many scientist have reported substantial levels of flavonoids, anthocyanins and hemagglutinin, including sambunigrin, viburnic acid, and vitamins A and C [36].

Bioactivity assays of wild fruits

The nutraceutical properties of the tested extracts were evaluated using different bioassays providing different information: DPPH about the hydrogen-donating ability of samples, Fe^{2+}-chelating assay about the capacity of extracts to chelate transition metal ions involved in the reactions generating ROS, Fe^{3+}-reducing assay about the capacity of an antioxidant agent to reduce Fe^{3+}, NO-scavenging assay about the ability of extracts to compete with oxygen, leading to reduced production of nitrite ions, and finally antityrosinase assay about the capacity of extracts to reduce melanin formation through tyrosinase activity.

The results obtained in the different bioassays and the TPC of each tested extract are reported in Fig. 1. BL and CC extracts had the highest and the lowest TPC values showing an average content of 14.77 and 7.55 g GAE/kg FW, respectively. The TPC values obtained for CC are slightly higher than those registered for samples collected in different regions in Turkey and Iran [37, 38], but lower than the results obtained for samples from Greece [39]; this variability confirms the need to conduct further studies among native CC population (no commercial CC cultivar exists) to determine those higher in nutraceutical properties. HW showed TPC values in the range 5.45–11.27 g GAE/kg, i.e., a mean content higher than that reported by [40] for ethanolic extracts. Conversely to our results, [41] reported higher TPC for HW than for CC. Therefore, we can conclude that our results agree only in part with those reported in literature for the different wild fruits, as the phenolic profiles depend on cultivars and genotype, and on the environmental conditions and the TPC values are solvent and botanical organ dependent. Considering the bioactivity assays, BL extracts were the most active as DPPH radical scavengers with an average value of 227.08 mM TE/mL, followed by CC (115.96 mM TE/mL) and HW (119.89 mM TE/mL), while EL extracts showed the lowest activity (37.13 mM TE/mL extract). The strongest activity of BL against DPPH could be ascribed to its high TPC content, but also to its high FRC. Total phenolic content is expected to be strongly correlated to the contents of flavonoids and phenolic acids, and to change in dependence on cultivars, environmental conditions and solvent used for the extraction. Various solvent extracts (dichloromethane, ethyl acetate, ethanol, and aqueous solvent) of branches, leaves and fruits from blackthorn (*P. spinosa*) were analyzed spectrophotometrically and the results of TPC and TAC showed solvent and botanical organ dependence [42].

Conversely, no activity in scavenging NO was detected independently from the considered fruit. All samples showed Tyr with values ranging from 20.90 to 74.23%. CC samples generally were the most active with the exception of CC4, CC5, and CC9 which showed Tyr values similar to those of EL samples. Intermediate values have been registered for BL and HW fruits. This capacity is interesting considering the fact that tyrosinase enzyme is involved in human melanization process and is also important in the developmental processes in insects. Therefore, potentially, inhibitors could be considered useful in food industry as food additive, in cosmetic industry as whitening agents, and also as insect control agents. The capacity to inhibit tyrosinase mediating melanogeneses was previously reported only for Tunisian *Crataegus monogyna* leaf constituents [43] and for proanthocyanidins isolated from Chinese HW fruit stone [44]. As shown in Fig. 1, FCC for all the extracts was in the range of 58.81–97.68%, with the following trend: CC > EL > BL > HW. It is worthy to be mentioned that CC extracts exhibiting the strongest FCC showed also the highest Tyr activity. This result can be supported by some earlier findings on the significance of the metal chelation ability when the inhibition of tyrosinase is considered [45].

Finally, data reported in Fig. 1 showed divergent bioactivities of the extracts belonging to the same species, but such a behavior is quite usual as differences in the phytochemical profiles exist. Our findings are in agreement with [46] who reported differences in the total phenolic, total

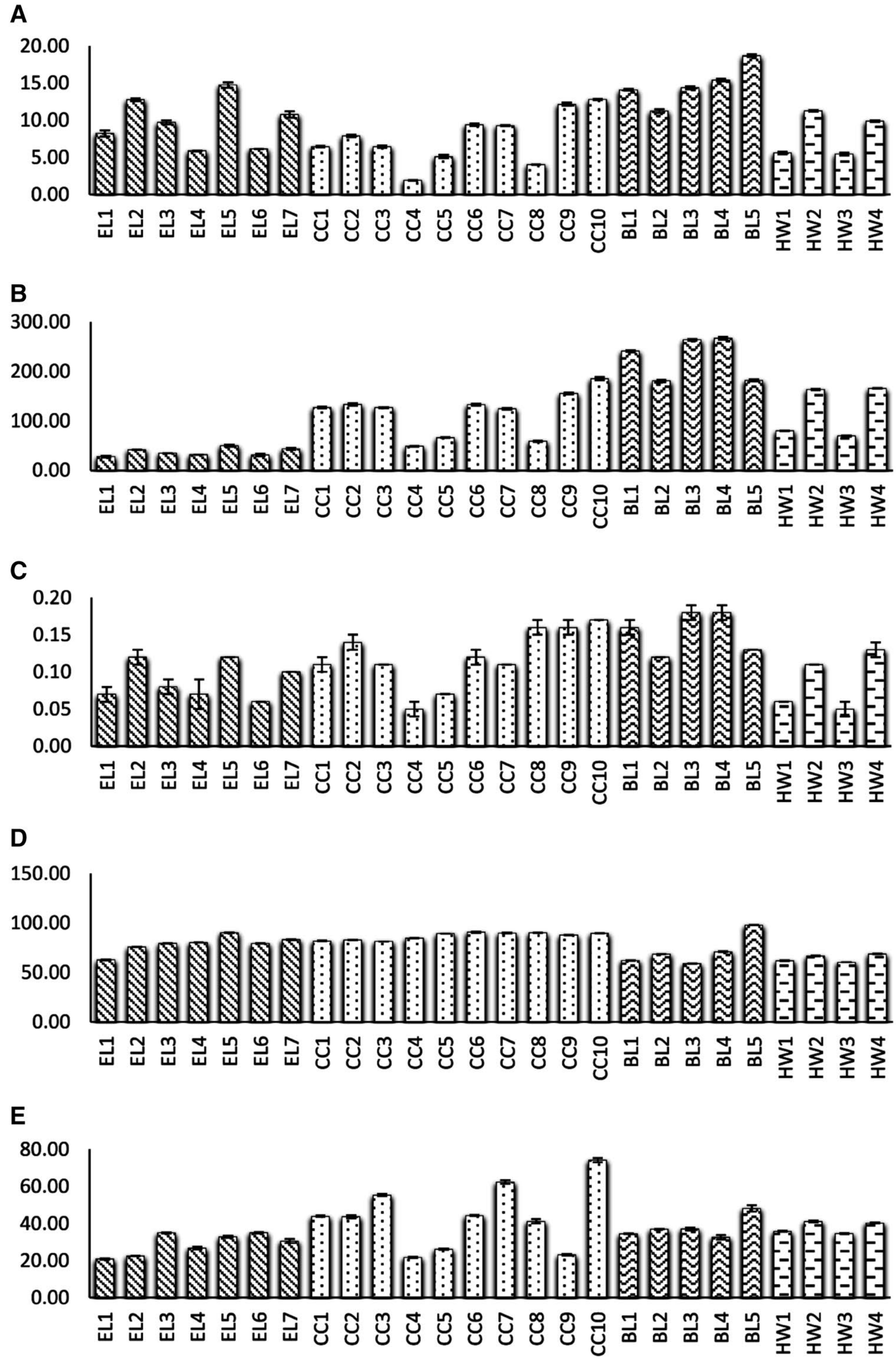

Fig. 1 TPC (**a**) and bioactivity results obtained in DPPH (**b**), Fe^{2+}-chelating capacity (**c**), Fe^{3+}-reducing capacity (**d**), and antityrosinase activity (**e**) assays for the tested samples

flavonoids, phenolic compounds, the mineral content, and the antioxidant activity of fruit extracts of seven wild species (*Crataegus monogyna* Jacq., *Prunus spinosa* L., *Rosa canina* L., *Hippophaë rhamnoides* L., *Rubus fruticosus* L., *Prunus padus, Cornus mas* L.). The analysis of phenolics resulted in quantification of numerous phenolic acids and flavonoids, and it was observed that the activity was more related to the type of individual phenolic compounds than to the total phenolic contents.

Quantification of the polyphenols

The quantitative mean data of individual phenolic compounds are summarized in Table 2. A set of 12 phenolic

Table 2 Quantitative phenolic composition of the wild fruit extracts obtained by UHPLC–DAD–HESI–MS/MS (mg/kg)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
EL1	23.12	–	7.21	1.71	14.16	5.69	0.38	–	–	2.21	0.60	1.39	–	255.41	14.30	–	0.50	2.42	0.33	0.54	0.63	0.93	0.10
EL2	–	–	16.25	2.84	3.98	0.56	–	–	–	1.08	0.36	3.73	–	195.66	28.76	–	0.53	2.99	1.15	0.38	0.30	0.48	–
EL3	30.21	–	25.75	2.42	11.98	46.77	3.41	0.72	–	2.86	0.45	7.27	–	269.70	22.53	–	0.66	3.54	0.38	–	0.08	0.12	–
EL4	30.77	–	15.35	2.09	12.31	25.36	0.53	–	–	1.22	0.38	4.33	–	325.29	10.56	–	0.46	0.94	0.56	–	0.13	0.20	–
EL5	26.08	–	21.21	3.28	6.99	155.39	2.38	–	–	2.14	–	4.34	–	471.51	33.13	–	0.64	1.40	1.93	0.36	0.26	0.40	–
EL6	31.99	–	20.03	3.46	3.13	67.76	2.77	–	–	1.27	0.28	2.26	–	253.26	23.35	0.08	0.89	2.61	0.62	–	0.31	–	0.21
EL7	–	–	15.19	2.51	8.38	1.18	–	–	–	1.96	0.17	2.68	–	187.30	29.39	–	0.25	2.59	1.52	0.45	0.24	0.42	–
CC1	–	6.56	0.83	1.81	0.00	16.17	0.85	–	1.11	0.88	15.17	0.18	–	10.62	1.81	–	0.27	–	1.16	–	–	–	0.09
CC2	–	8.51	1.81	2.63	0.76	20.50	–	–	0.55	0.39	25.56	0.17	–	5.78	2.32	–	0.27	–	1.82	–	0.13	–	0.10
CC3	–	5.80	0.73	1.88	0.34	14.79	–	–	1.35	0.58	36.52	–	–	18.13	3.17	–	0.24	–	1.30	–	0.13	–	–
CC4	–	2.54	0.91	2.72	2.98	5.64	1.25	–		0.35	15.93	–	2.99	1.27	1.01	–	0.26	–	0.26	–	0.27	0.59	0.49
CC5	–	15.41	3.75	2.70	0.82	20.74	–	–	0.94	0.54	84.92	–	–	2.74	2.40	–	0.31	–	0.96	–	0.10	–	0.09
CC6	–	30.94	1.31	4.76	–	38.37	–	–	1.74	1.17	27.29	0.26	–	3.03	1.25	–	0.37	–	1.29	–	0.10	–	–
CC7	–	13.93	–	2.20	–	4.44	–	–	1.90	1.19	26.07	0.28	–	1.96	0.81	–	0.26	–	0.33	–	0.02	–	–
CC8	–	8.15	2.09	3.34	0.97	2.51	–	–	1.06	0.33	18.91	–	2.45	1.44	2.67	–	0.17	–	0.98	–	0.22	–	0.27
CC9	–	18.88	4.20	2.07	1.14	13.67	–	–		0.68	41.08	0.28	–	3.64	3.84	–	0.29	–	–	–	0.33	–	–
CC10	–	111.14	1.40	3.55	0.76	28.60	–	–	1.52	0.75	77.73	0.31	–	3.80	1.83	–	0.21	–	0.89	–	0.15	0.10	0.16
BL1	–	–	1.55	6.44	–	1.27	–	1.87	–	–	1.31	0.25	–	52.56	4.73	–	4.16	6.82	0.04	–	0.05	0.06	–
BL2	–	–	1.77	5.56	–	1.60	0.15	1.67	–	–	0.94	0.24	–	59.02	5.90	–	3.88	7.11	–	–	0.01	0.02	–
BL3	–	–	1.43	5.42	–	1.78	–	1.67	–	–	0.66	0.22	–		6.60	0.09	4.05	8.62	–	–	0.03	0.03	–
BL4	–	–	3.02	6.33	–	2.82	–	1.22	–	–	0.66	0.22	–	58.94	7.87	0.09	3.53	7.43	–	–	0.01	–	–
BL5	–	–	1.14	6.67	–	–	–	3.17	–	–	1.87	–	–	33.92	3.30	0.07	1.83	6.16	–	–	0.01	0.01	–
HW1	–	–	1.95	6.24	2.07	23.07	2.37	4.66	0.58	0.95	5.58	0.50	–	16.75	81.17	–	0.35	–	0.98	–	0.24	0.31	1.73
HW2	–	–	0.78	3.79	1.56	4.36	1.35	6.66	–	0.56	2.49	0.52	–	30.92	77.31	0.06	–	–	0.32	–	0.04	0.04	0.59
HW3	12.28	–	–	14.69		20.71	0.61	–	–	0.14	0.63	0.11	3.29	66.54	117.35	–	0.41	29.49	3.91	–	0.06	0.09	0.25
HW4	–	–	–	4.84	0.35	9.70	0.37	–	–	–	0.31	0.12	0.05	52.66	95.16	–	0.39	43.86	0.75	–	–	0.02	0.11

1 Arbutin, *2* gallic acid, *3* protocatechuic acid, *4* aesculin, *5* *p*-hydroxybenzoic acid, *6, 5* *O*-caffeoylquinic acid, *7* caffeic acid, *8* vanilic acid, *9* syringic acid, *10* *p*-coumaric acid, *11* ellagic acid, *12* ferulic acid, *13* catechin, *14* rutin, *15* quercetin 3-*O*-galactoside, *16* apigenin 7-*O*-apioglucoside, *17* naringin, *18* kaempferol 3-*O*-glucoside, *19* phlorizin, *20* luteolin, *21* apigenin, *22* naringeni, *23* kaempferol acids and 11 flavonoids were determined. The phenolic profiles are quite different both with regard to the composition and the concentration of phenolic acids and flavonoids. BL extracts showed a characteristic profile with a lower number of phenolic acids and flavonoids when compared to other fruit extracts. In fact, among phenolic acids, gallic acid, *p*-hydroxybenzoic acid, caffeic acid (with the exception of BL2 sample), syringic acid, and *p*-coumaric acid were not confirmed as well as arbutin. Syringic acid was not detected, differently from [47] who identified it in the hydro-alcoholic extract of Southeast Serbian fruits. Interestingly, vanillic acid was detected in the tested samples in the range 1.22–3.17 mg/kg FW: this acid has been never identified in BL extracts before (but only in cranberry [48]). Catechin, luteolin, kaempferol, and phlorizin (with the exception of BL1 sample) were not detected in BL extracts. Similarly, catechin and kaempferol were not detected in EL extracts which were rich in rutin (187.30–471.51 mg/kg FW), differently from CC, BL, and HW samples. Arbutin was confirmed only in EL extracts in the concentration range 23.12–31.99 mg/kg FW. This is, most probably, the first report on the presence of arbutin in EL: this compound has been previously reported only in strawberry tree [49]. Arbutin, as a tyrosinase inhibitor, is extensively used as skin-lightening agent in human cosmetic products. However, EL extracts did not show high Tyr values, differently from HW and CC extracts. Such results could be explained by the fact that apart from arbutin, other polyphenols known as tyrosinase inhibitors, such as ellagic and vanillic acid, were present in larger quantities in CC. Aesculin, a natural substance possessing sunscreen, antioxidant, and antitumor activities [50], was present in all samples in a concentration range of 1.71–14.69 mg/kg FW. All berry fruits (with the exception of HW2 sample) stored naringin (useful for the treatment of several metabolic disorders [51]) and quercetin 3-*O*-galactoside with the highest concentration in BL and HW, respectively. Three HW samples stored *p*-coumaric acid. Interestingly, arbutin was identified in HW3 sample, which was additionally characterized by a slightly higher content of aesculin, rutin, quercetin 3-*O*-galactoside, and phlorizin. The presence of polyphenols in HW fruits was previously reported by [52]. *p*-Coumaric acid in EL samples was in the range 1.08–2.86 mg/kg FW, differently from CC, that contains a lower amount (from 0.33 to 1.19 mg/kg FW), and BL extracts, in which this acid was absent, in accordance with [49]. Gallic acid, so far reported to be present in blackberry and cloudberry fruit [53], was identified only in CC as well as syringic acid. The only exception was HW1 sample, containing 0.58 mg/kg FW. Recently, syringic acid attracted particular attention since it showed blood sugar regulating properties and was demonstrated to inhibit the development of diabetic cataract [54]. Finally, ellagic acid was identified in all the extracts, with CC showing a notably higher concentration than in all the other extracts.

Principal component analysis

Multivariate statistical analysis in polyphenol profiling enables clustering of extracts according to individual flavonoids, phenolic acids, and other non-flavonoid components. The score plots display trends, groupings and outliers. In this work, PCA on the quantitative data was employed to analyze wild fruits extracts. The content of each determined phenolic compound and each bioactivity assay served as input data. The results of PCA suggested that a seven-component model explained 84.37% of total variance (PC1, PC2, PC3, PC4, PC5, PC6, and PC7 accounted for 29.95%, 16.24%, 13.58%, 8.54%, 7.45%, 5.22%, and 3.39%, respectively). The scores plot and loadings plot for the first three principal components are shown in Fig. 2. The PCA scores plot discriminated the analyzed wild fruit samples into four separate clusters. From the loadings plot of PCA, it was possible to partly express the influence of the original variables on the separation among samples. EL samples formed separate cluster along PC1 which is in agreement with the distinctive phenolic profile when compared to the other samples investigated. EL fruits were characterized by higher contents of several phenolic acids (compounds 3, 5, 10, and 12) and notably higher content of rutin (14). Compounds 2, 9, and 11 and Tyr were important variables for the separation of CC along PC2. Finally, score plot revealed formation of two clusters of HW and BL samples along PC3. HW was characterized by notably higher contents of compound 15 than other samples, while BL demonstrated the highest DPPH$^{\cdot}$ scavenger activity, TPC values and contents of compound 17.

No evidence of relationships was found for most of the studied genotypes according to their geographic location. Our results are not unexpected considering the fact that hawthorns and blackthorn are self-sterile, while Cornelian cherry is partially self-fertile, so characterized by a high degree of heterozygosity. For that reason in each locality, different genotypes can be found.

Conclusion

Serbian wild fruits are considered to be a great source of phytonutrients and natural antioxidant compounds. Our investigation showed how every fruit could be considered unique in terms of its phytonutrient content. The HPLC profile of BL revealed the presence of at least 11 different phenolic compounds, including vanillic acid, reported for the first time in this paper. Alongside vanillic acid, the

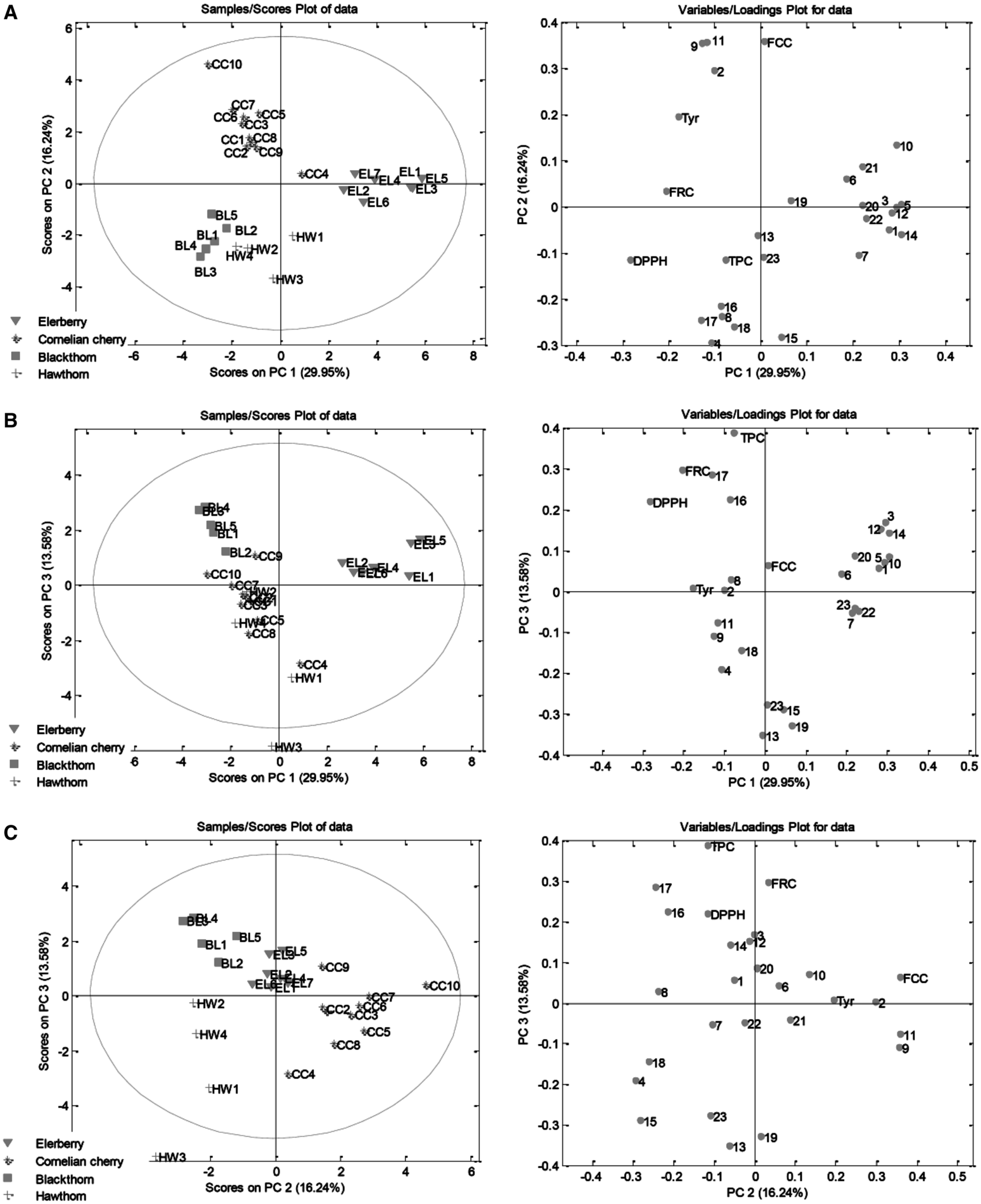

Fig. 2 Principal component analysis: scores plot of the first three principal components showing the sample clustering and loadings plot reflecting the influence of a particular parameter. Numbers correspond to the detected compound as given in Table 2

abundance of naringin was the highest among all the extracts investigated. EL extracts were unique due to the presence of arbutin, a well-known skin protector, while CC was characterized by syringic and gallic acids. PCA managed to classify berry fruit samples on the basis of variations in the content of individual flavonoids, phenolic acids and antioxidant capacity into four separate clusters. In conclusion, this work proved wild fruits to be an interesting field in the search for compounds with potential nutraceutical properties as they contain phytonutrients that could be used as potential benefits in blood sugar regulation and skin protection. However, additional bioactivity testing is needed, especially bearing in mind the synergistic effect among polyphenols as an important factor determining the functional properties.

Acknowledgements This work was supported by the Serbian Ministry of Education, Science and Technological Development (Projects 172017 and TR 31063). Partly, the research was supported by the CICOPS scholarship foreseen for research collaboration at the University of Pavia. The authors thank Prof. Milanese for her help in English revision.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with animal or human subjects.

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