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## Elderberry (*Sambucus nigra* L.) juice as a novel functional product rich in health-promoting compounds

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The medicinal herbs of the Balkan Peninsula are highly represented in traditional medicine. The connection between traditional and modern life and health is reflected in the creation of new food products with added value. In this study, the plant species *Sambucus nigra* L. was used to obtain freshly pressed juice, whose chemical composition and various biological activities were evaluated. The most abundant compounds were phenolic acids: protocatechuic and chlorogenic acid, as well as flavonoids: quercetin-3-O-hexoside, quercetin, and rutin. The analyzed juice was very rich in total phenolic compounds (1945 mg GAE per mL juice), and a significant anthocyanin concentration was observed (30.85 mg Cy-3-GE per mL juice). Bioactivity testing revealed that elderberry juice was an extremely potent agent in the process of neutralizing NO free radicals (53.06 g TE per L juice), while in reducing over-enzyme activity, the best result was achieved in the inhibition of tyrosinase enzyme (54.70 mg KAE per g of juice).

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### Introduction

Research in the field of food technology shows that functional food is becoming increasingly popular around the world and occupies an important place in human nutrition.<sup>1</sup> The global functional food market has expanded dramatically over the last decade and is estimated to grow steadily to reach \$255.10 million by 2024,<sup>2</sup> increasing the competition among producers to make the consumer demand even more intense.<sup>3</sup> This growth has been induced not only by innovation in the food industry, but also by increasing consumer awareness of their own health and the benefits of food products. Innovations within the food industry include obtaining new and improving and enhancing the quality of existing products by applying alternative solutions that are mostly related to the use of plants, which are easily available on the Balkan Peninsula.<sup>4</sup> Wild-growing plant species of the Balkan Peninsula have exceptional natural potential and have long been used to treat flu, colds, inflammation, and similar ailments. Numerous studies are based on the examination of the antioxidant potential of wild-growing plants in order to regulate metabolic processes.<sup>5</sup> In addition to the antioxidant potential, researches on various plant species also aim to examine the regulation of enzyme overactivity.

Excessive activity of enzymes leads to disorders in the functioning of the organism and causes the development of neurodegenerative diseases, melanoma, and diabetes.<sup>6</sup> Biologically active compounds present in wild-growing plant species are carriers of biological activity, especially phenolic compounds, minerals, phytosterols, terpenes. Wild-growing berry fruit is a very good source of natural molecules that show neuroprotective, anti-inflammatory, antioxidant, antidiabetic, and antimicrobial properties.<sup>7</sup> *Sambucus nigra* L. is a challenge for the food industry because there are still no commercially available food products based on the elderberry. Thanks to its healing properties, the elderberry is very attractive for scientific research, which is why it was selected for the topic of this study.

Elderberry is a wild-growing plant, widely distributed in Europe, Africa, Asia, and North America.<sup>8</sup> The development of products based on berry fruits increasingly attracts the attention of different research groups. Therefore, we put the focus of our research on the health and industrial potential of wild-growing fruit of elderberry (family Adoxaceae), insufficiently explored and unexploited plant material in the food industry.

Elderberry fruits are small, black berries, whose color and extent resemble aronia berries and blueberries. The content of primary metabolites (sugars and organic acids) gives elderberry the typical sweet and astringent taste, while its characteristic aroma is related to the presence of different volatile and phenolic compounds.<sup>9</sup> The purple color of fruit originates from anthocyanins and color pigments, so it could be used in many food commodities and nutritional supplements. The use of elderberry fruits is sometimes limited due to the presence of cyanogenic glycosides in this plant species. However, ripe

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elderberry fruits are safe for consumption and processing, due to the fact that an increasing degree of fruit ripeness causes, the content of these compounds in the fruit to decrease, thus not causing decreases toxic effects on the body.<sup>10</sup> Senica *et al.* have shown that the ripe fruits contains the least amount of cyanogenic glycosides compared to the flower and leaves.<sup>10</sup> Berry fruits are very attractive for juice production, due to the high amount of phytochemicals and natural color. The use of natural juices as food beverages occupies a high position in human nutrition, as they are very good sources of nutrients. The processes of obtaining juices are different, but they are mainly based on the application of different temperature treatments, which greatly affects their quality, *i.e.* the degradation of biomolecules that are not resistant to higher temperature.<sup>11</sup> In a study conducted by Senica *et al.*,<sup>12</sup> elderberry juice was obtained by the influence of temperature, while Busso Casati *et al.* obtained juice from berries dried at low temperatures.<sup>13</sup> In our research, the juice was obtained by traditional pressing, without temperature treatment of ripened elderberry fruit.<sup>14</sup> Therefore, the main objectives of this study were analysis of biochemical profile, biological abilities, and sensory characteristics of the traditionally obtained juice as a novel functional product rich in health-promoting compounds. The results of this research are of practical importance since they provide data on biopotential and sensory characteristics of the examined product, which can be a rational basis for elderberry usage at an industrial level.

## Materials and methods

### Plant material

Plant material of *S. nigra* was collected in August 2017 in Pljevlja (Republic of Montenegro). The specimen voucher was prepared and identified by Dr Milica Rat and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad, Faculty of Sciences, Republic of Serbia. The number of specimen voucher of *S. nigra* was 2-1512.

The juice was made by hand pressing of 1 kg of berries. After pressing, 0.5 L of mother elderberry juice was obtained, and the juice was filtered through sterile gauze to remove residual impurities. Gravimetric dry matter measurement of the juice was performed by heating the juice in an oven at  $105 \pm 0.5$  °C until the constant mass was formed. The dry matter content expressed as a percentage in the original juice sample was 7.70%. The degree of ripeness of the fruits was assessed by determining the sugar content of the refractometer, using an Oechsle scale.

The sugar content for the degree of ripeness of the fruit was calculated according to the formula:  $(Oe^\circ \times 0.266) - 3$ . Read value at the Oechsle scale was 50 Oe<sup>°</sup>, and the content of sugar was 10.3%, indicating a high degree of fruits maturity.

### Chemical composition of elderberry juice

The content of organic acids was determined using Waters Alliance HPLC system with a Waters 2414 refractive index detector. The column was "Carbohydrate analysis column" – Waters (3.9 × 300 mm) with an ambient temperature of the

oven. The mobile phase was ACN/H<sub>2</sub>O (80/20%) with a flow of 1.5 mL min<sup>-1</sup>. Final sugar content was expressed as gram per liter of juice (g L<sup>-1</sup> juice). The content of sugars was determined using Waters Alliance HPLC system with a UV/VIS detector. The column was Atlantis C18 (4.6 × 150 mm, 5 µm) with the oven temperature of 45 °C. The mobile phase was NaH<sub>2</sub>PO<sub>4</sub> × H<sub>2</sub>O (20 mmol L<sup>-1</sup>) with a flow of 0.5 mL min<sup>-1</sup>, while the UV detector was set at 210 nm. The volume of the sample was 10 µL. The final content was expressed as gram per liter of juice (g L<sup>-1</sup> juice).

pH was determined at room temperature with a pH meter (Metrohm, Switzerland).

### Nutritional profile

The carbohydrate content was determined by the method described by Trajković *et al.*<sup>15</sup> The content of fat in elderberry juice was determined by applying the method prescribed by the Regulation,<sup>16</sup> according to which the extraction of fat is carried out by applying an organic solvent on an apparatus according to Soxhlet, while the protein content was determined by the method described by Regulation.<sup>17</sup> The energy value was calculated based on the obtained content of protein, fat, and carbohydrate.

### Mineral composition

The mineral composition was established using a previously described method (Đurović *et al.*<sup>18</sup>) by ICP-OES analytical technique. The content of each element was expressed as milligram per liter of juice (mg L<sup>-1</sup> juice).

### Determination of bioactive components

Total phenolic (TPC) and total flavonoid (TFC) contents were determined using the previously described methods.<sup>19</sup> Final contents for TPC was expressed as milligram of gallic acid equivalents per L of juice (mg GAE per L juice), while results for TFC were expressed as milligrams of catechin equivalents per L of juice (mg CE per L juice).

### Determination of total monomeric anthocyanins

Anthocyanins were determined according to the previously described procedure (Majkić *et al.*<sup>20</sup>) using the pH differential method. Total anthocyanins content (TAC) was expressed as mg equivalents cyanidin-3-O-glucoside per L of juice (mg Cy-3-GE per L juice).

### Determination of total tannins

The content of total tannins (TTC) in elderberry was determined spectrophotometrically, using the characteristic of polyvinylpolypyrrolidone (PVPP) to bind tannins. The total tannins content was expressed as mg catechin per L of juice (mg CE per L juice).<sup>21</sup>

### HPLC-MS/MS analysis of phenolic compounds

The quantification of the 44 phenolic compounds and quinic acid in elderberry juice was carried out using the LC-MS/MS



method published by Orčić *et al.*<sup>22</sup> with additional compounds: morin, ellagic, and ursolic acid.

## Determination of biological activity

**Antioxidant properties.** In the present research, antioxidant properties of elderberry juice were tested by using radical scavenging (ABTS<sup>•+</sup>, DPPH<sup>•</sup> and 'NO), reducing power (FRAP and CUPRAC), metal chelating, total antioxidant activity and Fe<sup>2+</sup>/ascorbate induced lipid peroxidation (LP) according to previously described procedures.<sup>20</sup> The antioxidant activities of all samples were expressed as trolox equivalents (TE), with the exception of a metal chelating assay where activity was expressed as g EDTA equivalent per L of juice (g TE per L of juice and g EDTAE per L of juice).

## Enzyme inhibitory activity

In this study, the inhibitory activity of elderberry juice towards acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase,  $\alpha$ -amylase, and  $\alpha$ -glucosidase enzymes involved in some key metabolic pathways were investigated using the previously described methods.<sup>19</sup> The inhibitory effects were expressed as standard inhibitor equivalents (galanthamine (mg GALE per g juice) for AChE and BChE; kojic acid (mg KAE per g juice) for tyrosinase; and acarbose (mmol ACAE per g juice) for  $\alpha$ -amylase and  $\alpha$ -glucosidase).

## Color of elderberry juice

Instrumental color parameters (eight replicates):  $L^*$  (lightness),  $a^*$  (redness),  $b^*$  (yellowness),  $C^*$  (chroma – saturation index),  $h$  (hue angle), and  $\lambda$  [dominant wavelength (nm)] were determined by using a Konica Minolta Chroma Meter CR-400, using glass cell 20 mm CM-A99, D-65 lighting, a 2° standard observer angle and an 8 mm aperture in the measuring head. Also, the elderberry juice was subjected to visual color evaluation by 10 trained sensory panelists using the NCS color atlas.<sup>23</sup>

## Sensory profile of elderberry juice

The elderberry juice was sensory evaluated by quantitative descriptive analysis. The sensory analysis was carried out by a trained panel consisting of 12 members, per two sessions. The panelists were trained according to methods described in ISO 8586,<sup>24</sup> in a sensory laboratory equipped according to ISO 8589.<sup>25</sup> During calibration sessions, a panel discussion was held to develop a consensus vocabulary for sensory attributes for elderberry juice. The panelists were asked to identify references that would serve for each attribute term ISO 11035.<sup>26</sup> A sensory lexicon of the elderberry juice was created based on the languages generated from calibration sessions (Table 4). Individual elderberry juice was accessed for the intensity of the studied sensory attributes using a 100 mm-long line with line anchors of 0 – no intensity or extremely low intensity and 10 – extremely high intensity.

The elderberry juice sensory acceptance test was performed by 70 students of the Faculty of Technology Novi Sad, Serbia. Consumer acceptability was measured on a 9-point hedonic

scale anchored as 1 being 'dislike extremely' and 9 being 'like extremely'. The panelists were asked to evaluate appearance liking, flavor liking, mouthfeel liking, aftertaste liking, and overall liking.

Sensory evaluation of the color of the elderberry juice was also performed by trained evaluators (a panel of 12 evaluators) using NCS color atlas Natural Color System® – The international language of colour communication™, Scandinavian Colour Institute AB, Stockholm, Sweden, <http://www.ncscolour.com>.

## Results and discussion

### Chemical composition of elderberry juice

The content of organic acids, sugars, pH values, nutritional profile, and mineral composition of elderberry juice are shown in Table 1. Malic acid was the most abundant organic acid in the juice (3.75 g L<sup>-1</sup>), followed by citric acid (1.63 g L<sup>-1</sup>), while the tartaric acid content was 0.50 g L<sup>-1</sup>. As expected, glucose was the predominant sugar (29.12 g L<sup>-1</sup>), while the fructose content was 22.60 g L<sup>-1</sup>, which is in agreement with literature data.<sup>13,27</sup> Also, the obtained results were comparable with the results of different research group, which reported the dominance of glucose over fructose in *S. nigra*.<sup>13</sup> In the previous study,<sup>28</sup> sucrose was also detected, but in a significantly lower concentration than glucose and fructose, which differs from our research, since we did not record its presence in our sample. The pH of the juice was 4.11, which was higher than the pH of blueberry, elderberry, and blackcurrant juices.<sup>13</sup> This could be a consequence of the uneven degree of ripeness of the fruit, different content of organic acids and sugars in the juices, as well as different treatments in juice preparation, which all affect the acceptability of the product by consumers. An assessment of the nutritional composition showed that elderberry juice had an extremely low fat and protein content (0.02 g per 100 g), and up to total sugar content of 3.74 g per 100 g. The calorific value of 15.22 was significantly lower than that of commercial juices. It is assumed that this result is a consequence of the application of a traditional juice production technique that did not involve temperature treatment to assist the extraction of primary metabolites from the plant matrix.

Berries, such as strawberries, blueberries, blackberries, raspberries, and cranberries, are low in calories ( $\sim$ 0.3 to 0.6 kcal g<sup>-1</sup>) and are a very good source of nutrients such as vitamin C, potassium, and manganese, as well as other vitamins and minerals.<sup>29</sup>

As it can be seen from the results, the juice of matured elderberry berries contains a particularly high concentration of macroelements K and Mg (3955.56 and 280.40 mg L<sup>-1</sup>, respectively), while Ca and Na are present at somewhat lower amounts (163.80 and 7.69 mg L<sup>-1</sup>, respectively). The relatively large content of K and Mg is characteristic of the plant species *S. nigra*, and thus their large presence in elderberry fruit was expected.<sup>27</sup> The content of trace elements in the examined elderberry juice was in the range of 0.01–1.27 mg L<sup>-1</sup>, where Fe and Mn were present in the highest amounts (1.27 and 1.16 mg L<sup>-1</sup>, respectively), while Cr and Ni were quantified in the lowest



Table 1 Chemical composition of elderberry juice

Organic acids <sup>a</sup> (g L <sup>-1</sup> )			Sugars <sup>b</sup> (g L <sup>-1</sup> )						
Malic acid	Tartaric acid	Citric acid	Glucose	Fructose	pH				
3.75 ± 0.13	0.50 ± 0.01	1.63 ± 0.02	29.12 ± 1.15	22.60 ± 1.06	4.11 ± 0.01				
Nutritional profile									
Carbs (g/100 g)		Fat (g/100 g)		Proteins (g/100 g)					
3.74 ± 0.02		0.02 ± 0.00		0.02 ± 0.00					
Calories (kcal/100 g)									
15.22 ± 0.21									
Mineral composition									
Macro elements	Content <sup>c</sup> (mg L <sup>-1</sup> juice)	Trace elements	Content <sup>c</sup> (mg L <sup>-1</sup> juice)	Toxic elements	Content <sup>c</sup> (mg L <sup>-1</sup> juice)				
Na	7.69 ± 0.18	Fe	1.27 ± 0.01	Pb	<0.001				
K	3956 ± 2.56	Cu	0.13 ± 0.01	Cd	<0.001				
Mg	280.40 ± 0.91	Mn	1.16 ± 0.02	Hg	<0.001				
Ca	163.80 ± 2.01	Zn	0.48 ± 0.01	As	<0.001				
—	—	Cr	0.01 ± 0.00	—	—				
—	—	Ni	0.01 ± 0.00	—	—				

<sup>a</sup> g organic acid per liter of juice. <sup>b</sup> g sugar per liter of juice. <sup>c</sup> mg mineral per liter of juice.

concentrations (0.01 mg L<sup>-1</sup>). The safety of the produced juice was analysed by utilizing toxic elements presence: Pb, Cd, Hg and As were not detected in elderberry juice (the amount in the juice was below the detection limit). The results of the toxic element analysis pointed out that the examined elderberry was growing in an ecological area and non-contaminated soil.

The results of the research conducted by Konić-Ristić *et al.* confirmed that minerals K and Fe are dominant in the juice of different berry fruits.<sup>30</sup> The content of the minerals in the elderberry juice was higher than in the fruit juices that were examined by Konić-Ristić *et al.*<sup>30</sup> The assessment of the content of macro and trace elements is particularly important in the case of Fe and K since these elements are significant factors in the prevention of various diseases. The Recommended Dietary Allowances of K for men is 3400 mg, while for women it is somewhat lower – 2600 mg. The content of K in the analysed juice is a bit greater than the Recommended Dietary Allowances, so a 1 dL glass of elderberry juice is sufficient to provide the daily doses of K. The recommended daily doses of Fe for men are 8 mg and 18 mg for women.<sup>30</sup> The content of Fe in elderberry juice in a 1 dL glass is not sufficient to provide the daily doses Fe for the organism, but can be used as a source of this trace element. Overall, the results of this study indicate that elderberry juice is a very potent source of minerals.

### Total bioactive compounds and phenolic components

Polyphenols are pharmacologically active compounds and could be used as active food supplements. Flavonoids are a large group of polyphenols that are very common among plants and participate in processes such as plant pigmentation, antioxidant capacity, protection from biotic and abiotic stresses, and plant–environment interactions. Anthocyanin pigments are responsible for the orange, red, and blue colors of

plants.<sup>31</sup> Berry fruits contain tannins, which are commonly found in the skin, stems, and seeds and have a significant impact on the physical, chemical and sensory characteristics of juices.<sup>8</sup> The content of bioactive compounds is presented in Table 2.

The content of total phenols in the juice obtained by the traditional method of squeezing elderberry fruits was 1945 mg GAE per L juice, while the content of total flavonoids was 906.30 mg CE per mL of juice. As for the content of total monomeric anthocyanins in elderberry juice, it was 30.85 mg of CGE per mL of juice. The tannin content in the analysed juice was significantly higher: 1189.62 mg CE per mL juice. By comparing the obtained results with the results of previous study,<sup>13</sup> where juices of blueberries, elderberry, blackcurrant, and maqui berries were analysed, a significant difference in the content of total phenols and monomeric anthocyanins was noticed. The traditional elderberry juice had a lower content of these biomolecules than the juices analysed by Busso Casati *et al.*<sup>13</sup>

These results could be explained by different ways of berry fruits processing and juice preparation. Juices from blueberries, elderberry, blackcurrant, and maqui berries were obtained using lyophilized fruit pulps, which were afterwards dissolved in water.<sup>13</sup> So, lyophilization provides safe removal of water and concentration of nutrients, which results in their higher final content.

Also, the results of this study were compared to the content of total polyphenolic compounds of blueberry juice, which was determined by Siddiq *et al.*<sup>32</sup> It can be seen that the total content of bioactive molecules was higher in the traditionally obtained elderberry juice. In this case, one of the reasons for the difference in the results was the method used for juice preparation. In the previous study,<sup>32</sup> the temperature and enzyme treatment



**Table 2** The content of total phenolics, flavonoid, anthocyanins and tannins and polyphenolic profile of elderberry juice

Content of biologically active compounds	
TPC <sup>a</sup> (mg GAE per L juice)	1945 ± 33.10
TFC <sup>b</sup> (mg CE per L juice)	906.30 ± 2.31
TAC <sup>c</sup> (mg Cy-3-GE per mL juice)	30.85 ± 2.46
TTC <sup>d</sup> (mg CE per mL juice)	1189.62 ± 21.71
Phenolic profile	
Compounds	Content <sup>e</sup> (mg L <sup>-1</sup> )
p-Hydroxybenzoic acid	3.45 ± 0.03
Protocatechuic acid	42.7 ± 0.85
p-Coumaric acid	1.82 ± 0.01
Gallic acid	3.93 ± 0.29
Chlorogenic acid	7.52 ± 0.23
Caffeic acid	3.07 ± 0.06
Quinic acid	310 ± 1.86
Quercetin	6.17 ± 0.20
Kaempferol 3-O-glucoside	0.46 ± 0.02
Quercetin 3-O-hexoside	18.0 ± 0.90
Amentoflavone	0.36 ± 0.01
Rutin	5.11 ± 0.05
Esculetin	0.27 ± 0.02

<sup>a</sup> mg gallic acid equivalent per L of juice. <sup>b</sup> mg catechin equivalent per L juice. <sup>c</sup> µg cyanidin 3-O-glucoside equivalent per mL of juice. <sup>d</sup> µg catechin equivalent per mL of juice. <sup>e</sup> mg of detected compound per L of juice.

was applied to prepare the blueberry juice, and it resulted in a lower content of total polyphenolic compounds. Other reasons could be disparity in plant species, the geographical area where these plants grow, or interactions with other phytochemicals (non-phenolics) and Folin–Ciocalteu reagent used in this analysis.

To conduct a more detailed analysis, identification and quantification of the bioactive compounds (selected phenolic acids and flavonoids) in elderberry juice were performed using LC-MS/MS technique. The results are shown in Table 2, while corresponding chromatograms are shown in Fig. 1.

In the examined juice, six phenolic acids were determined. Phenolic acids, which were present in the highest concentration, belong to the group of benzoic acids, while the phenolic acids in lower concentrations were cinnamic acid derivatives. The dominant phenolic acids in elderberry juice were protocatechuic and chlorogenic (42.70 and 7.52 mg L<sup>-1</sup>, respectively). The elderberry juice was a good source of gallic and p-hydroxybenzoic acid (3.93 and 3.45 mg L<sup>-1</sup>, respectively). The presence of cinnamic acids in elderberry juice was characterized by caffeic (3.07 mg L<sup>-1</sup>) and p-coumaric acid (1.82 mg L<sup>-1</sup>). The high content of protocatechuic and chlorogenic acid is of great importance for the biological potential of the analysed juice. It has been proven that foods rich in berries and anthocyanins reduce the risk of developing metabolic disorders, so it is widely advocated to increase the intake of berry fruits.<sup>33</sup> Scientific studies have shown that protocatechuic and chlorogenic acid contribute to the prevention of the development of

neurodegenerative diseases, show strong antioxidant, cytotoxic, antidiabetic, and anti-inflammatory activity, thus making elderberry juice a very potent product which could protect the body against various diseases.<sup>34</sup> For example, *in vivo* studies showed that there was significant increase in the blood glucose level, glycosidase enzymes, and some lipids and lipid metabolites (TAG, LDL, lipid peroxidation) after 14 days treatment of Wistar rats with 38.75 mg kg<sup>-1</sup> protocatechuic acid.<sup>35</sup> Having in mind that examined juice is a good source of protocatechuic acid, but also that this acid is the major metabolite of cyanidin 3-O-glucoside in humans,<sup>36</sup> which is probably also present in our sample, it can be assumed that examined juice can be a good source of protocatechuic acid and that its consumption can, at least partially, contribute to the prevention of glucose and lipid metabolism disorders.

In addition to phenolic acids, six flavonoid compounds were identified in elderberry juice. The dominant flavonoids were quercetin-3-O-hexoside, quercetin, and rutin (18.00, 6.17, 5.11 mg L<sup>-1</sup>, respectively). These bioflavonoids protect the brain cells from oxidative stress, which damages the tissue and leads to Alzheimer's and other neurological disorders. Quercetin could prevent allergic reactions by inhibiting the release of histamine, and consequently contributing to the treatment and prevention of asthma and bronchitis.<sup>37</sup> The presence of quercetin-3-O-hexoside, quercetin, and rutin, as the dominant flavonoids, gives special significance to elderberry juice because of their potential in terms of health benefits.<sup>38</sup>

Considering the polyphenolic profile of the elderberry juice from this study, and study by Senica *et al.*,<sup>12</sup> chlorogenic acid was present in both tested samples, but was somewhat higher in ours. In the juice<sup>11</sup> there was no presence of protocatechuic, nor gallic and p-hydroxybenzoic acids. Also, a higher content of caffeic acid was identified in the elderberry juice in this study, while p-coumaric acid was recorded at a concentration of 6.6 mg kg<sup>-1</sup> in the analysis of Senica *et al.*,<sup>12</sup> which was about 3.6 times higher than in ours. The difference between two juice samples also reflected in the flavonoid content. Catechin and epicatechin were detected in juice analysed by Senica *et al.*, while the presence of these flavonoids compounds was not detected in this research. The differences in the phytochemical composition that occurred between juices could be explained by the influence of environmental factors on the development of *S. nigra* fruits, the composition of the soil on which the plant grows, as well as the techniques and methods used for obtaining and analysing of juice. However, the results obtained in this study are in agreement with the research conducted by Natic *et al.*<sup>39</sup> and Caruso *et al.*<sup>40</sup>

Based on this research, it could be suggested that dominant phenolic acids and flavonoids which are present in great concentrations in the elderberry juice, in synergism with another phenolic compound could positively affect the health of the consumer and the functional characteristics of the product itself.

### Biological activity

**Antioxidant activity.** The antiradical activity of the berry juices has a complex mechanism due to the various interactions



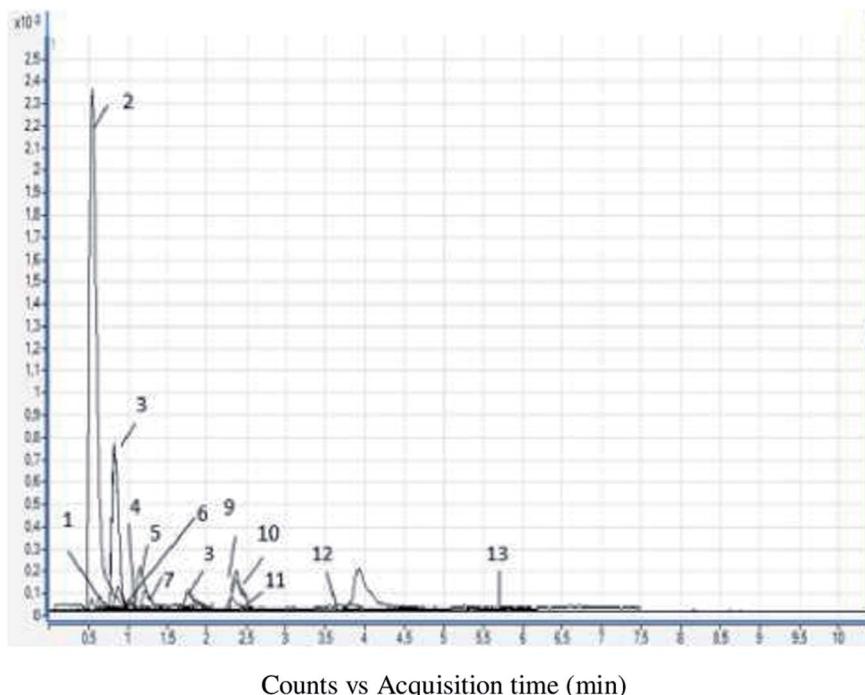


Fig. 1 LC-MS/MS chromatogram of elderberry juice 1: gallic acid, 2: quinic acid, 3: protocatechuic acid, 4: esculetin, 5: *p*-hydroxybenzoic acid, 6: chlorogenic acid, 7: caffeic acid, 8: *p*-coumaric acid, 9: kaempferol 3-*O*-glucoside, 10: quercetin 3-*O*-hexoside, 11: rutin, 12: quercetin, 13: amentoflavone.

Table 3 Biological activities of elderberry juice

Antioxidant assays	
ABTS <sup>a</sup> (g TE per L juice)	5.38 ± 0.30
DPPH <sup>a</sup> (g TE per L juice)	2.16 ± 0.79
LP <sup>a</sup> (g TE per L juice)	0.18 ± 0.04
NO <sup>a</sup> (g TE per L juice)	53.06 ± 17.40
FRAP <sup>a</sup> (g TE per L juice)	7.26 ± 1.63
CUPRAC <sup>a</sup> (g TE per L juice)	10.98 ± 0.46
Phosphomolybdenum <sup>a</sup> (g TE per L juice)	29.63 ± 2.31
Metal chelating <sup>b</sup> (g EDTAE per L juice)	0.11 ± 0.02

#### Enzymes inhibitor assays

AChE <sup>c</sup> (mg GALAE per g juice)	5.08 ± 0.12
BChE <sup>c</sup> (mg GALAE per g juice)	1.45 ± 0.48
Tyrosinase <sup>d</sup> (mg KAE per g juice)	54.70 ± 3.05
α-Amylase <sup>e</sup> (mmol ACAE per g juice)	0.12 ± 0.01
α-Glucosidase <sup>e</sup> (mmol ACAE per g juice)	0.05 ± 0.01

<sup>a</sup> g equivalent Trolox per liter of juice. <sup>b</sup> g equivalent EDTA per liter of juice. <sup>c</sup> mg galantamine equivalent per g of juice. <sup>d</sup> mg kojic acid equivalent per g of juice. <sup>e</sup> mmol acarbose equivalent per g of juice.

between the phenolic compounds present in the juice and radicals generated in the *in vitro* or *in vivo* systems.<sup>31</sup> The antioxidant capacity of elderberry juice was determined by several *in vitro* assays based on the radical scavenging activity (ABTS<sup>+</sup> and DPPH<sup>·</sup>), lipid peroxidation potential (LP), scavenger capacity of free radicals ('NO), reducing power (FRAP and CUPRAC), total

antioxidant and metal chelating capability. The results of this research are shown in Table 3.

Elderberry juice has extremely strong antioxidant activity, according to all applied assays. In this study, 'NO radical scavenging ability of elderberry juice was investigated for the first time. Based on the achieved value, which was very high (53.06 g TE per L juice), it was proven that the analysed juice was a very potent agent in the 'NO radical scavenging process. Reactive nitrogen species (RNS), as endogenous intermediates that continuously occur in living cells, play an essential role in the regulation of physiological processes, which is why different biological activities of 'NO are conditioned by its radical character.<sup>41</sup> The elderberry juice also achieved strong total antioxidant and metal chelating potential. Significant radical scavenging and a reducing power capability of elderberry juice were reached, and the obtained values ranged from 2.16–5.38 to 7.26–10.98 g TE per L juice. Compared to blueberry juice,<sup>32</sup> elderberry juice exhibited stronger reduction potential.

The very good ability to chelate metal ions of elderberry juice is probably conditioned by its phytochemical (polyphenol) composition. Namely, as we determined, the examined juice is a good source of phenolics, and the ability to chelate metal ions shown by phenolic compounds is a function of their characteristic chemical structure, number, and position of hydroxyl groups.<sup>41</sup>

It is important to point out that within this research, the ability of elderberry juice to inhibit lipid peroxidation, *i.e.* to prevent damage to cell membranes caused by free radicals, was also examined. The ability of the juice to inhibit the process of



Table 4 Lists, description of sensory attributes and reference materials used for the descriptive analysis of elderberry juice

Attribute	Description	Reference material
Beet	The damp, musty/earthy, and slightly sweet aromatics commonly associated with beets	Beet juice
Blackberry	Sweet, sour, and fruity aromatics associated with blackberries	Blackberry juice
Cherry	The sour, fruity, and slightly bitter aromatics commonly associated with cherries	Cherry juice
Vinegar	Sour, astringent, and slightly pungent aromatics associated with vinegar	Apple vinegar
Grapefruit	Characteristic aromatics associated with grapefruit	Fresh grapefruit
Coconut	Characteristic aromatics associated with coconut	Coconut milk
Clove	Characteristic aromatics associated with clove	Clove – dried flower
Jasmine, lilac	Sweet, light, and slightly perfume impression associated with flowers	Benzyl acetate
Dried plums	Characteristic aromatics associated with dried plums	Dried plums
Bitter	The fundamental taste factor associated with a caffeine solution	Caffeine
Sweet	The fundamental taste factor associated with a sucrose solution	Sucrose
Salty	The fundamental taste factor associated with a sodium chloride solution	Sodium chloride
Sour	The fundamental taste factor associated with a citric acid solution	Citric acid
Astringent	The dry puckering mouthfeel associated with an alum solution	Alum
Throatetch	A sensation of abrasion and drying of the throat	Fresh quince
Persistency	Perception of unclean sensation within the oral cavity after swallowing sample	

lipid peroxidation was 0.18 g TE per L of juice. In this way, it has been implicated that elderberry juice, as a potentially new functional product, can reduce the risk of developing different diseases of modern society caused by oxidative stress.

The plant species *S. nigra* is characterized as a “natural laboratory” of phytopharmacological molecules that are carriers of different biological activities.<sup>42</sup> The pronounced overall antioxidant activity of elderberry juice, which was determined by applying listed antioxidant assay, is most likely a consequence of the occurrence of various phenolic compounds, particularly dominantly present protocatechuic acid, quercetin-3-O-hexoside, chlorogenic acid, and rutin. Compared to the antioxidant potential of commercial berry fruits juice, the examined juice showed slightly lower radical scavenging activity because it has no additional enzymes or additives to achieve better bioactivity of the product.<sup>43</sup>

#### Enzyme-inhibitory activity of elderberry juice

Enzymes, as valuable pharmaceutical targets for controlling global health problems, were examined within the frame of this study, and the results are presented in Table 3.

The development of Alzheimer's disease is associated with altered enzyme activity, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). These enzymes play a role in regulation of the levels of the neurotransmitter acetylcholine (ACh) on the synaptic gap. In this regard, inhibiting the overactivity of these enzymes is a valuable therapeutic way of alleviating Alzheimer's symptoms. The obtained results show that elderberry juice inhibits AChE and BChE well (5.08 and 1.45 mg GALAE per g, respectively). Previous studies have found that phenolic acids, or molecules with a phenolic ring and hydrophobic

residues in their structure, are considered to be potential inhibitors of AChE and BChE.<sup>19</sup> The phenolic acids identified in elderberry juice significantly contribute to the realized activity.

Tyrosinase is a key enzyme in the synthesis of melanin and some other pigments. An increased concentration of this enzyme in the body could lead to melanoma. Therefore, tyrosinase inhibition can be very important for controlling various forms of skin disorders, especially pigmentation disorders. Hence, the need to find and isolate the natural inhibitors of this enzyme has been imposed.<sup>44</sup>

The inhibitory power of elderberry juice was also tested on the tyrosinase enzyme. Analyses have shown that examined juice was a very potent tyrosinase inhibitor (juice 54.70 mg KAE per g). Antityrosinase potential of the examined juice could be explained by the extremely rich phytochemical composition. The most abundant compounds, such as protocatechuic acid, quercetin, quercetin-3-O-hexoside, chlorogenic acid, and rutin can contribute to the strong antityrosinase potential of elderberry juice.<sup>19</sup> Namely, flavanones and flavanonols have the ability to inhibit tyrosinase reversibly and competitively. Flavanones are copper chelators which disrupt tyrosinase structure by hydrophobic interactions and chelate a copper ion, coordinating with 3 histidine residues (HIS61, HIS85, and HIS259).<sup>45</sup> So, it can be concluded that compounds with flavonoid structure, detected in the elderberry juice, could affect the chelation of copper ions and thus reduce the overactivity of tyrosinase, which further reduces the possibility of various diseases development. Also, this ability is supported by previously discussed ability of phenol-rich elderberry juice to chelate metal ions (Table 3).

Table 5 Instrumental defined color parameters of elderberry juice

<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i> *	<i>h</i>	$\lambda$
19.68 ± 0.11	0.63 ± 0.38	1.66 ± 0.09	1.81 ± 0.17	69.84 ± 10.58	581.71 ± 3.38





Fig. 2 Defined color of elderberry juice (card S 5040-R20B).

$\alpha$ -Amylase and  $\alpha$ -glucosidase are enzymes involved in carbohydrate metabolism, and the overactivity of these enzymes leads to the accumulation of glucose in the blood, which is an initial step in the development of diabetes, one of the most widespread diseases of modern society. Inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is considered as an effective way of controlling blood glucose levels in patients with diabetes.<sup>46</sup> Elderberry juice inhibited both enzymes at concentrations of 0.12 and 0.05 mmol ACAE per g juice, respectively. These results indicated that elderberry juice obtained by the traditional pressing technique is a product that could potentially interfere with the evolution of diabetes.<sup>47</sup>

The results of this research support the concept of functional food – the food which provides not only essential nutrients but also compounds that contribute to normal function and can help prevent/alleviate diseases.<sup>48</sup> Thus, through molecular and physiological mechanisms, the potential health benefits of elderberry juice can be linked to the chemical characteristics of present phenolic acids and flavonoids and their ability to remove free radicals and/or chelate redox-active metals or to interact with specific enzymes included in different metabolic processes. Elderberry as a wild-growing plant that has not been technologically changed could be characterized as a plant 'from the field to health'.<sup>49</sup>

### Color of elderberry juice

Average values of lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), chroma – saturation index ( $C^*$ ), hue angle ( $h$ ) and dominant wavelength ( $\lambda$ ) for elderberry juice are shown in Table 5. According to the NCS color atlas (the consensus method), the color of the elderberry juice was visually identified as the color of the S 5040-R20B color card (Fig. 2). The color of elderberry juice was compared with the color of blueberry juice examined by Siddiqi *et al.*<sup>30</sup> In this study, it was determined that elderberry juice was darker, while the redness of blueberry juice was higher. The biggest difference was observed in the value of the

angle of hue, which is much higher in elderberry juice, 69.84, while in blueberry juice it was only 6.88. The results were also compared to the results of the Busso-Casati *et al.* study,<sup>13</sup> where the color of juices and extracts obtained from various berries (blueberry, elderberry, black currant, and maqui berry) was determined. An extensive analysis showed that the juice tested in this study has a lower color intensity than the juices and extracts tested by Busso-Casati *et al.*,<sup>13</sup> which is a consequence of the application of different methods of product preparation, as well as production techniques. In the mentioned study, after collection, the elderberries were lyophilized, and methanol was used as a solvent to obtain extracts, which greatly influenced the final result.

It is important to point out that Porras-Mija<sup>28</sup> research group performed color determination of elderberry fruits ethanol extracts too. Also, in this case, a slightly stronger shade of color was observed in relation to the color of the juice examined in our study. The color differences between the two juices obtained from the berries come from the degree of ripeness of the fruits, and the content of plant pigments that is responsible for the color of the juices. The extraction of plant pigments anthocyanins depends on the applied technological procedure during product preparation. In the first step, the application of lyophilisation as a modern drying technique influenced the preservation of the chemical composition of berries, and secondly, the extraction with methanol and ethanol provided higher amounts of pigments and consequently a darker color of the products.<sup>13,28</sup> Our traditional way of obtaining juice, without the influence of temperature, ensured high quality of the product, and on the other hand, it led to incomplete isolation of anthocyanins, due to which the examined juice had lighter color.

### Sensory profile and consumer's appearance of elderberry juice

The mean scores for 16 identified sensory attributes are shown in Fig. 3. The elderberry juice had the highest intensities of

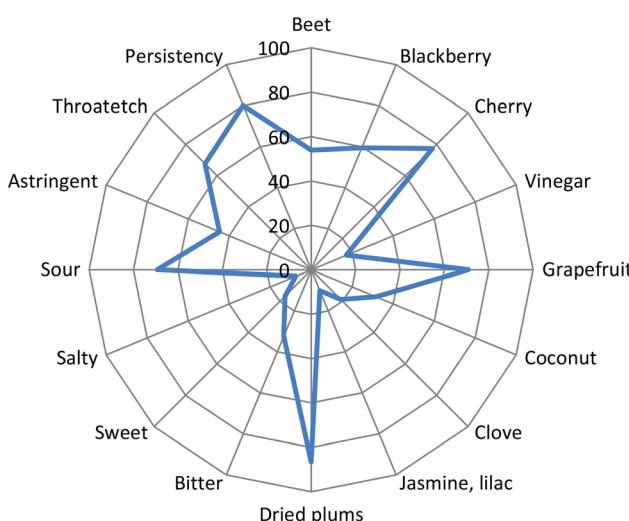


Fig. 3 Sensory profile of the elderberry juice.



Table 6 Sensory scores of elderberry juice

Appearance liking	Flavour liking	Mouthfeel liking	Aftertaste liking	Overall liking
7.40	5.70	6.10	5.90	6.10

dried plums (86.5), cherry (77.3), grapefruit (70.9), sour (69.3) and throatatch (67.6) notes. Further, the elderberry juice had a blackberry (59.6), beet (54.0) and astringent (44.7) note. Finally, the juice had lower notes of bitter (32.2), coconut (31.7), clove (18.8), vinegar (17.3), sweet (16.7), jasmine (lilac) (10.1) and salty (7.5). The persistence of the elderberry juice was 80.1.

The mean scores for appearance liking, flavor liking, mouthfeel liking, aftertaste liking, and overall liking are shown in Table 6 and values were 7.4 (between 'like moderately' and 'like very much'), 5.7 (between 'neither like nor dislike' and 'like slightly'), 6.1 (slightly higher than 'like slightly'), 5.9 (slightly lower than 'like slightly') and 6.1 (slightly higher than 'like slightly'), respectively.

## Conclusions

We investigated elderberry juice, obtained by a traditional pressing method, to validate its potential as a novel functional product, which is not present on the market. For the first time, the nutritional composition of the elderberry juice, its neuro-protective, antityrosinase and antidiabetic activities, as well as sensory properties were examined, which gives particular value to this research.

The results obtained by analysing the polyphenol profile of elderberry juice showed that a significant amount of primary and secondary metabolites is present in the juice. The analysis demonstrated the great presence of protocatechuic acid and quercetin-3-O-hexoside as a dominant phenolics. Elderberry juice expressed strong biological potential, especially the antioxidant and antityrosinase activity. Moreover, the evaluated juice exhibited very good neuroprotective ability. Consumption of this juice could be of importance for adequate daily intake of the micronutrients, especially K and Fe, because these minerals are present in immense concentration. Also, a positive assessment of the acceptability of this product by consumers is of great importance. The obtained results could be used as guidelines and selection criteria for the further use of elderberry juice as a new potential functional product that could become concurrent with existing juices on the market.

## Conflicts of interest

The authors declare no conflict of interest.

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