**Antioxidant Activity and Chemical Characteristics of Sambucus nigra L. Blossom from Different Regions in Bulgaria**

Galia Gentscheva 1,*, Iliana Milkova-Tomova 2, Krastena Nikolova 3,*, Dragomira Buhalova 2, Velichka Andonova 4, Viliana Gugleva 4, Nadezhda Petkova 5, Ina Yotkovska 1 and Nadezhda Ivanova 4

1 Department of Chemistry and Biochemistry, Medical University, 5800 Pleven, Bulgaria; ina.iotkovska@mu-pleven.bg
2 Department of Nutrition and Tourism, University of Food Technologies, 4002 Plovdiv, Bulgaria; iliana_tomova@abv.bg (I.M.-T.); dra.buhalova@gmail.com (D.B.)
3 Department of Physics and Biophysics, Faculty of Pharmacy, Medical University, 9000 Varna, Bulgaria
4 Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University, 9000 Varna, Bulgaria; velichka.andonova@mu-varna.bg (V.A.); viliana.gugleva@mu-varna.bg (V.G.);
nadejda.ivanova@mu-varna.bg (N.I.)
5 Department of Organic Chemistry and Inorganic Chemistry, University of Food Technologies, 4002 Plovdiv, Bulgaria; petkovanadejda@abv.bg

* Correspondence: gentscheva@mu-pleven.bg (G.G.); kr.nikolova@abv.bg (K.N.); Tel.: +359-64-884-259 (G.G.);
+359-897-832-753 (K.N.)

**Abstract:** The aim of the current study was to investigate some bioactive compounds from Sambucus nigra L. blossoms and to evaluate the antioxidant potential of the obtained extracts. In this study, samples from four different regions of Bulgaria /Rhodopes, Plovdiv, Strandzha and Dobrich region/ from Sambucus nigra L. were collected and analyzed for total phenols, flavonoids, sugars and amino acids. The antioxidant activity of the extracts was evaluated by four assays based on different mechanisms. The sweetness index and total sweetness index of the extracts were also evaluated. The carbohydrate composition of the leaves and the blossoms was determined, with glucose and fructose predominating in both cases, as their contents were not above 3%. Nineteen amino acids have been identified in the composition of Sambucus nigra L., and glutamic, leucine and asparagine acids are predominant. The highest antioxidant activity and total content of phenols (49.2 ± 1 mg GAE/g) and flavonols (18.6 ± 0.5 mgQE/g) were found in the sample from the Rhodope region. Therefore, the higher altitude and lower temperature in mountains could influence the accumulation of secondary metabolites in blossoms of Sambucus nigra L., which improves the antioxidant potential of the samples.

**Keywords:** Sambucus nigra L.; medicinal plants; antioxidant activity; flavonoids; phenols; carbohydrates; amino acids

1. **Introduction**

Elder or black elder (Sambucus nigra L.) is a perennial, deciduous shrub or small tree with shallow roots, propagated by seed. The tree reaches up to 8–10 m in height and 20–30 cm in diameter. It blooms from May to June, depending on the altitude, with white-cream colored or greenish-yellow individual flowers gathered in flat, complex, umbrella-like umbels, approximately 10–20 cm in diameter, containing 3–5 small seeds. The blossoms are characterized by a fresh, intense, fruity-sweet aroma.

Black elderflower tea may help in a variety of cases, such as hoarse voice (dysphonia) and upper respiratory tract inflammation [1], hemorrhoids, ascites, high blood pressure, impotence, urinary tract inflammation, kidney and bladder inflammation, prostatitis, hematuria, shortness of breath (dyspnea), and obesity [2–4]. It has been found that elderflowers promote secretion from sweat glands, while elderberries stimulate renal activity. Up to this day, the blossoms of Sambucus nigra L. are used to treat diseases, such as fever, cold...
and influenza infections [5]. In the composition of blossoms, a large number of bioactive ingredients can be found, including terpenes, sterols, polyphenols, proteins, vitamins and minerals [6–9].

The health benefits of different parts of the plant *Sambucus nigra* L. have been investigated by different authors [10,11] and the data are summarized in Table 1.

Table 1. Healing properties of *Sambucus nigra* L.

| Parts of the Tree | Components’ Properties                                                                 |
|------------------|---------------------------------------------------------------------------------------|
| Blossoms         | Promotes sweating, antipyretic agent, expectorant, anti-inflammatory agent, immunostimulant, antibacterial activity; |
| Fruits           | Strengthening the immune system, antineuralgic, antiviral, antibacterial action, antioxidant, laxative, diuretic agent; |
| Leaves           | Diuretic agent, blood purifying properties, laxative agent, detoxifying properties;    |
| Bark             | Diuretic agent, laxative, inflammatory agent;                                          |

Polyphenolic compounds are chemical compounds with a positive effect on human health and various pharmacological effects: antiviral, antibacterial and anti-cancer functions [9]. Some publications have shown that elderflower extract contains bioactive compounds that are able to metabolize glucose and lipids, which leads to a reduction in fat accumulation [12,13]. Other studies have reported strong antimicrobial effects of elderflower on various nosocomial pathogens, especially on methicillin-resistant *Staphylococcus aureus* MRSA (clinically significant pathogen) [14].

The healthy effects of *Sambucus nigra* L. on the human body based on the high antioxidants, bioactive flavonoids and phenolic acid content, mineral salts, and fibers, as well the aim to enrich a healthy diet with phytocomponents, motivates the research for the possibilities for inclusion of *Sambucus nigra* L. blossom in foods and beverages, as well its use in food supplements [15,16].

There are many studies on the bioactive compounds present in medical herbs; however, regarding the *Sambucus nigra* L. blossoms, the information is relatively insufficient. While the chemical composition of elderberries has been comprehensively studied and a lot of applications in pharmacy, in beverage production are known and food, there is still not enough information on leaves and blossoms from the Bulgarian region with respect to the bioactive compounds content. Given the above, our study focuses on the blossom of *Sambucus nigra* L. from four different regions in Bulgaria (Rhodopes, Plovdiv, Strandzha, and Dobrich) and creating a profile of the chemical composition using spectrophotometric and HPLC methods to determine antioxidant activity, the content of carbohydrates, amino acids, total phenols and flavonoids.

2. Materials and Methods

2.1. Plant Material

Fresh blossoms of *Sambucus nigra* L. from different regions of Bulgaria (Strandzha, Plovdiv, the Rhodope Mountain and Dobrich region) were selected for this study. The blossom clusters of *Sambucus nigra* L. with attached leaves are harvested by cutting when the tree is in full bloom [17]. The intensive flowering period is in May–July, depending on the altitude of the region. The identification of plant material was performed in the Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (IBER-BAS) in Sofia. The department’s herbarium is the most representative source of information on the biological diversity of the flora of the Balkans and Bulgaria. It is registered in the Index Herbariorum as an internationally recognized Herbarium with the acronym SOM (Herbarium of Vascular Plants).
2.2. Methods

2.2.1. Drying

Fresh blossoms of *Sambucus nigra* L., intended for the study, were dried in a thin layer in the shade and turned periodically to a constant mass of the sample. The dried samples were homogenized, ground, packed in canvas bags, and stored at 18–20 °C, in the absence of light.

2.2.2. Determination of Antioxidant Activity (AOA)

The samples were extracted with water in a solid to solvent ratio (1:10 w/v) using an ultrasonic bath operating at 40 kHz at 40 °C for 20 min. The antioxidant activity of the extracts was evaluated by four methods based on different mechanisms.

DPPH Assay

The method provides information on the ability to scavenge the radical DPPH (1,1-Diphenyl-2-picrylhydrazyl), based on the mechanisms of mixed transfer of the hydrogen atom (HAT) and single electron transfer. The analyzed sample (0.15 mL) was mixed with 2.85 mL freshly made 0.1 mM DPPH solution (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) in methanol (Merck KGaA, Darmstadt, Germany). The sample was incubated for 15 min at 37 °C in the dark. The reduction in the absorbance at 517 nm was measured by spectrophotometer a VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom) in comparison to control sample containing methanol and the % inhibition was calculated [18]. A standard curve with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) at a concentration of 0.005–1.0 mM was generated. The results of the assay are presented as millimoles of Trolox equivalents (mM TE)/g dry weight sample.

FRAP Assay

The antioxidant power of ferric ion reduction is based on the mechanism of single electronic transfer. The sample was treated according to [19], with minor modifications. FRAP reagent was prepared by mixing 100 mL of 0.3 M acetate buffer (pH 3.6) (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany), 10 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl (Merck KGaA, Darmstadt, Germany) and 10 mL of 20 mM FeCl₃·6H₂O (Merck KGaA, Darmstadt, Germany) in distilled water. The reaction was carried out by mixing 3.0 mL of FRAP reagent with 0.1 mL of the studied extract. The reaction time was 10 min at 37 °C in the dark and the absorbance was measured at a wavelength of 593 nm by a VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom) versus a control sample prepared with methanol (Merck KGaA, Darmstadt, Germany). The antioxidant activity is expressed in mM Trolox® equivalents (TE) per g dry weight.

ABTS Assay

The ABTS radical was prepared by mixing in equimolar amounts ABTS (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) (7 mM in distilled water) and potassium persulphate (2.45 mM in water), which were then left for 16 h in the dark. Prior to the experiment, 2 mL of the solution was mixed with 60 mL methanol (Merck KGaA, Darmstadt, Germany) to obtain a final adsorption of 1.0 ÷ 1.1 at 734 nm by a VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom). For the analysis, 0.15 mL of the studied sample was mixed with 2.85 mL of freshly made ABTS radical solution. The reaction mixture was incubated in the dark for 15 min at 37 °C. The adsorption reduction was determined spectrophotometrically at a wavelength of 734 nm [20].

CUPRAC Assay

The reaction was initiated by mixing 1 mL of 10 mM CuCl₂ × 2H₂O, 1 mL Neocuproin (7.5 mM in methanol) (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany), 1 mL 0.1 M
ammonium acetate buffer (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany), pH 7, 0.1 mL of tested sample and 1 mL of distilled water. The reaction mixture was incubated for 20 min at 50 °C in the dark. After cooling the mixture, the absorbance was measured at 450 nm using a VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom) [20]. The results of the assay are presented as millimoles of Trolox equivalents (mM TE)/g dry weight sample.

2.2.3. Determination of Phenolic Content

The total phenolic content in the tested samples was determined by the method of Folin–Ciocalteu [21]. Aqueous extract (0.2 mL) was added to 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) diluted five times and then 0.8 mL of 7.5% sodium carbonate solution was added. After 20 min in the dark, the absorption was measured at a wavelength of 765 nm by a UV/VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom) against a blank prepared with distilled water (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany). The concentration of total phenolic content is expressed as milligrams of gallic acid equivalent—mg GAE/g dry weight. The calibration curve is linear in the range of 0.02–0.10 mg gallic acid (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) used as a standard [19].

2.2.4. Determination of Total Flavonoids

Measurement of flavonoid concentration is based on the method described by [22], and the results are expressed as quercetin equivalents. The plant material was extracted with distilled water in a solid to solvent ratio (1:10 w/v). The extraction was performed in duplicate for 20 min at 45 °C in a 35 kHz ultrasonic bath (Isolab Laborgeräte GmbH, Eschau, Germany). The sample was filtered through filter paper and 1 mL of the solution was added to test tubes containing 0.1 mL of 10% aluminum nitrate (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany), 0.1 mL of 1 M potassium acetate (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) and 3.8 mL of 95% ethanol (Merck KGaA, Darmstadt, Germany). After 40 min at room temperature, the absorbance was determined at 415 nm using a VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, the United Kingdom). The results were expressed as mg quercetin equivalent (QE)/g dry sample.

2.2.5. Determination of Carbohydrate Content (HPLC)

The dried Sambucus nigra L. flowers and leaves were extracted with distilled water (solid to liquid ratio 1:5 (w/v)) in an ultrasonic bath (Isolab Laborgeräte GmbH, Eschau, Germany) with a frequency of 40 kHz and 60 W power at 50 °C in duplicate. The samples were filtered through a 0.45 µm syringe filter, PTFE45/25 mm (Isolab Laborgeräte GmbH, Eschau, Germany), before injection. The contents of glucose, fructose and sucrose were determined using a Shimadzu HPLC, coupled with an LC-20 ADpump, and a Shimadzu RID-10A refractive index detector (RID). The separation was performed on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) column coupled with a Shodex SP-Gguard column (5 µm, 6 mm × 50 mm) (Shodex Co., Tokyo, Japan) operating at 85 °C. The mobile phase was ultrapurified water (water purification system Adrona B30 Integrity + HPLC, Riga, Latvia) with a flow rate of 0.5 mL/min. The injection volume was 20 µL [23]. The results were expressed as g/100 g dry weight.

2.2.6. Sweetness Index and Total Sweetness Index

The Sweetness Index (SI) and Total Sweetness Index (TSI) were calculated to determine the sweetness perception. The calculations were performed according to Magwaza and Opara [24], as follows:

\[
SI = (1.00 \times [\text{glucose}]) + (2.30 \times [\text{fructose}]) + (1.35 \times [\text{sucrose}])
\]

\[
TSI = (1.00 \times [\text{sucrose}]) + (0.76 \times [\text{glucose}]) + (1.50 \times [\text{fructose}])
\]
2.2.7. Amino Acid Analysis

Dried *Sambucus nigra* L. flowers were subjected to acid hydrolysis using 6N HCl for 24 h at 105 °C. An aliquot of the hydrolysate was derivatized using an AccQ-Fluor reagent kit (Waters, Milford, MA, USA). The derivate was separated on an RP AccQ-Tag™ silica-bonded amino acid column C18, 3.9 mm × 150 mm (Waters, Milford, MA, USA) conditioned at 37 °C using an ELITE LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan). A sample of 20 µL was injected and the elution of the amino acids was performed by a gradient system: eluent A, buffer WAT052890 (Waters, Milford, MA, USA) and eluent B, 60% acetonitrile (Sigma-Aldrich, Merck, Darmstadt, Germany) with a constant flow rate of 1.0 mL/min. The amino acids were detected using a diode array detector (DAD) at 254 nm. The amino acid peaks were then analyzed using EZChromElite™ software and were calculated based on the amino acid standard calibration curve (amino acid standard H, Thermo Fisher Scientific, Waltham, MA, USA). The results are expressed as mg AA/g dry weight (dw) [25].

2.2.8. Mathematical Processing of Results

Five samples of *Sambucus nigra* L. were collected in the area at the indicated geographical point. Five parallel measurements were made for each of the studied parameters. The data on phenolic content, antioxidant activity, sugar content and amino acids were processed to obtain the mean value and standard deviation of the mean value (SD). The analysis of dispersion was used to compare the mean values with a significance level of \( p < 0.05 \). A one-way analysis of variance and a subsequent Duncan test for multidirectional comparisons based on the investigated parameters were performed for elderberry from different regions of Bulgaria. Statistical analysis was performed using the IBM SPSS Statistic 26, computer program, US.

3. Results and Discussion

3.1. Characteristics of *Sambucus nigra* L.

Identification of Plant Material

Fresh raw material of *Sambucus nigra* L. from four regions of Bulgaria: Western Rhodopes, Velingrad from the area of Golyam Beglik–Karatepe–Sutka dam (Figure 1a); Plovdiv municipality, village of Staro Zhelezare (Figure 1b); Gramatikovo village, Strandzha region (Figure 1c); Dobrich region (Figure 1d) was studied for authenticity. For simplicity, in the text, those regions are cited as follows: (a) Rhodopes region, (b) Plovdiv region, (c) Strandzha region and (d) Dobrich region.

The identification of plant material makes it possible to draw conclusions about the species, genera, family and evolutionary development of a plant species. According to the requirements of IBEI-BAS, the herbaria of *Sambucus nigra* L. from four regions of Bulgaria were prepared to determine the botanical identity. The presence of morphologically similar species determines the necessity of authenticity evaluation. The locality was described in detail and the geographical coordinates were determined (Table 2).

As can be seen from the photos of different herbaria, there are differences in the morphology of the plants *Sambucus nigra* L. The Elder from the Strandzha region is characterized by the smallest leaves, whereas the Elder from the Rhodopes region has the largest flowers. The plants from the Plovdiv and Dobrich regions have comparable leaf sizes; however, the elder flowers from the Dobrich region are slightly larger than those from the Plovdiv region. It can be concluded that, based on the performed identification, plant species were established, and the identification numbers of each specimen were determined.

Table 3 presents the average carbohydrate composition of the blossoms and leaves of *Sambucus nigra* L. Three sugars were detected in the blossoms and leaves of *Sambucus nigra* L. In leaves, glucose and fructose were the dominating sugars, as glucose presents 49% of the total sugar content. In blossoms, the most abundant sugar was glucose, which occupied 58% of the total sugars in the samples. Our findings in the current research confirmed the reported data [26] for the highest glucose content (as glucose represented from 60%
to 85% total analyzed sugars in elderberry flower). Contrary to the fructose to glucose ratio (1:1) determined in elderberry fruits [27], the glucose/fructose ratio was 1.92 for blossoms and 1.18 for leaves (Table 3). Glucose/fructose ratio coincided with data for peaches [28] and was lower than the glucose-fructose ratio in strawberry and blueberry leaves (1.57–1.65) [29]. This can be explained by the fact that glucose is an essential sugar in plant metabolism, not just for fruit ripening, but also for some other structural, nuclear and biochemical processes in plants (signaling, growth, development and respiration).

![Sambucus nigra L. from different regions of Bulgaria. (a) Western Rhodopes, Velin-grad, Golyam Beglik–Karatepe–Sutka; (b) Staro Zhelezare village, Plovdiv region; (c) Gramatikovo village, Strandzha; (d) Dobrich region.](image)

**Figure 1.** *Sambucus nigra* L. from different regions of Bulgaria. (a) Western Rhodopes, Velin-grad, Golyam Beglik–Karatepe–Sutka; (b) Staro Zhelezare village, Plovdiv region; (c) Gramatikovo village, Strandzha; (d) Dobrich region.

It is known that the reduction of sucrose content is extremely important during the elaboration of new food compositions (the World Health Organization recommends limiting added sugars to 5–10% of daily calorie consumption), and very often the aim is to increase the content of monosaccharides (glucose and fructose). Glucose and fructose have the same calories per gram (4 kcal). Glucose is used immediately to generate energy for the body or to be converted into glycogen, which will be stored in the muscles or liver for future use. Similar to glucose, fructose is absorbed directly into the blood across the small intestine. In contrast to glucose, fructose gradually raises blood sugar levels and has an immediate effect on insulin levels.
Table 2. Necessary data for the identification of the studied plant species.

| Plant Material/Herbs, Identification Number | Location | Geographical Coordinates | Altitude | Date |
|--------------------------------------------|----------|--------------------------|----------|------|
| Sambucus nigra L. SOM 1406 n (number of samples) 5 | Bulgaria, Western Rhodopes, Velingrad, Golyam Beglik-Karatepe-Sutka | 35TKG5111032486 Lat. 41.80444 Lon. 24.159444 | UTM/MGRS KG53 1271 m | 06.07.2021 |
| Sambucus nigra L. SOM 1407 n = 5 | Bulgaria, Plovdiv, Staro Zhelezare village | 35TKH 54468 37565 Lat. 42.750555 Lon. 24.000004 | UTM/MGRS KH53 294 m | 06.05.2021 |
| Sambucus nigra L. SOM 1405 n = 5 | Bulgaria, Gramatikovo village, Strandzha region | 35TNG0009261486 Lat. 42.104 7222 Lon. 27.001111 | UTM/MGRS NG06 295 m | 18.06.2021 |
| Sambucus nigra L. SOM 1404 n = 5 | Bulgaria, Dobrich region | 35TPJ03240 39007 Lat. 43.696111 Lon. 28.281111 | UTM/MGRS PJ03 205 m | 09.05.2021 |

Table 3. Carbohydrate content in samples of Sambucus nigra L.

| Sample | Leaves | Blossoms |
|--------|--------|----------|
| Sucrose, g/100 g | 0.55 ± 0.05 a | 0.26 ± 0.03 b |
| Glucose, g/100 g | 3.19 ± 0.02 a | 1.50 ± 0.05 b |
| Fructose, g/100 g | 2.70 ± 0.06 a | 0.79 ± 0.05 b |
| Total sugars | 6.44 ± 0.06 a | 2.55 ± 0.04 b |
| Sucrose/Glucose | 0.17 | 0.17 |
| Glucose/Fructose | 1.18 | 1.92 |
| Sweetness index | 10.1 | 3.7 |
| Total sweetness index | 7.0 | 2.6 |

Means in a column with a common superscript letter (a,b) differ (p < 0.05) as analyzed by one-way ANOVA.

As shown in Table 3, the flowers and leaves of Sambucus nigra L. contain monosaccharides glucose and fructose, and the glucose values are approximately 5.8 times higher than sucrose values in both samples. The leaves have a higher carbohydrate content in comparison to blossoms (6.44% versus 2.55%, respectively). The content of glucose and fructose in the blossoms of Sambucus nigra L. in Bulgaria was found to be lower than that established in other European countries [30]. However, the sucrose content is similar to that published in [30]. In our study, sugar content in leaves and flowers decreased in the following order: glucose > fructose > sucrose (Table 3). A similar tendency was found in elderberry fruits, while in elderflowers, it was the opposite [31]. The detected values of sucrose were lower than the reported values for Portuguese elderberry varieties [30].

The lower sucrose content had an impact on the sweet perception of analyzed blossoms and leaves of Sambucus nigra L. Leaves demonstrated twice the sweetness and total sweetness indices than blossoms (Table 3). Sweetness indices were 10.1 and 3.7 for the leaves and blossoms of Sambucus nigra L., respectively. The values were closer to published values for the sweetness index of strawberry [32].

The distribution of amino acids in g/100 g of the raw materials of Sambucus nigra L. is presented in Table 4. The average results for samples of the regions used are presented, as the differences are within the experimental error of the method (±3%).

There are proven statistical differences for elderberry blossoms from the different Bulgarian regions of the respective studied characteristic, at p < 0.05. Values for p are not given in the table, where the samples from the respective regions are indistinguishable by that parameter.
As a result of the Duncan test for most of the amino acids, statistical differences were proved for the blossoms of elderberry from different regions of Bulgaria, except for histidine, gystine, hydroxyproline, tryptophan, methionine and alanine.

It is noteworthy that aspartic, leucine and glutamic acids exhibit the highest content (0.06 ± 0.1 g/100 g). It is known that these two amino acids make the greatest contribution to the formation of taste. In addition, Elder contains the full range of eight essential amino acids with leucine, methionine, phenylalanine, valine and lysine and semi-essential arginine, reaching significant levels (between 0.03 g/100 g and 0.05 g/100 g), which determines the beneficial health effect. Vulic et al. defined the amino acid composition of elderberry fruit [33]. The samples collected from mountain regions (Rhodopes and Strandzha) contained the highest level of essential amino acids in a comparison to the samples collected from the valleys (Plovdiv and Dobrich region).

Antioxidant activity was evaluated by four different methods—DPPH; ABTS; FRAP; and CUPRAC assays. The results in mM TE/g dry biomass are shown in Table 5.

### Table 4. Amino acid composition of *Sambucus nigra* L. blossoms from different regions in Bulgaria.

| Amino Acids, g/100 g | Rhodopes Region | Plovdiv Region | Strandza Region | Dobrich Region | p Value |
|----------------------|-----------------|----------------|-----------------|----------------|---------|
| Alanine              | 0.030 ± 1.10⁻³  | 0.030 ± 1.10⁻³ | 0.030 ± 1.10⁻³  | 0.030 ± 1.10⁻³ | -       |
| Arginine             | 0.051 ± 2.10⁻³  | 0.049 ± 2.10⁻³ | 0.049 ± 2.10⁻³  | 0.048 ± 2.10⁻³ | 0.028   |
| Aspartic acid        | 0.063 ± 5.10⁻³  | 0.058 ± 4.10⁻³ | 0.059 ± 4.10⁻³  | 0.059 ± 4.10⁻³ | 0.000   |
| Glutamine            | 0.030 ± 5.10⁻³  | 0.029 ± 2.10⁻³ | 0.030 ± 1.10⁻³  | 0.028 ± 2.10⁻³ | 0.003   |
| Threonine            | 0.042 ± 5.10⁻³  | 0.041 ± 5.10⁻³ | 0.042 ± 5.10⁻³  | 0.038 ± 5.10⁻³ | 0.000   |
| Histidine            | 0.113 ± 1.10⁻³  | 0.098 ± 1.10⁻³ | 0.100 ± 1.10⁻³  | 0.098 ± 1.10⁻³ | 0.000   |
| Tyrosine             | 0.030 ± 1.10⁻³  | 0.029 ± 1.10⁻³ | 0.030 ± 1.10⁻³  | 0.029 ± 1.10⁻³ | 0.030   |
| Isoleucine           | 0.062 ± 7.10⁻³  | 0.060 ± 5.10⁻³ | 0.060 ± 5.10⁻³  | 0.059 ± 4.10⁻³ | 0.021   |
| Leucine              | 0.031 ± 2.10⁻³  | 0.029 ± 1.10⁻³ | 0.030 ± 2.10⁻³  | 0.028 ± 1.10⁻³ | 0.000   |
| Methionine           | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | -       |
| Proline              | 0.031 ± 3.10⁻³  | 0.029 ± 2.10⁻³ | 0.030 ± 3.10⁻³  | 0.029 ± 2.10⁻³ | 0.01    |
| Serine               | 0.030 ± 2.10⁻³  | 0.030 ± 2.10⁻³ | 0.030 ± 2.10⁻³  | 0.030 ± 2.10⁻³ | -       |
| Tyrosine             | 0.052 ± 6.10⁻³  | 0.049 ± 5.10⁻³ | 0.050 ± 6.10⁻³  | 0.048 ± 4.10⁻³ | 0.000   |
| Threonine            | 0.031 ± 3.10⁻³  | 0.029 ± 2.10⁻³ | 0.030 ± 3.10⁻³  | 0.030 ± 3.10⁻³ | 0.013   |
| Tryptophan           | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | -       |
| Hydroxyproline       | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | 0.010 ± 1.10⁻³  | 0.010 ± 1.10⁻³ | -       |
| Phenylalanine        | 0.041 ± 6.10⁻³  | 0.039 ± 3.10⁻³ | 0.040 ± 5.10⁻³  | 0.038 ± 4.10⁻³ | 0.020   |
| Histidine            | 0.020 ± 1.10⁻³  | 0.020 ± 1.10⁻³ | 0.020 ± 1.10⁻³  | 0.020 ± 1.10⁻³ | -       |

Means in a row with a common superscript letter (a–c) differ (p < 0.05) as analyzed by one-way ANOVA.

### Table 5. Antioxidant activity in samples of *Sambucus nigra* L. blossoms from different regions in Bulgaria, mM TE/g dry weight sample (mean ± SD, n = 5).

| Sample            | DPPH     | ABTS     | FRAP     | CUPRAC   |
|-------------------|----------|----------|----------|----------|
| Rhodopes region   | 236.5 ± 5  | 324.5 ± 5 | 193.0 ± 2 | 748.7 ± 12 |
| Plovdiv region    | 160.3 ± 3  | 249.8 ± 6 | 131.7 ± 2 | 554.4 ± 11 |
| Strandzha region  | 153.2 ± 5  | 240.0 ± 6 | 124.4 ± 2 | 540.4 ± 23 |
| Dobrich region    | 135.1 ± 7  | 199.1 ± 7 | 101.7 ± 2 | 365.1 ± 25 |

Means in a column with a common superscript letter (a–d) differ (p < 0.05) as analyzed by one-way ANOVA.
The highest antioxidant activity (AOA) was found in *Sambucus nigra* L. blossoms from the region of Rhodopes, irrespective of the assay method used, followed by elder blossoms from the regions of Plovdiv and Strandzha. The lowest values were obtained for the blossoms of *Sambucus nigra* L. from the Dobrich region. Therefore, the AOA of the studied samples may depend on the soil and climate. The AOA according to ABTS assay of the samples of dry blossoms from the Dobrich region was in the range (167–212.74) mM Trolox/g extract, reported also for *Sambucus nigra* L. blossoms from other regions of Europe [34]. The antioxidant activity of elderberry blossoms evaluated by the DPPH method was higher than reported values [35].

A similar trend was also found for the content of biologically active compounds (polyphenols and flavonoids—Table 6). The total phenolic content of *Sambucus nigra* L. blossoms samples from the Dobrich and Plovdiv regions were similar to those published by Dzugan and Viapiana [9,36]. For the blossom samples collected from mountainous regions—Western Rhodopes and Strandzha, it was higher than the published values for samples from other regions of Europe and the world. Moreover, the elderberry blossom in our study demonstrated higher values than those reported for Polish samples (25.34 ± 5.41 mg GAE/g) [36]. The latter may be explained by the climatic conditions and the environment in which the particular plant species grows. A similar conclusion was also reported in another study [37]. According to the authors, the total phenolic content of blossom samples of cultivated *Sambucus nigra* L. is higher than that of the wild species.

**Table 6.** Total polyphenolic and flavonoid content in samples of *Sambucus nigra* L. from different regions in Bulgaria.

| Sample           | Total Polyphenols, mg GAE/g Dry Biomass | Total Flavonoids, mg QE/g Dry Biomass |
|------------------|----------------------------------------|---------------------------------------|
| Rhodopes region  | 49.2 ± 1.0 a                           | 18.6 ± 0.5 a                          |
| Plovdiv region   | 36.8 ± 0.5 b                           | 12.5 ± 0.5 b                          |
| Strandzha region | 39.8 ± 2.1 c                           | 12.4 ± 0.5 b                          |
| Dobrich region   | 29.3 ± 1.0 d                           | 6.4 ± 0.5 c                           |

Means in a column with a common superscript letter (a–d) differ (p < 0.05) as analyzed by one-way ANOVA.

A correlation was found between the total polyphenol content and antioxidant activity using the four methods. Similar correlations were also reported in [38]. The results for the established regression models are presented in Table 7.

**Table 7.** Correlation dependences between TPC and antioxidant activity for blossoms of *Sambucus nigra* L. from Bulgaria.

| Correlation Dependence | R²  |
|------------------------|-----|
| DPPH = 5.05 TPC + 24.66| 0.86|
| ABTS = 6.15 TPC + 14.84| 0.94|
| FRAP = 4.51 TPC + 37.2 | 0.90|
| CUPRAC = 18.73 TPC + 174.26| 0.97|

The relationships between AOA by CUPRAC and the ABTS method for determining the antioxidant activity and total phenolic content are characterized with highest correlation coefficient.

4. Conclusions

The following conclusions can be drawn on the basis of the obtained results:

- The blossoms of *Sambucus nigra* L from the Rhodope region have the highest antioxidant activity, total phenol content (49.2 ± 1 mgGAE/g) and total flavonoid content (18.6 ± 0.5 mgQE/g). The blossoms of *Sambucus nigra* L from the Dobrich region have the lowest antioxidant activity, total phenols and flavonoid content. Probably,
the higher altitude and lower temperature in mountains could influence the accumulation of secondary metabolites in blossoms of *Sambucus nigra* L., which improves the antioxidant potential of the samples.

- The predominant amino acids in all blossoms are glutamic acid, leucine and aspartic acid. The samples collected from mountain regions (Rhodopes and Strandzha) contained the highest level of essential amino acids in comparison to the samples collected from the valleys (Plovdiv and Dobrich region).

- For the first time, the sweetness index for taste perception of elderberry blossoms was evaluated. The concentrations of sugars in the leaves are higher than those in the blossoms. Both parts contained more glucose and fructose than sucrose. The leaves showed twice the indices of sweetness and overall sweetness of the blossoms.

The obtained results were valuable for the nutritional potential of elderberry blossoms as edible flowers with antioxidant potential.

**Author Contributions:** K.N. and I.M.-T. constructed and conceived the project, V.A. and G.G. designed the study. D.B., V.G., N.I. and I.Y. performed the study. V.A., I.M.-T. and N.P. analyzed the data and interpreted the results. K.N., G.G. and N.P. wrote the article. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** Special thanks to the Medical University-Pleven for the financial support provided for the publication of the paper.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Berry, P.E. Encyclopedia, Britannica, Encyclopedia/Plants. 2021. Available online: https://www.britannica.com/plant/Dipsacales (accessed on 5 January 2022).

2. Bozhilova, E.; Koeva, J.; Pavlova, D.; Atanasova, Y.; Tonkov, S.; Kozhukharova, E.; Nedelcheva, A. *Guide to the Taxonomy of Higher Plants*; Pensoft: Sofia, Bulgaria, 2003; pp. 65–68.

3. Bukovinova, V. *Herbal Medicine*; Signs Publishing House: Sofia, Bulgaria, 2019; pp. 100–110.

4. Georgiev, M. (Ed.) *Bulgarian Folk Medicine: Encyclopedia*; Academic Publishing House of BAS “Prof. Marin Drinov”: Sofia, Bulgaria, 2013; pp. 70–75.

5. Frohn, B. *Lexikon der Heilpflanzen und ihrer Wirkstoffe*; Weltbild: Buchverlag, Germany, 2007; pp. 200–250.

6. Najar, B.; Ferri, B.; Cioni, P.L.; Pistelli, L. Volatile emission and essential oil composition of *Sambucus nigra* L. organs during different developmental stages. *Plant Biosyst.* 2021, 155, 721–729. [CrossRef]

7. Agalar, H.G. *Nonvitamin and Nonmineral Nutritional Supplements*; Academic Press: Cambridge, MA, USA, 2018; Chapter 3.14; pp. 211–213.

8. Christensen, L.P.; Knaack, K.; Frette, X.C. 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.* 2007, 227, 293–305. [CrossRef]

9. Viapiana, A.; Wesolowski, M. The Phenolic Contents and Antioxidant Activities of Infusions of *Sambucus nigra* L. Plant Foods Hum. Nutr. 2017, 72, 82–87. [CrossRef]

10. Liebert, K. *Holunder*; Demmler Verlag: Ribnitz-Damgarten, Germany, 2009.

11. Pilaske, R. Natürliche Hausapotheke. In *Heilkraft der Bäume*; Fachverlag Fraud: Mainz, Germany, 2002; pp. 25–30.

12. Ho, G.T.T.; Kase, E.T.; Wangensteen, H.; Barsett, H. Effect of Phenolic Compounds from Elderflowers on Glucose- and Fatty Acid Uptake in Human Myotubes and HepG2-Cells. *Molecules* 2017, 22, 90. [CrossRef] [PubMed]

13. Bhattacharya, S.; Christensen, K.B.; Olsen, L.C.B.; Christensen, L.P.; Greven, K.; Færgeman, N.J.; Kristiansen, K.; Young, J.F.; Oksbjerg, N. 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.* 2013, 61, 11033–11040. [CrossRef] [PubMed]

14. Hearst, C.; McCollum, G.; Nelson, D.; Ballard, L.M.; Millar, B.C.; Colin, E.; Goldsmith, E.; Rooney, P.J.; Loughrey, A.; John, E.; et al. Antibacterial activity of elder (*Sambucus nigra* L.) flower or berry against hospital pathogens. *J. Med. Plants Res.* 2010, 4, 1805–1809.
15. Gentscheva, G.; Milkova–Tomova, I.; Buhalova, D.; Pehlivanov, I.; Stefanov, S.; Nikolaov, K.; Andonova, V.; Panova, N.; Gavrailov, G.; Dikova, Z.; et al. Incorporation of the dry blossom flour of *Sambucus nigra* L. in the production of sponge cakes. *Molecules* 2022, 27, 1124. [CrossRef] [PubMed]

16. Młynarczyk, K.; Walkowiak, D.; Tomczak, P.; Grzegorz, P.; Lysiak, G. The Content of Selected Minerals, Bioactive Components, and Antioxidant Activity of the Flowers of *Sambucus nigra* L., *Prunus spinosa* L., and *Fumaria officinalis* L. *Food Sci. Technol.* 2020, 42, 1023–1033. [CrossRef]

17. Kivrac, I.; Duru, M.E.; Ozturk, M.; Mercan, N.; Harmandar, M.; Topcu, G. Antioxidant, anticholinesterase and antimicrobial activities of the leaves of *Salvia officinalis*. *Phytother. Res.* 2016, 30, 1277–1282. [CrossRef]

18. Akšić, M.F.; Tosti, T.; Sredojević, M.; Milivojević, J.; Meland, M.; Natić, M. Comparison of Sugar Profile between Leaves and Fruits of *Sambucus nigra* L. as a functional ingredient for food and pharmaceutical industry. *J. Funct. Foods* 2018, 40, 377–390. [CrossRef] [PubMed]

19. Marchev, A.; Petrova, A.; Nedelcheva, D.; Lazarova, I.; Trucheva, B.; Kostova, N.; Bankova, V.; Pavlov, A. GS/MS profiles and amino acid composition of a bacteriocin produced by Bacillus methylotrophicus Strain BM47. *Food Technol. Biotechnol.* 2018, 56, 546–552. [CrossRef] [PubMed]

20. Manolov, I.; Petkova, N.; Hrusavov, D.; Hristov, D.; Ivanov, I. Isolation, Characterization and Amino Acid Composition of a Bacteriocin Produced by Bacillus methylotrophicus Strain BM47. *Food Technol. Biotechnol.* 2018, 56, 546–552. [CrossRef] [PubMed]

21. Seljåsen, R.; Slimestad, R. Fructooligosaccharides and phenolics in flesh and peel of spring harvested helianthus tuberosus. *Acta Hort.* 2007, 744, 447–450. [CrossRef]

22. Petru, G.S.; Muste, S.; Muresan, C.; Păucean, A.; Mureșan, A.E.; Nagy, M. Chemical Profiles and Antioxidant Activity of Black Elder (*Sambucus nigra* L.)—A Review. *Bull. Univ. Agric. Sci. Veter.-Med. Cluj-Napoca. Sci. Phytopharma.* 2017, 118, 9–16. [CrossRef]

23. Sadilova, V.; Stintzing, F.C.; Kammerer, D.R.; Carle, R. Matrix dependent impact of sugar and ascorbic acid addition on color and anthocyanin stability of apple, elderberry and strawberry single strength and from concentrate juices upon thermal treatment. *J. Food Res. Treat.* 2009, 42, 1023–1033. [CrossRef]

24. Magwaza, L.S.; Opara, U.L. Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Sci. Hortic.* 2015, 184, 179–192. [CrossRef]

25. Tumbarski, Y.; Deseva, I.; Mihaylova, D.; Stoyanova, M.; Krastev, L.; Nikolova, R.; Yanakieva, V.; Ivanov, I. Isolation, Characterization and Antioxidative Properties of Fruits and Flowers of Different Cultivars and Wildly Growing Plants of *Sambucus nigra* L. *Molecules* 2019, 24, 2674. [CrossRef] [PubMed]

26. Paparozzi, E.T.; Meyer, G.E.; Schlegel, V.; Blankenship, E.E.; Adams, S.A.; Conley, M.E.; Loseke, B.; Read, P. Strawberry cultivars vary in productivity, sugars and phytonutrient content when grown in a greenhouse during the winter. *Sci. Hortic.* 2017, 212, 546–550. [CrossRef] [PubMed]

27. Benzie, I.F.; Strain, J.J. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* 1999, 269, 70–76. [CrossRef] [PubMed]

28. Magwaza, L.S.; Opara, U.L. Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Acta Hortic.* 2015, 184, 179–192. [CrossRef]

29. Sambucus nigra L. as a functional ingredient for food and pharmaceutical industry. *J. Funct. Foods* 2018, 40, 377–390. [CrossRef] [PubMed]

30. Mikulic-Petkovsek, M.; Ivancic, A.; Schmitzer, V.; Veberic, R.; Stampar, F. Comparison of Major Taste Compounds and Antioxidative Properties of Fruits and Flowers of Different *Sambucus Species* and Interspecific Hybrids. *Food Chem.* 2016, 200, 134–140. [CrossRef] [PubMed]

31. Petrov, A.; Popova, A.; Desseva, I.; Manolov, I.; Petkova, N.; Vrancheva, R.; Peltekov, A.; Slavov, A.; Zhivondov, A. Comprehensive Evaluation of Late Season Peach Varieties (*Prunus persica* L.): Fruit Nutritional Quality and Phytochemicals. *Molecules* 2021, 26, 2818. [CrossRef]

32. Akšić, M.F.; Tosti, T.; Sredojević, M.; Milivojević, J.; Meland, M.; Natić, M. Comparison of Sugar Profile between Leaves and Fruits of Blueberry and Strawberry Cultivars Grown in Organic and Integrated Production System. *Plants* 2019, 8, 205. [CrossRef] [PubMed]

33. Mikulic-Petkovsek, M.; Ivanic, A.; Schmitter, V.; Veberic, R.; Stampar, F. Comparison of Major Taste Compounds and Antioxidative Properties of Fruits and Flowers of Different *Sambucus Species* and Interspecific Hybrids. *Food Chem.* 2016, 200, 134–140. [CrossRef] [PubMed]

34. Sandrine, S.; Ferreira, M.; Silva, A.M.; Nunes, F.M.; Sambucussnigra, L. Fruits and Flowers: Chemical Composition and Related Bioactivities. *Food Rev. Int.* 2020, 7, 1–29. [CrossRef]

35. Paparozzi, E.T.; Meyer, G.E.; Schlegel, V.; Blankenship, E.E.; Adams, S.A.; Conley, M.E.; Loseke, B.; Read, P. Strawberry cultivars vary in productivity, sugars and phytonutrient content when grown in a greenhouse during the winter. *Sci. Hortic.* 2018, 227, 1–9. [CrossRef]

36. Vulić, J.; Vračar, L.O.; Šumić, Z.M. Chemical Characteristics of Cultivated Elderberry Fruit. *Acta Period. Technol.* 2008, 39, 85–90. [CrossRef]

37. Džugan, M.; Pizović, A.; Tomczyk, M.; Kapusta, I. A New Black Elderberry Dye Enriched in Antioxidants Designed for Healthy Sweets Production. *Antioxidants* 2019, 8, 257. [CrossRef]

38. Ruiz-Rodriguez, B.M.; de Ancos, B.; Sanchez-Moreno, C.; Fernandez-Ruiz, V.; de Cortes Sanchez-Mata, M.; Camara, M.; Tardio, J. Wild blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) fruits as valuable sources of antioxidants. *Fruits* 2014, 69, 61–73.