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Agriculture and Environment

Influence of the extraction method on phytochemicals content and antioxidant activity of *Sambucus nigra* flowers

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(Manuscript received 27 June 2022; accepted for publication 26 October 2023)

Abstract. *Sambucus nigra* L. (elder) is one of the most common plant species in Europe, widely used for its health and healing properties and with a long botanical history. In the present study, elderberry flowers were subjected to different extraction methods (infusion, decoction, microwave- and ultrasound- assisted extraction) and their phytochemical content and antioxidant potential were evaluated. As a result, the total phenolic content in the extracts varied between 34.21 ± 0.42 and 47.46 ± 1.87 mg GAE/g dw and the total flavonoid content was found to be in the range of 11.69 ± 0.16 and 16.18 ± 0.23 mg QE/g dw. The highest values were reported for decoction. Organic acid, phenolic acid and sugar profiles of content were evaluated and compared. Extraction method had a profound effect on the content of sugars, phenolic and organic acids extractability from the plant matter, decoction being the most efficient extraction method. In addition, the correlation between the analyses was studied, outlining the contribution of the contained phytochemicals. In conclusion, aqueous extracts of elderflowers can be considered a promising source of natural antioxidants and should be further investigated for the specific profile of phytochemicals present and promoted for consumption.

Keywords: antioxidant activity, phenolic and flavonoid content, elder flowers, green extraction, phytochemicals

Introduction

The consumption of plants dates back to ancient times. People use plants for different aspects – food, medicine, building materials, clothing, furniture, shelter, pollution control, etc. Without a doubt, one globally significant use is to enhance human wellbeing through food additives. One of the most important and worldwide distributed aspect of use is for improvement of the wellbeing of people as a food additive. Furthermore, the World Health Organization (WHO) strategy, 2014–2023, aims to strengthen the role of traditional medicine, emphasizing the importance of

promoting and integrating the use of medicinal plants in the health systems of Member Countries (WHO, 2013).

Recently, the flowers of *Sambucus nigra* L. (elder) have garnered increased attention. *Sambucus* flowers are used to flavor wine and to make tea and nonalcoholic cordial (Charlebois et al., 2010). Among the various parts of *S. nigra*, only the flowers and fruits are considered medicinal. Specifically, elder flowers are approved by the German commission E for colds (Blumenthal et al., 2000). Meanwhile, fruits, leaves and bark are not approved by the WHO, ESCOP and the German Commission E (Ulbricht et al., 2014) possibly due to the potentially toxic cyanogenic

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glycoside sambunigrin present in the bark, leaves, seeds and immature fruits. The amount of cyanogenic glycoside depends on the growing conditions and the flowers have a lower cyanogenic glycoside content than other parts of the plant (Senica et al., 2017).

The flowers of the plant contain a large number of bioactive compounds, including proteins, vitamins, minerals, terpenes, sterols and polyphenols (Loizzo et al., 2015; Thanh et al., 2017; Petkova et al., 2021; Gentscheva et al., 2022). The chemical constituents determine the biological properties of *S. nigra*. For example, polyphenolic compounds and organic acids, known for their antioxidant activity, are the dominant classes of phytochemicals that characterize the plant (Tundis et al., 2019). Ferreira et al. (2020) reviewed the reported health-promoting effects of elderberries and elderflowers in the literature, and summarized the following beneficial activities towards cardiovascular, inflammatory diseases, and cancer. In addition, antioxidant, anti-inflammatory, immune-stimulating, chemo-preventive, and atheroprotective effects were also cited. A recent study claimed analgetic and diuretic, anti-inflammatory and anti-tumor effects of 1% water extracts of *Sambucus nigra* L. fruits and flowers (Ignatov et al., 2021).

Many research papers are released each year proving the potential of the chemical compounds found in the studied samples as health beneficial. Complicated extraction techniques are often applied. More research on practical extraction techniques is essential to make science more accessible in everyday life. Moreover, in the last decades there is an increasing demand for new green and non-conventional methods to shorten the extraction time, reduce organic solvent consumption, and create a selective recovery. In recent years, the number of research papers using water as a green extraction solvent has increased significantly due to its non-toxic nature for both health and environment (Castro-Puyana et al., 2017; Mihaylova and Lante, 2019; Avanza et al., 2021).

In this context, the aim of this paper is not only to evaluate the phytochemical profile and antioxidant activity of *Sambucus nigra* L. flowers, but also to give a clear methodology for easy-to-prepare biologically active extracts.

Material and methods

Plant material

All reagents used in this study were of analytical grade and purchased from Merck Chemicals (Germany) and Sigma-Aldrich (Germany).

Sample preparation

Flowers of *Sambucus nigra* L. from Bulgaria were subjected to extraction and analyses. The plant material was obtained from a local shop in fragmented and dry condition. The flowers were further dried to constant weight by drying in a vacuum-oven at 60°C. They were subsequently ground using electric grinder (Moulinex, France) to obtain a fine powder and stored at ambient temperature in air-tight containers before extraction.

The following extraction procedures were conducted:

- The infusion was prepared as follows: 5 g of plant material was combined with 100 ml of boiling water and allowed to cool (Inf).

- The decoction was prepared as follows: 3 g of plant material was combined with 60 ml of water, which were brought to a boil at heat-reflux for 30 minutes (Dec).

- Microwave-assisted extract was prepared as follows: 3 g of plant material was mixed with 60 ml of water and put in a microwave oven (LG MB4047C) for 80 s, at 800 W output power (Mic).

- Ultrasound-assisted extract was prepared when mixing 3 g of plant material with 60 ml of water and extracted on ultrasonic bath (UST 5.7150 Siel, Gabrovo, Bulgaria) at a frequency of 35 kHz with a maximum input power of 240 W, for 30 min, at 50°C (Ult).

All the extracts were then filtered and stored at 4°C without adding any preservatives until analyses.

Total polyphenol content analysis (TPC)

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala et al. (2000). The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight (dw).

Total flavonoid content (TFC)

The total flavonoid content was evaluated according to the method described by Kivrak et al. (2009). Quercetin was used as a standard and the results were expressed as mg quercetin equivalent (QE)/g dw.

Identification and quantification of sugars content

HPLC determination of sugars was performed on Agilent 1220 HPLC system (Agilent Technology, USA), equipped with binary pump and Refractive Index Detector. Separation was performed using Aminex HPX – 87H column (300 × 7.8 mm, BioRad), eluent 4 mM H₂SO₄, flow 0.5 ml/min, temperature 25°C. The standard compounds (glucose, fructose, galactose, rhamnose, arabinose, sucrose, maltose, cellobiose and sorbitol) were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as mg/g dw.

Identification and quantification of organic acids

HPLC determination of organic acids was performed on Agilent 1220 HPLC system (Agilent Technology, USA), equipped with binary pump and UV-Vis detector. Wavelength of 210 nm was used. Organic acid separation was performed using Agilent TC-C18 column (5 µm, 4.6 mm × 250 mm) at 25°C. An isocratic elution with 25 mM phosphate (K₂HPO₄/H₃PO₄) buffer (pH 2.4), flowing at 0.8 ml/min was used (Ognyanov et al., 2022). Quinic acid, malic acid, ascorbic acid, shikimic acid, citric acid, α-ketoglutaric acid, oxalic acid, succinic acid and tartaric acid used as standards were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as mg/g dw.

Identification and quantification of phenolic acids

The qualitative and quantitative determination of phenolic acids in the extracts was performed by using a Hitachi LaChrom Elite® HPLC System (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA), coupled with diode-array detector (DAD, L-2455) and EZChrom Elite™ software. Separation of the phenolic acids was performed by a Supelco Discovery HS C18 column (5 µm, 25 cm × 4.6 mm), operated at 30°C under gradient conditions with mobile phase consisting of 2% (v/v) acetic acid (solvent A) and acetonitrile (solvent B), as reported by Mihaylova et al. (2021 a, b). The gradient program used was: 0–1 min: 95% A and 5% B; 1–40 min: 50% A and 50% B; 40–45 min: 100% B; 46–50 min: 95% A and 5% B. The detection of phenolic acids was carried out at 280 nm for gallic, protocatechuic, and cinnamic acids and at 320 nm for chlorogenic, caffeic, ferulic, p-coumaric, sinapic, rosmarinic, and chicoric acids at a flow rate of 0.8 ml/min. The results were expressed in mg/g dw.

Antioxidant activity (AOA)

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams et al. (1995) (Mihaylova et al., 2021 a, b). The DPPH radical scavenging activity was presented as a function of the concentration of Trolox having equivalent AOA expressed as the µmol (µM) Trolox per g dw.

ABTS radical cation decolorization assay

The radicals scavenging activity of the extracts against radical cation (ABTS^{•+}) was estimated according to a previously reported procedure with some modifications (Re et al., 1999). The results were expressed as µM TE/g dw.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain (1999). The results were expressed as µM TE/g dw.

Cupric ion reducing antioxidant capacity assay (CUPRAC)

CUPRAC assay was performed according to the method of Apak et al. (2004). The results were expressed as µM TE/g dw.

Oxygen radical absorbance capacity assay (ORAC)

The method developed by Ou et al. (2002) was used with some modifications. The results were expressed as µM TE/g dw. The excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

Statistical analysis

Analytical determinations were performed in triplicate and the results were expressed as mean ± SD (MS Excel 2017 software). Statistical analysis of the data was carried out using one-way ANOVA. The Tukey-Kramer post-hoc test (α=0.05) was employed as described by Assaad et al. (2014).

Results and discussion

The present study focuses on phytochemical composition and antioxidant potential of *S. nigra* flowers. Four extraction techniques were chosen based on their daily applicability. Water was chosen as the solvent to meet the green concept of reducing environmental impact. Some authors also focused their research on an environmentally friendly approach to replace ethanol for the extraction of *S. nigra* plant polyphenols (Vladimir-Knežević et al., 2022) when using green solvents, including deep eutectic solvents achieving similar efficiency.

The content and composition of soluble sugars in the plant foods and medicinal plants are important for their nutritional value and palatability. Glucose and fructose are the simplest forms of sugar that can be absorbed into the bloodstream. These sugars are naturally present in foods such as grains, fruits and vegetables. They are also the major ingredients in many sweeteners and processed foods. With regard to the nutritional and health perspective, fruit with increased fructose levels should be favored by the consumers as this sugar tastes sweeter than glucose or sucrose and has a much lower glycemic index compared to other analyzed sugars (Atkinson et al., 2008).

In general, several sugars were found in all extracts, fructose and glucose being the predominant ones (Table 1). It is evident from the results that extraction method has a profound effect on sugar extractability from *S. nigra* flowers. Decoction was the most efficient method for sugar analysis, extracting the highest amounts of glucose, rhamnose, arabinose and sucrose and cellobiose/maltose. Respectively, the highest amount of total sugars was extracted by this method (61.76 mg/g dw). Gentscheva et al. (2022) reported total sugars content in *S. nigra* flowers of 2.55 ± 0.04 g/100g with the presence

of glucose, fructose and sucrose as glucose was the predominant. Kan (2019) reported fructose as the main sugar compound followed by glucose in black elderberry genotypes fruits. Moreover, Kan recommended elderberry fruit as beneficial supplement to the human diet based on the lower levels of total sugars than other popular fruits as apples and sweet cherry (Kan, 2019).

Sucrose was also detected in the *S. nigra* extracts, ranging from 4.39 ± 0.37 to 9.23 ± 0.59 mg/g dw. Its content is comparable to glucose and significantly lower than fructose concentration.

Table 1. Sugar composition (mg/g dw) of *S. nigra* flower extracts, obtained by different extraction methods*

Sample/compound	Fructose	Glucose	Galactose	Rhamnose	Arabinose	Sucrose	Cellobiose/Maltose	Total
Dec	17.91 ± 0.77^a	9.19 ± 0.39^a	4.30 ± 0.18^a	6.91 ± 0.38^a	7.80 ± 0.39^a	6.05 ± 0.51^b	9.60 ± 0.49^a	61.76
Mic	15.35 ± 0.67^b	4.73 ± 0.37^c	3.89 ± 0.22^a	6.68 ± 0.40^a	4.60 ± 0.35^b	9.23 ± 0.59^a	8.21 ± 0.40^b	52.69
Inf	14.41 ± 0.88^b	7.51 ± 0.45^b	4.01 ± 0.23^a	4.14 ± 0.36^b	5.41 ± 0.23^b	4.39 ± 0.37^c	5.27 ± 0.31^d	46.14
Ult	18.14 ± 1.30^a	7.22 ± 0.43^b	4.29 ± 0.21^a	4.83 ± 0.35^b	4.28 ± 0.26^b	3.03 ± 0.22^d	6.12 ± 0.37^c	47.92

* Values are means \pm SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the TUKEY test.

The amount of organic acids is another important parameter for medicinal plants and foodstuffs. It is known that many of them reveal health-promoting effects. The content and composition of organic acids in the obtained extracts are shown in Table 2. Similarly, as with sugar content, decoction was the most efficient method for organic acids extraction. There was no difference in ascorbic acid extractability via the different methods used. The total amount of the extracted organic acids by decoction reached

42.00 mg/g, which was 10% more than the second most efficient method - microwave-assisted extraction. The most predominant were quinic and citric acids. In elderberry berries citric, malic, shikimic, and fumaric were the most abundant organic acids reported (Mocanu and Amariei, 2022). According to Uzlasir et al. (2020), elderflowers are rich in malic acid as the major organic acid present, and in smaller amounts, elderflowers also contain citric acid, tartaric acid, shikimic acid, and fumaric acid.

Table 2. Organic acids content (mg/g dw) of *S. nigra* flower extracts, obtained by different extraction methods*

Sample/compound	Quinic	Malic	Ascorbic	Citric	Oxalic	Succinic	Total
Dec	11.66 ± 0.21^a	10.93 ± 0.40^a	2.47 ± 0.20^a	11.25 ± 0.40^a	2.82 ± 0.17^a	2.86 ± 0.17^a	42.00
Mic	6.31 ± 0.29^b	8.65 ± 0.29^b	2.01 ± 0.19^a	12.25 ± 0.61^a	3.13 ± 0.17^a	2.44 ± 0.14^a	37.80
Inf	11.16 ± 0.30^a	8.67 ± 0.32^b	2.21 ± 0.11^a	9.57 ± 0.25^b	2.53 ± 0.11^a	1.31 ± 0.09^b	35.45
Ult	11.47 ± 0.17^a	8.85 ± 0.22^b	2.34 ± 0.13^a	9.92 ± 0.39^b	2.98 ± 0.20^a	1.08 ± 0.10^c	36.64

* Values are means \pm SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the TUKEY test.

Various pharmacological activities inherent to medicinal plants have been attributed to their phenolic composition which is why it is not surprising that the consumption of plant infusions for prevention and treatment of health disorders is a worldwide practice (Marques and Farah, 2009). Figure 1 presents the results for the total phenolic and total flavonoid content of the studied extracts. TPC ranged between 34.21 ± 0.42 and 47.46 ± 1.87 mg GAE/g dw with the highest being found for decoction and the lowest for ultrasound extract. Infusion and microwave

extracts did not differ significantly with respect to TPC ($p < 0.05$). The results correspond well with the established highest total phenolic content of water extract at the highest temperature (90°C) – more than 45 mg CAE/g (Ferreira-Santos et al., 2021). Viapiana and Wesolowski (2017), on other hand, reported TPC of elderflowers infusions in the range from 15.23 to 35.57 mg GAE/g dw, which is lower compared to the established for infusions in the present study (41.74 ± 0.73 mgGAE/g dw), but higher when compared to elderberries. These results are

consistent with other previous studies, indicating that high temperatures tended to improve the recovery of phenolic compounds and increase their content in the final extract (Dawidowicz et al., 2006). The reason for that may be the thermal destruction of cell walls and subcellular compartments during heating, favoring the release of these compounds (Juániz et al., 2016). Viapiana and Wesolowski (2017) reported that the TPC of infusions from thirteen *S. nigra* flowers ranged from 15.2 to 35.6 mg CAE/g flower (and flavonoids content from 5.27 to 13.19 mg rutin equivalent/g). The results comparison revealed the influence of the plant material origin, pretreatment and the methods of extraction. The differences in chemical composition are due to the period and method of harvesting the plant (Petrisor et al., 2022).

Flavonoids are a subset of phenolic compounds, secondary metabolites, present in plants. The biological effects of many plants are due to these compounds (Cai et al., 2004). In our study, the trend for total flavonoid content of the samples followed the one for the TPC. The

highest values TFC were established for the decoction (16.18 ± 0.23 mg QE/g dw) and the lowest ones for the ultrasound (11.69 ± 0.16 mg QE/g dw) extract. A possible influence to the better flavonoid content in decoction could be the higher temperature applied when decoction is performed and due to the highest phenolic compounds in the extract itself, as well. In contrast, Gentscheva et al. (2022) reported TFC values between 6.4 ± 0.5 and 18.6 ± 0.5 mg QE/g dw for *S. nigra* blossoms from different regions in Bulgaria extracted by ultrasound at 40 kHz at 40°C for 20 min. Differences in TFC values indicate the influence of the plant material origin and the parameters of the extraction procedure. In order to obtain the desired components, the extraction procedure is a crucial step. In this regard, there are several factors that might affect extraction process, such as matrix properties (plant part), temperature, pH, pressure and time (Ferreira-Santos et al., 2020). However, the ultrasound water extraction resulted in quite similar TFC values between the two studies.

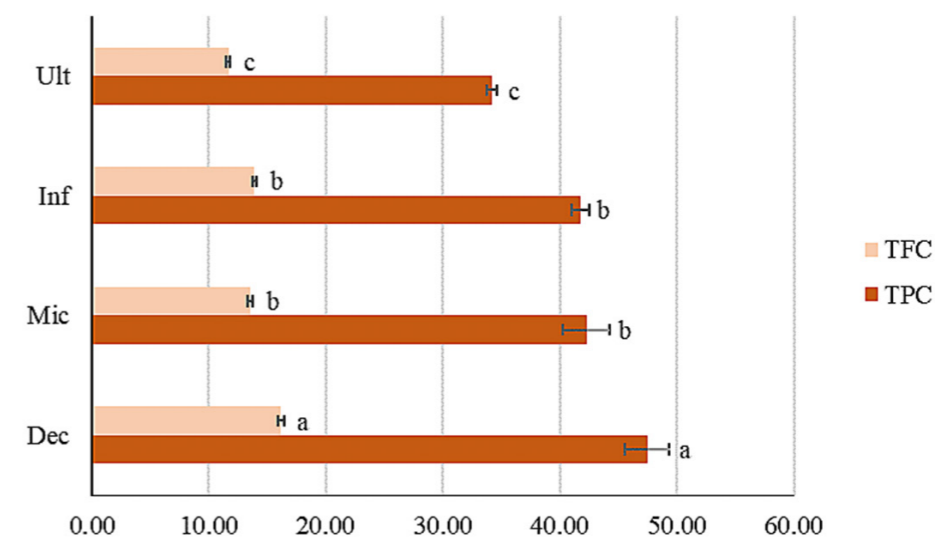


Figure 1. Total phenolic content (mg GAE/g dw) and total flavonoid content (mg QE/g dw) of *S. nigra* flower extracts, obtained by different extraction methods*

* Values are means \pm SEM, n = 3 per treatment group. Means in a chart columns without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the TUKEY test. Ult – Ultrasound extract; Inf – Infusion; Mic- Microwave extract; Dec – decoction.

The total content of phenolic acids found varied between 18.62 and 31.52 mg/g dw, with chlorogenic acid making the largest contribution (Table 3). Decoction of elderflower seems to be the most suitable method for extraction in this case. Our results are in agreement with the ones of Radojković et al. (2021) and Ferreira-Santos et al. (2021) that also found that chlorogenic acid, followed by ferulic acid, was the predominant phenolic acid in all studied extracts. Przybylska-Balcerek et al., 2021

reported sinapic acid together with chlorogenic acid in elderberry to prevail.

Chlorogenic acid is considered one of the most common beneficial polyphenols in plants and is well known as a dietary antioxidant in plant-based foods (Kundu and Vadassery, 2019). Importantly, phenolic substances, such as chlorogenic acid, do not influence the metabolism of probiotic bacteria, making them even more suitable for the food industry (Chang et al., 2006).

Table 3. Phenolic acids content (mg/g dw) of *S. nigra* flower extracts, obtained by different extraction methods*

Sample/Compound	Protocatechuic acid	Chlorogenic acid	Caffeic acid	Ferulic acid	p-Coumaric acid	Sinapic acid	Cinnamic acid	Total
Dec	3.59±0.06 ^b	18.89±0.07 ^a	0.98±0.00 ^a	5.29±0.02 ^a	1.03±0.01 ^a	1.72±0.00 ^a	0.02±0.00 ^a	31.52
Mic	3.08±0.05 ^b	11.51±0.06 ^c	0.36±0.00 ^d	1.57±0.01 ^d	0.47±0.00 ^d	1.61±0.01 ^b	0.02±0.00 ^a	18.62
Inf	3.02±0.01 ^b	14.96±0.05 ^b	0.86±0.00 ^c	4.29±0.01 ^c	0.87±0.00 ^b	1.20±0.01 ^c	0.02±0.00 ^a	25.22
Ult	2.19±0.01 ^c	12.04±0.03 ^c	0.91±0.00 ^b	4.93±0.02 ^b	0.75±0.00 ^c	0.73±0.00 ^d	0.01±0.00 ^b	21.56

* Values are means ± SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the TUKEY test.

Antioxidant potential is an important and crucial parameter for determining the health benefits of food products. Various compounds were reported to contribute to the antioxidant potential of plants and *Sambucus* sp. in particular (Anton et al., 2013). Water was described as one of the best solvents to produce *S. nigra* extracts with high free radical scavenging activity by Duymuş et al. (2014). Gentscheva et al. (2022) reported similar results as regards the antioxidant potential of *S. nigra* flowers water ultrasound-assisted extract. Following the phytochemical content, the antioxidant potential of *S. nigra* flowers water extracts was assessed (Table 4). In order to more comprehensively assess the antioxidant potential of the samples, five methods based on the two main mechanisms of in vitro analysis were applied, namely hydrogen atom transfer methods, which donate a hydrogen ion from a stable molecule, thus allowing an antioxidant to clear reactive oxygen species (ABTS, ORAC), and single electron transfer, which depends on the potential of the antioxidant to reduce certain molecules and compounds by electron transfer (FRAP, DPPH, CUPRAC) (Sidleeg et al., 2021).

According to FRAP analysis, the antioxidant potential of the *S. nigra* flowers extracts ranged from 250.00 ± 2.77 to 392.41 ± 28.13 µM TE/g dw. Decoction extracts displayed the highest antioxidant potential. The results of the CUPRAC assay were again in favor of decoction (459.92 ± 1.93 µM TE/g dw), indicating the efficacy of this type of technique and the potential of such extracts.

The AOA toward the synthetic DPPH-radical confirmed that decoction was the most active extract of *S. nigra* flowers. Ferreira-Santos et al. (2021) demonstrated the influence of temperature on the antioxidant capacity of *S. nigra* extracts and reported activity toward DPPH free radical around 0.15 mM TE/g dw of water extracts under different temperatures. When tested, different extraction

methods affected (extraction with organic solvent and water by heat or ultrasound) the phytochemical profile and antioxidant activity of other edible flowers (*Clitoria ternatea*) Jeyaraj et al. (2021) found to have equally potent antioxidant activity according to the DPPH analysis for both extracts.

Several studies have correlated total phenolic compounds with antioxidant potential (Piluzza and Bullitta, 2011; Ahmad et al., 2017; Mrduljaš et al., 2017; Santos et al., 2020). In this regard, Gonçalves et al. (2013) established high relationship between the antioxidant activity (DPPH assay) and TPC of infusions prepared with Mediterranean medicinal plants. Not surprisingly, the highest AOA value in the present study according to the DPPH-assay was established for the highest in TPC, the decoction.

As regards the other synthetic free radical ABTS, the values were between 127.99 ± 0.40 and 159.74 ± 1.07 µM TE/g dw in prevalence for decoction again.

ORAC assay followed a similar trend and decoction was the most potential extract with 1337.78 ± 23.34 µM TE/g dw. The unanimity of the five conducted antioxidant analyses outlines the highest potential of decoction and generally demonstrates that *S. nigra* flowers are an easily accessible source of antioxidant compounds.

All this allows to highlight the decoction approach as the most suitable for using water as the extractant.

Several authors have successfully correlated the antioxidant activity and phenolic compounds as the latter are molecules with good scavenging activity and metal chelators, due to the hydroxyl groups in their structure (Santos et al., 2020; Kim and Lee, 2020; Mufflihah, 2021).

The results of this study outlined the *S. nigra* water extracts as potential dietary source of bioactive phenolic compounds that contribute to healthy nutrition and improving life quality.

Table 4. Antioxidant activity according to FRAP, CUPRAC, DPPH, ABTS and ORAC assays (µM TE/g dw) of *S. nigra* flower extracts, obtained by different extraction methods*

Sample/Assay	FRAP	CUPRAC	DPPH	ABTS	ORAC
Dec	392.41±28.13 ^a	459.92±1.93 ^a	270.53±3.63 ^a	159.74±1.07 ^a	1337.78±23.34 ^a
Mic	312.05±1.88 ^c	416.64±4.86 ^c	233.24±7.13 ^b	153.45±0.53 ^b	1270.02±20.12 ^b
Inf	322.93±1.28 ^b	437.12±1.11 ^b	233.83±4.96 ^b	148.85±1.24 ^c	936.74±10.16 ^d
Ult	250.00±2.77 ^d	325.71±4.02 ^d	179.65±5.67 ^c	127.99±0.40 ^d	1068.22±15.34 ^c

* Values are means ± SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the TUKEY test.

Conclusion

The present study highlights the potential of *Sambucus nigra* flowers as a rich source of valuable phytochemicals. For this reason, four extraction methods that are easily reproduced were tested. Water was used as solvent. Sugars, phytochemicals (including phenolic acids, TPC, and TFC) were subsequently analyzed, along with the resulting antioxidant activity. Among all the tested methods, decoction proved to be the most efficient. Therefore, this study underscores the potential of aqueous extracts from elderflowers as beneficial daily phytonutrients and outlines the need for further research to reveal the phytochemical profile of the extracts and to promote the consumption.

Acknowledgment

This research was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577/17.08.2018.

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