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Original Article

Antioxidant Properties and Antibacterial Activity of Water Extracts from *Sambucus Nigra L.* under Different Conditions

Iliana Milkova-Tomova[1], Zornica Kazakova[1], Dragomira Buhalova[1], Galia Gentscheva[2], Krastena Nikolova[3], Stefka Minkova[3]

[1] *Department of Catering and Tourism, University of Food Technologies, Plovdiv, Bulgaria*

[2] *Department of Chemistry and Biochemistry, Medical University, Pleven, Bulgaria*

[3] *Department of Physics and Biophysics, Medical University, Varna, Bulgaria*

Corresponding author: Krastena Nikolova, Department of Physics and Biophysics, Medical University, Varna, Bulgaria; Email: Krastena.Nikolova@mu-varna.bg

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Abstract

Introduction: In folk medicine, dried white flowers of *Sambucus nigra L.* are used to make infusions, decoctions, and juices.

Aim: The present article aims to study and compare the antioxidant activity of aqueous solutions of leaves and flowers of *Sambucus nigra L* obtained at different exposure times and assess the antibacterial activity of these solutions against *Escherichia coli ATCC 8739, Salmonella NCTC 6017, Listeria monocytogenes NCTC 11994,* and *Staphylococcus aureus ATCC 25093.*

Materials and methods: We studied the physicochemical properties of aqueous extracts of leaves (fresh) and flowers (fresh and dry) of *Sambucus nigra L* collected from the Rhodope region of Bulgaria. The samples from *Sambucus nigra L* were analyzed to determine their total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The diameters (in millimeters) of the growth inhibition zones of four pathogens were measured, and a comparative assessment of their antibacterial activity was made.

Results: The infusions of fresh blossoms and fresh leaves of *Sambucus nigra* L had the highest antioxidant activity at the total contact time of 30 minutes (82.7 mmol TE/100 ml) and 35 minutes (36.5 mmol TE/100 ml), respectively. The phenol-richest infusions were those made from dried flowers of *Sambucus nigra L* after a 30-minute contact time (86.7 mg GAE/ml). Of the four pathogens we studied, we found that the extracts affected partially only the pathogenic bacteria of *Salmonella*.

Conclusions: The highest content of bioactive components was obtained from dried blossoms of *Sambucus ni*gra L. for infusions with a total contact time of 30 minutes and for decoctions at a contact time of 45 minutes.

Keywords

antioxidant activity, antibacterial activity, decoctions, flavonoids, infusion, *Sambucus nigra L*

INTRODUCTION

Black elderberry is a member of the family *Caprifoliaceae Vent,* genus *Sambucus*. The genus has about forty species, but the fruits of only three (*Sambucus nigra L, Sambucus Canadensis Hasse, and Sambucus cerulean Ral.*) are edible. On the Balkan Peninsula, one of the most common plants is the black elderberry (*Sambucus nigra L.*). Folk medicine uses all parts of this plant. Its flower, bark, leaves, and fruits are high in carbohydrates, lipids, terpenoids, flavonoids, phenolic acids, alkaloids, and other compounds.[1] Dried white flowers of *Sambucus nigra L* are used for preparation of infusions, decoctions, and juices. Water extracts and decoctions of the flowers are recommended to use to relieve the symptoms of colds, runny nose, sore throat, cough, inflammation of the urinary tract, and some more.[2-4] The water-soluble substances contained in the blossoms of *Sambucus nigra L* can directly induce insulin secretion and increase glucose metabolism.[5,6] Elder blossoms also have antimicrobial activity.[7] Standardized plant fruit extracts inhibit the reproduction of influenza B virus[8] and influenza A virus[9]. Ethanolic extracts of Sambucus nigra L. blooms and fruits have been shown to inhibit 13 pathogens, including *Staphylococcus sp*., *Bacillus cereus*, *Salmonella poona*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[10]

Water extracts are frequently used in a variety of food, cosmetic[3], and pharmaceutical products[11,12] due to their high concentration of biologically active components.

The majority *of Sambucus nigra L* research is focused on the preparation of extracts using hydrophobic solvents, with only a few studies focusing on water extracts.

AIM

The present study aimed to study and compare (I) the antioxidant activity of water extracts of leaves and flowers of *Sambucus nigra L* obtained at different exposure times and temperatures about 100°C and (II) the antibacterial effect of these water extracts on *Escherichia coli ATCC 8739, Salmonella NCTC 6017, Listeria monocytogenes NCTC 11994,* and *Staphylococcus aureus ATCC 25093.*

MATERIALS AND METHODS

Samples

Black elder (*Sambucus nigra* L.) blossoms and leaves were taken from the ground at an altitude of 600–900 m in the Rhodope region during peak flowering (May-June). The blossoms were shade dried for 10 days by turning.

Methods for obtaining water extracts

Infusions

A quantity of 5 g of chopped material (fresh or dry leaves or blossoms) was soaked in a porcelain volume graduated vessel of hot water (98°–100°C) at a hydromodule of 1:20 (product: water, w/v). The dish was placed in a water bath at the indicated temperature for 15 minutes. After the specified time, the vessel was taken out of the water bath, and the samples were taken at 10, 15, and 20 minutes.

Decoctions

Five grams of chopped material were boiled in water (98°–100°C) at a hydromodule of 1:20 (product: water, w/v) for 30 minutes. Then, the decoctions were left at room temperature, and the samples were taken at 10, 15, and 20 minutes.

The obtained decoctions and infusions were filtered and stored at 4°C for measurement.

Determination of total phenolic (TPC) and total flavonoid contents (TFC)

The total phenolic content of the investigated samples was determined using the method of Folin-Ciocalteu.[6] Folin-Ciocalteu reagent (1 ml) diluted five times was mixed with a 0.2-ml sample and 0.8 ml of 20% Na_2CO_3 (Sigma-Aldrich, Germany). After staying for one hour in darkness, the absorbance was measured at 750 nm. TFC was determined spectrophotometrically using $Al(NO_3)_3$ in water extracts.[18]

The DPPH radical-scavenging ability

The analyzed sample (0.15 ml) was mixed with 2.85 ml freshly prepared 0.1 mM solution of DPPH in methanol. The sample was incubated for 15 minutes at 37°C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol, and the inhibition percentage was calculated.[13]

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain[13] with slight modification. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 minutes at 37°C in darkness, and the absorbance was measured at 593 nm against blank prepared with methanol.

Determination of antibacterial activity

The strains of microorganisms (*Listeria monocytogenes NCTC 11994, Escherichia coli ATCC 8739, Salmonella*

enterica subsp., Enterica serovar Abony NCTC 6017, and *Staphylococcus aureus ATCC 25093*) were supplied by the National Bank for Industrial Microorganisms and Cell Cultures. Selective bacteriological media were used for the microbiological test, respectively: Listeria Oxford Agar Base with an additive containing cycloheximide (Biolife); ENDO agar (Merck); LEIFSON Agar (Merck); Baird Parker Agar Base (Biolife) with yolk-tellurite supplement, and Plate Mount Agar (Merck), which were inoculated with pathogen suspensions prepared from a 24-hour culture. The antibacterial activity of the water extracts was assessed. The experiments were performed by using 24-h old bacterial suspensions. The extracts were tested using sterilized metal rings 5 mm in diameter. The discs were impregnated with 15 µl of the extract, kept until dry under laminar airflow and then placed into previously inoculated Petri dishes. Subsequently, the plates were incubated for 24 hours at 37°C. Comparative assessment of their antibacterial activity was made. For this purpose, the diameters of inhibition zones of pathogen growth were measured around the metal rings.

Statistical analysis

All measurements were repeated five times. The presence of reliable variance between the types of the samples in the analyzed indicators has been determined by the two-factor analysis of variance (ANOVA) and evaluation of averages according to Duncan's method.[14]

RESULTS

The antioxidant activity measured by DPPH and FRAP methods, the total phenolic and flavonoid content of infusions and decoctions from fresh and dried blossoms or leaves were evaluated. The results are presented in **Table 1**.

The results from the antibacterial activity are presented in **Fig. 1.**

Table 1. Total phenols, flavonoids, and antioxidant activity of infusions and decoctions from leaves and blossom from *Sambucus nigra L*

Samples	**Total phenolic content**	**Total flavonoids**	**Antioxidant activity mmol TE/100 ml±SD**	
Contact time	mg GAE/ml±SD	mg QE/ml±SD	DPPH method	FRAP method
		Infusions from fresh leaves		
25 min	1.6 ± 0.1^{f}	0.7 ± 0.1^{e}	13.4 ± 1.1^{f}	12.8 ± 0.3^{f}
30 min	2.0 ± 0.1^{e}	0.7 ± 0.1^{e}	23.3 ± 0.7^{e}	16.5 ± 0.2^{e}
35 min	4.1 ± 0.1^{d}	1.4 ± 0.1^{d}	36.5 ± 1.3^{d}	34.3 ± 1.4^{d}
		Infusions from fresh blossom		
25 min	$13.5\pm0.1^{d*}$	$6.8\pm0.2^{e*}$	$64.9\pm1.3^{e*}$	$80.7\pm1.1^{d*}$
30 min	$13.0\pm0.1^{d*}$	$5.5\pm0.1^{f*}$	$82.7\pm2.1^{d*}$	$70.8\pm0.9^{e*}$
35 min	$13.0\pm0.1^{d*}$	$8.7\pm0.2^{d*}$	$64.5\pm1.3^{e*}$	$74.4\pm1.6^{e*}$
		Decoctions from fresh leaves		
40 min	22.2 ± 0.1^{c}	10.2 ± 0.1^{b}	181.3 ± 0.8^{c}	146.6 ± 0.6^{c}
45 min	36.8 ± 0.2^{b}	8.5 ± 0.1^{c}	344.6 ± 1.1^{b}	265.6 ± 0.8^{b}
50 min	55.1 ± 0.1^{a}	14.6 ± 0.1^{a}	442.9 ± 0.7^{a}	372.9 ± 0.5^{a}
		Decoctions from fresh blossom		
40 min	21.4 ± 0.1^{f}	15.7 ± 0.2^{f}	136.7 ± 0.1^{f}	133.9 ± 0.5^{f}
45 min	34.0 ± 0.1^{e}	16.8 ± 0.1^{e}	180.2 ± 0.1^{e}	165.7 ± 0.9^{e}
50 min	43.5 ± 0.2^{d}	17.1 ± 0.1^{d}	249.2 ± 1.0^{d}	234.3 ± 0.9^{d}
		Infusions from dry blossom		
25 min	$45.6\pm0.4^{c*}$	$23.3\pm0.1^{c*}$	$324.4\pm6.2^{c*}$	$286.6\pm6.3^{c*}$
30 min	$86.7\pm0.5^{a*}$	$48.2\pm0.3^{a*}$	$648\pm9.7^{a*}$	$566.9\pm5.2^{a*}$
35 min	$78.8\pm0.3^{b*}$	$46.4\pm0.3^{b*}$	$596.6\pm6.7^{b*}$	$522.2\pm5.4^{b*}$
		Decoctions from dry blossom		
40 min	129.5 ± 0.3^{c}	66.0 ± 0.1^{b}	707.5 ± 1.3^{b}	662.4 ± 1.4^{c}
45 min	164.9 ± 0.2^{a}	81.0 ± 0.2^{a}	986.2 ± 1.3^{a}	832.1 ± 1.1^{a}
50 min	134.2 ± 0.3^{b}	64.3 ± 0.1^{c}	693.3 ± 2.1^{c}	722.5 ± 0.9^{b}

a, b, c - indexes for Duncan test of infusion and decoctions from fresh leaves; level of significance 0.5

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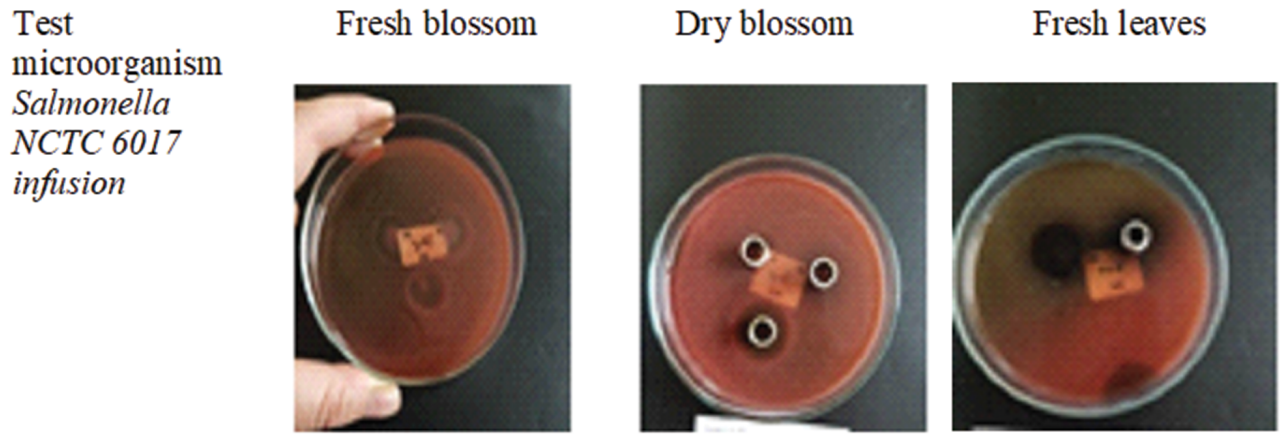

Figure 1. Zones of inhibition of the growth of pathogenic bacteria (mm) in selective media from various extracts of *Sambucus nigra L.*

DISCUSSION

All plants have different antioxidant potential which also depends on many external factors[15] such as soil type, climate, variety, wild or cultivated plants, storage, etc.[16]. Significant differences in concentrations of total phenols, flavonoids, and antioxidant activity were observed between infusions obtained from fresh leaves and blossoms depending on the time of extraction. The total phenol concentrations and antioxidant activity by both methods increased with the contact time, only the concentrations of total flavonoids required longer treatment to change their values of 0.7 mg QE/ml /25 and 30 min/twice at 35 min.

Total flavonoids in an infusion from fresh blossoms were 6 to 10 times greater than the content in the infusion from fresh leaves made under the same conditions. Dawidowicz et al. have obtained similar results.[17] There were no significant differences between the content of total flavonoids in decoctions from fresh leaves and blossoms. The highest concentrations of phenolic compounds were found in the decoctions of fresh leaves exposed to temperature for 50 minutes (55.1 mg GAE/ml), and the poorest infusions of fresh leaves at 25 minutes of treatment temperature (1.6 mg GAE/ml). The trend is similar for flavonoids in infusions and decoctions of fresh elder leaves.[18] The content of phenols in fresh blossom infusions is not affected by the duration of heat treatment. In infusions prepared from dry blossoms, the phenol content decreases with increasing time factor. The highest concentrations of phenols were found in the infusions of dried flowers of *Sambucus nigra L*, subjected to a 30-minute (86.7 mg GAE/ml) and 35-minute (78.8 mg GAE/ml) contact. Both the duration and the infusion of fresh/dry material had a proven effect on the content of flavonoids, and in the case of those of fresh flowers, they were many times lower. The greater the content of flavonoids, the higher the antioxidant ability of the extracts. The antioxidant activity determined by the DPPH method in most of the extracts of *Sambucus nigra L* was higher than that determined by the FRAP method. The antioxidant activity of fresh leaf decoctions during the total contact time of 50 minutes was 1.8 and 1.6 times higher than that of fresh flower decoctions determined by DPPH and FRAP methods, respectively. Infusions of fresh blossom *Sambucus nigra* L had the highest antioxidant activity during the total contact time of 30 minutes (82.7 mmol TE/100 ml). Fresh leaf infusions had the highest antioxidant activity at 35 minutes of total contact time (36.5 mmol TE/100 ml). Higher value of antioxidant activity determined by DPPH method for blossoms and leaves, respectively, was also reported by Dawidowicz et al.[4] The authors reported antioxidant activity of 94.15 mmol TE/100 ml for extracts from blossoms and 16.76 mmol TE/100 ml for extracts of leaves, respectively. This was most likely caused by the use of water-ethanol extracts. The change in antioxidant activity depends on the temperature, extraction time, and the raw material type. A significant difference was observed in decoctions and infusions of dry blossoms.

The study of phenols and flavones in infusions and decoctions is important because of their redox properties preventing the decomposition of hydroperoxides into free radicals.[5,19] *Sambucus nigra* L. dry blossoms infusions prepared at total contact time of 30 and 35 minutes are characterized by the highest content of total flavonoids. The trend is similar for decoctions. The total phenolic content for decoctions for dry blossoms is similar to that determined from Stoeva et al. (194.0 mg GAE/100 ml).[20] The antioxidant activity of decoctions and infusions is mainly related to the presence of flavonoids. Linear relationships are obtained between FRAP and TFC, as well as between DPPH and TFC, DPPH and TPC, FRAP and TPC with correlation coefficients greater than 0.91.

Of the four pathogens studied, only the pathogenic bacteria of *Salmonella* were partially affected by the extracts. Infusions of fresh leaves and dried and fresh flowers have an inhibitory effect, while decoctions do not show such an effect. The fresh blossom infusions with total contact times of 30 min and 35 minutes were the most effective against the Gram-negative bacteria of *Salmonella NCTC 6017* (18 mm inhibition zone, IZ). The inhibition zones of dry blossom infusion with total contact times of 25 and 30 minutes were quite similar (10 mm IZ), but the largest inhibition zone was found for the dry blossom infusions with a

35-min total contact time (22 mm). The inhibition zones of fresh leaves infusion with 35 minutes and 30 minutes had the same size (10 mm IZ). On the other hand, the decoctions of blossoms or leaves did not inhibit the test cultures.

Fig. 1 illustrates the growth inhibition zones of *Salmonella* from *Sambucus nigra L.* extracts. Similar results are reported by Hearst et al.[10] In this study, the aqueous extracts from *Sambucus nigra L.* demonstrated a notable inhibition of *Salmonella* (7 mm). It is known that the activity on the main components of aromatic products (essential oils, extracts) is arranged in the following sequence: phenols > alcohols > aldehydes > ketones > esthers > hydrocarbons.[21] It can be concluded that the antimicrobial activity of different part of the plants is influenced by the chemical composition of these plant parts and the conditions under which the extracts were obtained.

We also determined the total number of mesophilic aerobic and facultative anaerobic microorganisms, molds, and yeasts in fresh flowers and elder leaves. It was found that the total number of microorganisms in fresh flowers (8.4×10^5 CFU/g) was 2.6 times higher than that in fresh leaves (3.2×10^5 CFU/g). The content of mold and yeast in the leaves (2.5×10^5 CFU/g) was significantly higher than that in the flowers (5.3×10^4 CFU/g).

CONCLUSIONS

The highest content of bioactive components was obtained from as follows:

- from fresh leaves and fresh blossoms - infusions at 35 min and decoctions at 50 min of contact time
- from dry blossoms - infusions at 30 minutes and decoctions at 45 minutes of treatment

The extracts of *Sambucus nigra L. s*how antibacterial activity against the pathogenic bacteria of *Salmonella.* The results obtained for antioxidant activity, total phenolic content and total flavonoids can be used to select parts of the plant (leaves, blossoms) and method of preparation (infusion, decoction) depending on the desired application.

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Антиоксидантныесвойстваиантибактериальнаяактивность водных экстрактов *Sambucus Nigra L.* в различных условиях

Илиана Милкова-Томова[1], Зорница Казакова[1], Драгомира Бухалова[1], Галя Генчева[2], Крастена Николова[3], Стефка Минкова[3]

[1] *Кафедра „Общественное питание и туризм", Университет пищевых технологий, Пловдив, Болгария*

[2] *Кафедра химии и биохимии, Медицинский университет – Плевен, Плевен, Болгария*

[3] *Кафедра физики и биофизики, Медицинский университет – Варна, Варна, Болгария*

Адрес для корреспонденции: Крастена Николова, Кафедра физики и биофизики, Медицинский университет – Варна, Варна, Болгария; E-mail: Krastena.Nikolova@mu-varna.bg

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Резюме

Введение: В народной медицине сушёные белые цветки *Sambucus nigra L.* используют для приготовления настоев, отваров и соков.

Цель: Настоящая статья посвящена изучению и сравнению антиоксидантной активности водных растворов листьев и цветков *Sambucus nigra L*, полученных при разном времени воздействия, и оценке антибактериальной активности этих растворов в отношении *Escherichia coli ATCC 8739, Salmonella NCTC 6017, Listeria monocytogenes NCTC. 11994* и *Staphylococcus aureus ATCC 25093*.

Материалы и методы: Исследовали физико-химические свойства водных экстрактов листьев (свежих) и цветков (свежих и сухих) *Sambucus nigra L*, собранных в Родопском регионе Болгарии. Образцы *Sambucus nigra L* были проанализированы для определения их общего содержания фенолов (TPC), общего содержания флавоноидов (TFC) и антиоксидантной активности с использованием 1,1-дифенил-2-пикрилгидразила (DPPH) и антиоксидантной способности, восстанавливающей железо (FRAP). Измерены диаметры (в миллиметрах) зон задержки роста четырёх возбудителей и дана сравнительная оценка их антибактериальной активности.

Результаты: Настои свежих цветков и свежих листьев *Sambucus nigra L.* обладали наибольшей антиоксидантной активностью при общем времени контакта 30 минут (82.7 mmol TE/100 ml) и 35 минут (36.5 mmol TE/100 ml) соответственно. Наиболее богатые фенолом настои были приготовлены из высушенных цветков *Sambucus nigra* L после 30-минутного контакта (86.7 mg GAE/ ml). Из четырёх исследованных нами патогенов мы обнаружили, что экстракты частично воздействовали только на патогенные бактерии сальмонеллы.

Заключение: Наибольшее содержание биоактивных компонентов получено из высушенных цветков *Sambucus nigra L.* для настоев при общем времени контакта 30 минут и для отваров при времени контакта 45 минут.

Ключевые слова

антиоксидантная активность, антибактериальная активность, отвары, флавоноиды, настой, *Sambucus nigra L*