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Antioxidant and antibacterial properties of biologically active compounds from *Sambucus nigra* and *Crataegus sanguinea*

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Abstract

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The present study investigated the biochemical composition and antimicrobial potential of fruit extracts obtained from medicinal plant species collected from different regions of Armenia. Fresh and dried fruit samples were analysed to determine the contents of free organic acids, including malic acid, citric acid, and ascorbic acid, using titrimetric and spectrophotometric methods. The antimicrobial activity of aqueous tinctures and hydroethanolic extracts was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. The obtained results demonstrated that malic acid predominated in hawthorn samples, whereas citric acid was dominant in elderberry fruits. Higher concentrations of ascorbic acid were detected in hawthorn compared to elderberry. Hydroethanolic extracts exhibited stronger inhibitory effects against the tested microorganisms than aqueous tinctures. Statistical analysis revealed no significant regional differences in the analysed biochemical parameters ($p > 0.05$). Processed plant materials retained considerable biological activity and exhibited antibacterial effects against the tested microorganisms. The findings indicate that the studied species represent promising natural sources of bioactive compounds with potential pharmacological and nutraceutical applications and support their traditional medicinal use.

Keywords: ascorbic acid, citric acid, hydroethanolic extracts, medicinal plants, phenolic compounds, plant extracts.

INTRODUCTION

Medicinal plants have been widely used for centuries in both traditional and modern medicine due to their biologically active compounds, which possess antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. Numerous plant-derived metabolites, including flavonoids, phenolic compounds, terpenoids, alkaloids, vitamins and organic acids, exhibit important physiological and pharmacological effects and are regarded as promising natural alternatives to synthetic drugs (Akhtar et al., 2018; Obiștioiu et al., 2023). In recent years, growing interest has focused on medicinal plants as potential sources of natural antimicrobial and antioxidant agents capable of inhibiting pathogenic microorganisms and reducing oxidative stress in living organisms (Voon et al., 2012).

Sambucus nigra L. and *Crataegus sanguinea* Pall., two medicinal plants, are particularly well known for their high content of biologically active compounds and therapeutic potential. Different organs of *Sambucus nigra*, including fruits, flowers, leaves and bark, contain flavonoids, anthocyanins, phenolic acids, organic acids, terpenoids, carbohydrates and other biologically active compounds (Agalar, 2019; Młynarczyk et al., 2018). Several studies have demonstrated that extracts obtained from *Sambucus nigra* possess antibacterial activity against both gram-positive and gram-negative bacteria, including *Bacillus cereus* Frankland & Frankland, *Staphylococcus aureus* Rosenbach, *Pseudomonas aeruginosa* (Schröter) Migula, and *Escherichia coli* (Migula) Castellani & Chalmers (Hearst et al., 2010; Goud & Prasad, 2020). The antioxidant activity of elderberry is associated with its high levels of phenolic compounds such as rutin, isoquercitrin, and anthocyanins (Mikulic-Petkovsek et al., 2015; Sun & Shahrajabian, 2023). Previous research has also shown that *Sambucus nigra* flowers exhibit stronger antioxidant activity than leaves and fruits, largely due to their higher concentrations of phenolic constituents (Młynarczyk et al., 2018).

Species of the genus *Crataegus* L. are likewise important medicinal plants widely used in traditional and official medicine. The fruits, flowers, and leaves of *Crataegus sanguinea* contain flavonoids, anthocyanins, tannins, organic acids, and ascorbic acid, contributing to their antioxidant and cardioprotective properties (Kurkin et al., 2022; Martinelli et al., 2021). Preparations derived from various *Crataegus* species are widely used as cardiotonic agents and have been shown to possess antimicrobial, antioxidant, and anti-inflammatory activities (Naghiu et al., 2023). Organic acids, particularly malic and citric acids, are considered important biochemical indicators of fruit quality and biological activity in *Crataegus* species (Gundogdu et al., 2014).

Previous studies have shown that aqueous extracts and tinctures made from the flowers of *Sambucus nigra* are traditionally used to relieve respiratory and urinary tract disorders such as coughs, sore throats, nasal discharges and inflammatory conditions (Cejpek et al., 2009). Water-soluble compounds present in the flowers have also been reported to stimulate insulin secretion and improve glucose metabolism (Lu et al., 2016). In addition, several authors have described the antimicrobial activity of *Sambucus nigra* flower extracts (Arjoon et al., 2012). In traditional Chinese medicine, the fresh and dried fruits of *Crataegus pinnatifida* Bunge are widely used for their beneficial effects on the cardiovascular, digestive, and endocrine systems, as well as their inhibitory activity against pathogenic microorganisms (Wu et al., 2014).

The biochemical composition and biological activity of medicinal plants may vary considerably depending on environmental conditions, geographical origin, climatic factors, and post-harvest processing methods. Drying, storage conditions, and extraction procedures can influence the concentration of biologically active compounds and, consequently, affect the antimicrobial and antioxidant properties of plant materials. Earlier studies have shown that drying can reduce ascorbic acid and certain organic compounds, although the overall biological activity may remain substantial.

In Armenia, research on the bioecological, physiological, and antibacterial properties of *Sambucus nigra* and *Crataegus sanguinea* remains limited. Previous investigations conducted in the Lori Region described the bioecological characteristics of *Sambucus nigra* and revealed the antibacterial activity of juices and extracts obtained from its generative organs (Vardanyan et al., 2015, 2019, 2021, 2025). However, comprehensive comparative analyses of organic acids, ascorbic acid content, and antibacterial activity of *Sambucus nigra* and *Crataegus sanguinea* collected from different regions of Armenia are still lacking.

Armenia is characterised by diverse climatic and ecological conditions, which may influence the accumulation of biologically active compounds in medicinal plants. Therefore, comparative studies of plant materials collected from different regions are important for evaluating their biochemical and biological properties.

Therefore, the present study aimed to investigate the content of organic acids, including ascorbic acid, in fruits of *Sambucus nigra* and *Crataegus sanguinea* collected from different regions of Armenia, and to evaluate the antibacterial activity of tinctures and hydroethanolic extracts obtained from these samples. Special attention was also given to the effect of drying on the retention of biologically active compounds and the biological activity of the studied plant materials. The study addressed the following research questions: (a) Do *Sambucus nigra* and *Crataegus*

Table 1. The main differences in the climatic conditions of the Lori, Aragatsotn and Tavush Provinces of Armenia (Simonyan, 2003; Manasyan et al., 2003)

Province	Annual precipitation range (mm)	Summer temperature range (°C)	Winter temperature range (°C)	Elevation range (m a.s.l.)	Number of cloudy days	Sunshine duration range (h/year)
Lori	400–700	18	–4.8	1788	43	2000
Aragatsotn	200–500	32–39	–25 to –27	1254–1637	84	2502–2600
Tavush	500–600	18–24	–1 to +1	1594	64	1900–2100

sanguinea differ in their contents of organic acids and ascorbic acid? (b) Does geographical origin influence the biochemical composition of the studied plant materials? (c) How does drying affect the levels of biologically active compounds? (d) Do the obtained extracts exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*?

MATERIALS AND METHODS

Study species

The study was conducted on two medicinal plant species, *Sambucus nigra* and *Crataegus sanguinea*, which are widely distributed across different regions of Armenia and are traditionally used in folk medicine due to their rich content of biologically active compounds.

Sampling sites and bioclimatic conditions

Plant material was collected from natural populations in the Lori, Tavush, and Aragatsotn Provinces of Armenia. Sampling sites were selected to reflect contrasting climatic and ecological conditions across regions. In Lori Province, samples were collected in Gugark Region at an elevation of approximately 1325 m above sea level. In Tavush Province, material was obtained from Noyemberyan Region at altitudes ranging from 750 m to 850 m. Samples from Aragatsotn Province were collected in mountainous areas situated between 1254 m and 1637 m above sea level.

The studied regions differ markedly in their climatic characteristics (Table 1). Tavush Province is characterised by relatively high humidity, while Aragatsotn Province experiences lower annual precipitation and pronounced temperature fluctuations. Lori Province exhibits moderate and comparatively stable climatic conditions (Simonyan, 2003; Manasyan et al., 2003).

Table 2. Sample codes and sampling regions

Sample code	Species	Province
CA1	*Crataegus sanguinea*	Aragatsotn
CL2	*Crataegus sanguinea*	Lori
CL3	*Crataegus sanguinea*	Lori
CL4	*Crataegus sanguinea*	Lori
CA5	*Crataegus sanguinea*	Aragatsotn
CA6	*Crataegus sanguinea*	Aragatsotn
ST7	*Sambucus nigra*	Tavush
SL8	*Sambucus nigra*	Lori
ST9	*Sambucus nigra*	Tavush
SL10	*Sambucus nigra*	Lori
ST11	*Sambucus nigra*	Tavush
SL12	*Sambucus nigra*	Lori

For clarity and consistency in data presentation, each sample was assigned a code indicating the plant species and sampling region. Samples of *Crataegus sanguinea* collected from Aragatsotn Province were coded as CA1, CA5 and CA6, whereas samples collected from Lori Province were coded as CL2, CL3 and CL4. Samples of *Sambucus nigra* collected from Tavush Province were coded as ST7, ST9 and ST11, while samples collected from Lori Province were coded as SL8, SL10 and SL12.

Plant material collection and preparation

Fresh fruits of *Sambucus nigra* and *Crataegus sanguinea* were collected during the fruiting stage in August–September 2024 from the selected sampling sites. The collected plant material was cleaned of impurities and mechanically damaged fruits.

Part of the collected material was analysed in the fresh condition. In contrast, another part was dried under laboratory conditions at room temperature in a shaded, well-ventilated environment until constant weight was achieved. Dried samples were stored in paper containers in dry premises to prevent moisture accumulation.

For antibacterial activity assays, tinctures and hydroethanolic extracts were prepared from both fresh and dried fruits (Hovhannisyan, 2016).

Determination of organic acids

The content of organic acids was determined using titrimetric and spectrophotometric methods. Total acidity was measured by titration with sodium hydroxide (NaOH) using phenolphthalein as an indicator.

Approximately 5 g of homogenised fresh or dried fruit material was extracted with 50 mL of distilled water and stirred for 30–60 min at room temperature. The extracts were then filtered and centrifuged to remove suspended particles. The resulting supernatants were diluted appropriately before spectrophotometric analysis.

Individual organic acids were quantified using a UV-visible spectrophotometer at 200–220 nm, employing 1-cm quartz cuvettes (Krukowski et al., 2017).

Determination of ascorbic acid

Although ascorbic acid belongs to the group of organic acids, it was quantified separately owing to its specific biological function as ascorbic acid and its importance as a major antioxidant marker frequently used in the biochemical evaluation of medicinal plants.

The ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol (DCPIP) (GOST, 1989; Dave, 2016).

Approximately 5 g of homogenised plant material was extracted with 50 mL of a 3% metaphosphoric acid solution to stabilise ascorbic acid and prevent oxidation. The extracts were filtered and analysed immediately.

A standard DCPIP solution (0.01 g/100 mL) was prepared and standardised using pure ascorbic acid. 10 mL of each plant extract was titrated with the DCPIP solution until a persistent pink endpoint was observed. All analyses were performed in triplicate, and the results were expressed as mg/100 g fresh weight.

Antibacterial activity

Antibacterial activity of the prepared aqueous extracts and hydroethanolic extracts was evaluated using the agar well diffusion method on Mueller-Hinton M173 medium.

The antibacterial activity was tested against *Escherichia coli* K12DS498 and *Staphylococcus aureus* ATCC 6538 strains using 18–20 h bacterial cultures. The antibacterial activity was evaluated according to inhibition zone diameters as follows (CLSI, 2012): (a) 11–18 mm – moderate activity; (b) 20–27 mm – high activity; (c) ≥ 28 mm – very high activity.

Statistical analysis

All experiments were performed in triplicate. The results are presented as mean ± standard deviation. As the data sets were not normally distributed, non-parametric tests were used in the analysis. Comparisons between samples were performed using the Kruskal-Wallis test, while comparisons between pairs of samples were performed using the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$. The effect of factors on the content of the analysed compounds in the samples was assessed using a two-way permutational analysis of variance (PERMANOVA) with 999 permutations and the Euclidean distance metric. All analyses were performed using the PAST 5.0.2 software package (Hammer et al., 2001).

RESULTS

Organic acid content in *Crataegus sanguinea*

The content of free organic acids was determined in six fruit samples of *Crataegus sanguinea* collected from Lori and Aragatsotn Provinces. The obtained values ranged from 1.56 mg/100 g to 4.66 mg/100 g, with the highest content observed in samples collected from Aragatsotn Province (Table 3). The Kruskal-Wallis test revealed significant differences among all samples (H = 15.97, $p = 0.007$), but no differences were found between any pair of samples. A two-way permutation analysis revealed that the content of free organic acids was affected by region (F = 13.19, $p = 0.001$) and sampling site (F = 29.22, $p = 0.001$). However, the interaction between these factors was not significant.

The content of malic and citric acids was analysed in the studied samples of *Crataegus sanguinea*.

Malic acid content ranged from 16.75 mg/100 g to 80.4 mg/100 g, while citric acid content ranged from 16.0 mg/100 g to 76.8 mg/100 g. The highest concentrations of both organic acids were detected in samples collected from Aragatsotn Province (Table 3). The Kruskal-Wallis test revealed significant differences in malic acid content among all samples (H = 16.58, $p = 0.005$), but no differences were found between pairs of samples. Similar results were found for citric acid content (H = 16.57, $p = 0.005$). A two-way permutation analysis showed that the region (F = 344.19, $p = 0.001$ and F = 3128.40, $p = 0.001$) and the sampling site (F = 364.8, $p = 0.001$ and F = 3683.50, $p = 0.001$) had a significant effect on the content of malic acid and citric acid, respectively. However, the interaction between these factors did not affect the content of these acids in either case.

Organic acid content in *Sambucus nigra*

The content of free organic acids was also determined in fruit samples of *Sambucus nigra* collected from Lori and Tavush Provinces. The analysed samples exhibited free organic acid contents ranging from 1.16 mg/100 g to 3.78 mg/100 g. The highest value was recorded in samples collected from Lori Province (Table 4). The Kruskal-Wallis test revealed significant differences among all samples (H = 16.25, $p = 0.006$), though no differences were found between any two samples. A two-way permutation analysis showed that the content of free organic acids was influenced by both region (F = 57.84, $p = 0.001$) and sampling site (F = 225.35, $p = 0.001$). However, the interaction between these two factors was not significant.

It should be noted that the mean free organic acid content of the *Sambucus nigra* samples was significantly lower than that of the *Crataegus sanguinea* samples (H = 5.63, $p = 0.018$).

Citric acid was the predominant organic acid in the analysed samples of *Sambucus nigra*, ranging from 40.20 mg/100 g to 93.80 mg/100 g (Table 4). The highest citric acid concentration was detected in samples collected from Tavush Province. The Kruskal-Wallis test revealed significant differences among all samples (H = 16.58, $p = 0.005$), though no differences were found between any two samples. A two-way permutation analysis showed that citric acid content was affected by region (F = 257.66, $p = 0.001$) and sampling site (F = 359.80, $p = 0.001$), though the interaction between these factors was not significant. The citric acid content of the studied *Sambucus nigra* samples was significantly higher than that of the *Crataegus sanguinea* samples (H = 11.68, $p < 0.001$). The malic acid content in the studied *Crataegus sanguinea* samples was significantly lower (H = 11.68, $p < 0.001$) than in the *Sambucus nigra* samples.

Malic acid content ranged from 51.20 mg/100 g to

Table 3. Content of free organic acids, malic and citric acids in the studied samples of *Crataegus sanguinea*

Sample code	Amount of free organic acids (mg/100 g)	Malic acid (mg/100 g)	Citric acid (mg/100 g)
CA1	2.18 ± 0.30	16.75 ± 0.11	32.00 ± 0.30
CL2	2.54 ± 0.10	40.20 ± 0.80	38.40 ± 0.40
CL3	4.39 ± 0.30	60.30 ± 0.21	57.60 ± 0.47
CL4	1.56 ± 0.20	33.50 ± 0.70	16.00 ± 0.21
CA5	4.66 ± 0.29	80.40 ± 0.14	76.80 ± 0.49
CA6	3.59 ± 0.30	53.60 ± 0.13	51.20 ± 0.60
Mean	3.15 ± 1.28	47.46 ± 22.51	45.33 ± 21.51

Table 4. Content of free organic acids, malic and citric acids in *Sambucus nigra* samples

Sample code	Amount of free organic acids (mg/100 g)	Malic acid (mg/100 g)	Citric acid (mg/100 g)
ST7	1.68 ± 0.03	64.00 ± 1.20	67.00 ± 1.02
SL8	1.16 ± 0.02	51.20 ± 1.11	40.20 ± 0.98
ST9	2.03 ± 0.04	76.80 ± 1.17	73.60 ± 1.21
SL10	3.78 ± 0.11	73.60 ± 1.21	80.40 ± 1.23
ST11	2.62 ± 0.09	74.30 ± 1.30	93.80 ± 1.50
SL12	1.95 ± 0.10	57.60 ± 0.87	60.30 ± 1.31
Mean	2.20 ± 0.90	66.25 ± 10.23	69.22 ± 18.24

76.80 mg/100 g, with the highest value also observed in samples from Tavush Province. The Kruskal-Wallis test revealed significant differences among all samples (H = 16.16, p = 0.006), though no differences were found between any two samples. A two-way permutation analysis showed that malic acid content was affected by region (F = 50.33, p = 0.001) and sampling site (F = 121.44, p = 0.001), though the interaction between these factors was not significant.

Ascorbic acid content

The concentration of ascorbic acid in the fruit samples of *Crataegus sanguinea* and *Sambucus nigra* varied significantly (Table 5). The Kruskal-Wallis test comparing all *Crataegus sanguinea* samples revealed significant differences in ascorbic acid content (H = 16.58, p = 0.005). However, no differences were found between pairs of samples. Two-way permutation analysis showed that the region (F = 1301.3, p = 0.001) and the sampling site (F = 753.84, p = 0.001) had a significant effect on the ascorbic acid content of *Crataegus sanguinea*. Still, the interaction between these factors did not affect this content in either case.

The ascorbic acid content in the analysed samples ranged from 11.15 mg/100 g to 46.60 mg/100 g. Significantly higher (H = 17.31, p < 0.001) mean concentration was observed in *Crataegus sanguinea* samples (35.28 ± 8.44 mg/100 g) compared to *Sambucus nigra* (17.49 ± 9.37 mg/100 g). Overall, the ascorbic acid content was substantially higher in *Crataegus sanguinea* than in *Sambucus nigra*, indicating species-specific differences in ascorbic acid accumulation. The highest ascorbic acid content (46.60 mg/100 g) was detected in samples collected from Aragatsotn Province. In contrast, the lowest value (11.15 mg/100 g) was recorded in *Sambucus nigra* samples collected from Tavush Province. Two-way permutation analysis showed that species (F = 38.18, p = 0.001) and region (F = 16.58, p = 0.001) significantly affected ascorbic acid content. However, the interaction between these factors did not affect ascorbic acid content in either case.

Table 5. Ascorbic acid content in the samples of *Crataegus sanguinea* and *Sambucus nigra*

Sample code	Ascorbic acid content (mg/100 g)
Crataegus sanguinea	
CA1	37.80 ± 0.45
CL2	41.50 ± 0.51
CL3	27.80 ± 0.32
CL4	22.10 ± 0.31
CA5	35.90 ± 0.41
CA6	46.60 ± 0.37
Mean	35.28 ± 8.44
Sambucus nigra	
ST7	12.58 ± 0.21
SL8	15.87 ± 0.19
ST9	11.15 ± 0.11
SL10	14.50 ± 0.14
ST11	13.25 ± 0.12
SL12	37.58 ± 0.22
Mean	17.49 ± 9.37

Antibacterial activity

The antibacterial activities of aqueous tinctures and hydroethanolic extracts obtained from the fruits of *Crataegus sanguinea* and *Sambucus nigra* against *Escherichia coli* and *Staphylococcus aureus* were evaluated. The analysed extracts exhibited varying degrees of antibacterial activity against the tested microorganisms (Table 6).

Hydroethanolic extracts exhibited stronger antibacterial activity compared to aqueous tinctures. In both species, hydroethanolic extracts prepared from fresh fruits showed the highest antibacterial activity. In both plant species, higher antibacterial activity was observed against *Escherichia coli* than against *Staphylococcus aureus*.

Statistical analysis using the Mann-Whitney U test revealed no statistically significant differences in the contents of free organic acids, malic acid, citric acid, or ascorbic acid between the sampling regions for either *Crataegus sanguinea* or *Sambucus nigra*. All obtained p-values exceeded the 0.05 significance threshold, indicating that the observed regional variations were not statistically significant.

DISCUSSION

Organic acids are important biochemical constituents of medicinal plants and contribute to antioxidant, antimicrobial, acidifying, and preservative properties. Malic, citric, tartaric, and ascorbic ac-

Table 6. Antibacterial activity of tinctures and hydroethanolic extracts of *Crataegus sanguinea* and *Sambucus nigra*. Symbols: + – moderate activity; ++ – high activity; +++ – very high activity

Plant species	Sample	*Staphylococcus aureus*	*Escherichia coli*
Crataegus sanguinea	Tincture from dried fruits	+	+
	Tincture from fresh fruits	++	+
	Hydroethanolic extract from fresh fruits	++	++
Sambucus nigra	Tincture from dried fruits	+	+
	Tincture from fresh fruits	+	++
	Hydroethanolic extract from fresh fruits	+	++

ids are considered among the major organic acids present in fruits of *Crataegus* species and are important indicators of fruit quality and biological activity (Gundogdu et al., 2014).

Previous investigations have demonstrated that the composition and concentration of organic acids in *Crataegus* fruits may vary across species, geographical origin, and environmental conditions. Citric acid has frequently been reported as one of the dominant organic acids in hawthorn fruits (Gundogdu et al., 2014). However, other studies have shown that malic acid may predominate in several *Crataegus* species, including *Crataegus monogyna* and *Crataegus pentagyna* (Cosmulescu et al., 2020). The results of the present study are generally consistent with these observations, since relatively high concentrations of malic acid were detected in the analysed *Crataegus sanguinea* samples.

The analysed *Sambucus nigra* samples also contained considerable amounts of organic acids. Previous studies have reported that elderberry fruits primarily contain malic, citric, fumaric, and tartaric acids, with citric and malic acids usually being the predominant fractions (Młynarczyk et al., 2018; Vujanović et al., 2020). In the present study, citric acid predominated in most *Sambucus nigra* samples, which agrees with previously published data.

Statistical analysis using the Mann–Whitney U test revealed no significant differences in the contents of free organic acids, malic acid, citric acid, or ascorbic acid between the studied regions for either *Crataegus sanguinea* or *Sambucus nigra* ($p > 0.05$). Nevertheless, minor variations among individual samples were observed, suggesting that local environmental factors may contribute to differences in the accumulation of biologically active compounds. However, these effects were not statistically significant under the conditions of the present study.

The ascorbic acid content detected in *Crataegus sanguinea* fruits was generally higher than in *Sambucus nigra* fruits. According to the literature, the ascorbic acid concentration in *Crataegus* fruits may range from 6 mg/100 g to 50 mg/100 g depending on species and environmental conditions (Kaack & Austed, 1998; García-Mateos et al., 2013). The values obtained in the present study fall within these previously reported ranges.

Although statistical analysis did not reveal significant regional differences, *Crataegus sanguinea* samples collected in Aragatsotn Province tended to have higher ascorbic acid concentrations than samples from other regions. Similar trends have been reported in studies describing the influence of environmental and climatic conditions on antioxidant metabolism in fruit-bearing plant species.

Previous studies have reported that drying may reduce the concentration of certain biologically active compounds, particularly ascorbic acid, due to oxidation and degradation processes. Nevertheless, dried plant materials may retain considerable biological activity and remain valuable sources of bioactive compounds.

Both aqueous tinctures and hydroethanolic extracts demonstrated antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with hydroethanolic preparations showing stronger inhibitory effects. This enhanced activity may be associated with a higher ethanol extraction efficiency for phenolic compounds, flavonoids, and other antimicrobial constituents (Hearst et al., 2010; Goud & Prasad, 2020).

The observed antibacterial activity against *Escherichia coli* was generally stronger than against *Staphylococcus aureus*. Similar antibacterial patterns have previously been reported for elderberry and hawthorn extracts. They may be related to differences in the sensitivity of gram-negative and gram-positive bacteria to plant-derived bioactive compounds.

Notably, both fresh and dried extracts retained antibacterial properties, demonstrating that processing does not eliminate the antimicrobial potential of these species. This finding supports the potential use of processed plant materials in herbal preparations and natural antimicrobial products.

The observed antibacterial activity, together with the presence of organic acids and ascorbic acid, further supports the potential application of these species in the development of functional foods and plant-based health products.

The obtained findings confirm that *Crataegus sanguinea* and *Sambucus nigra* represent valuable natural sources of biologically active compounds with antioxidant and antibacterial potential. These species may therefore be considered promising raw materials for further pharmacological, nutraceutical, and antimicrobial investigations.

CONCLUSION

The results of the present study demonstrate that fruits of *Crataegus sanguinea* and *Sambucus nigra* collected from different regions of Armenia are rich sources of biologically active compounds, including organic acids and ascorbic acid. Malic acid predominated in Crataegus sanguinea fruits, whereas citric acid was the dominant organic acid in *Sambucus nigra* samples. The ascorbic acid content was generally higher in *Crataegus sanguinea* than in *Sambucus nigra*, and the obtained values were consistent with previously published data.

Statistical analysis revealed no significant regional differences in the biochemical composition of the studied species ($p > 0.05$), indicating relatively stable profiles of biologically active compounds across the investigated geographic regions of Armenia. These findings suggest that both species may serve as reliable natural sources of bioactive compounds regardless of their specific collection sites.

Both aqueous tinctures and hydroethanolic extracts prepared from fresh and dried fruits exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with hydroethanolic extracts demonstrating stronger antimicrobial effects.

The obtained findings provide scientific support for the traditional medicinal use of *Crataegus sanguinea* and *Sambucus nigra* and confirm their potential as natural sources of antioxidant and antimicrobial compounds. Preservation of biological activity after processing further indicates the suitability of these species for the development of herbal preparations, nutraceutical products, and natural antimicrobial formulations.

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Author contributions. ZV coordinated and supervised the research, collected and processed samples, and analysed and interpreted results; VH collected plant samples, conducted laboratory experiments, processed data and analysed results; HM collected plant samples and conducted laboratory experiments. All authors read and approved the final version of the article.

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