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Phytochemicals and antimicrobial properties of traditional medicinal plants in Bosnia and Herzegovina

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ABSTRACT: In traditional medicine, plants are widely utilized as sources of bioactive compounds for treating various diseases. This study aimed to evaluate the secondary metabolite composition, antioxidant properties, and antimicrobial effects of 38 medicinal plants commonly used in Bosnia and Herzegovina. Plants were collected from natural habitats, and dried plant material from different organs, selected based on their traditional medicinal use, was used for the extraction of bioactive compounds with 80% ethanol. The extracts were analysed for phenolic, flavonoid, and tannin content, as well as antioxidant capacity (using DPPH and FRAP assays) and antimicrobial activity. The antimicrobial activity of all 38 plants was initially screened using the disc diffusion method. For plants showing significant antimicrobial activity (inhibition zones > 20 mm), the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. All analysed plants exhibited high phenolic content, with *Melissa officinalis* leaf extract, *Filipendula vulgaris* flower extract, and *Rubus plicatus* leaf extract containing over 300 mg GAE/g DW. According to the DPPH assay, high antioxidant capacity was observed in extracts from the leaves of *Fragaria vesca*, *Prunus armeniaca*, *Rubus plicatus*, and *R. ideus*, as well as in *Rosa canina* fruit and *Filipendula vulgaris* flower extracts, with values reaching 702.39 mg TE/g DW. Among the 38 tested plants, 16 exhibited high antimicrobial activity with inhibition zones greater than 20 mm. To ensure both the efficacy and safety of these plants, further studies on their toxicity, particularly dose-dependent toxicity, are necessary.

KEYWORDS: Bosnia and Herzegovina; ethnopharmacy; secondary metabolites; antioxidant activity; antimicrobial activity

1. INTRODUCTION

The emergence of drug resistance and increasing number of poorly responsive infectious diseases to antibiotics is adding to already great numbers of people dying from infectious diseases [1]. For this reason, research is more and more oriented toward natural sources of bioactive compounds for identification of new drugs as well as for investigation of synergistic and antagonistic effects of plant extracts. Additionally, people in developing countries and rural areas are more oriented toward natural remedies and often avoid seeking western medicine due to lower availability, drug prices, lack of medical care or simply due to cultural background.

The rise of drug resistance and the increasing number of infectious diseases that respond poorly to antibiotics have significantly contributed to the already high mortality rates from these diseases. As a result, research has increasingly focused on natural sources of bioactive compounds for the development of new drugs, as well as on investigating the synergistic and antagonistic effects of plant extracts. Additionally, in developing countries and rural areas, people often rely on natural remedies and may avoid seeking Western medicine due to limited availability, high drug prices, lack of medical care, or cultural preferences.

The use of natural medicine dates back to the Neolithic period [2] and it is estimated that nearly 80% of the global population uses plants as part of their traditional medicine for treating various diseases [3]. The beneficial effects of plant extracts and infusions have been confirmed by numerous studies with many identified bioactive compounds now being well-established as drugs and dietary supplements [4]. In Europe, several species have been extensively studied in regions such as Turkey, Italy and Spain, including a large

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number of species from the Lamiaceae, Asteraceae and Rosaceae families [5,6]. Members of the Lamiaceae family, for example, have been associated with various beneficial and medicinal effects on central nervous system [5] and antimicrobial effects [7]. *Achillea millefolium* group is one of the most diverse sources of the crude drug *herba Milefolii* and is one of the most prominent medicinal plants from the Asteraceae family [8] with a long history of use across Balkans [9]. Similarly, many species in the Rosaceae family have been linked to improvement in human health [10], with berries serving as one of the main sources of polyphenols and other bioactive compounds [11, 12] commonly used in nutrition and traditional medicine.

The Balkan region has an exceptionally diverse flora, with more than 6300 vascular plant species [13]. In the small area of Bosnia and Herzegovina, which covers just 51,129 square kilometres, over 3,600 vascular plant species are found. Despite the long history of medicinal plant use in Bosnia and Herzegovina [9], there are surprisingly few ethnopharmacological studies for this region [14]. This study aims to validate the bioactive and medicinal potential of 38 medicinal plants commonly used by people in Bosnia and Herzegovina for treating various infections and other medical conditions. The species included in this study, along with their traditional medicinal uses, are listed in Table 1. The validation of these plants' bioactive and medicinal potential was conducted through an analysis of their polyphenol and tannin content, antioxidant capacity, and antimicrobial activity. The selection of these 38 plant species was carefully guided by their extensive use in traditional medicine within Bosnia and Herzegovina, as well as by existing ethnopharmacological literature highlighting their medicinal properties. The plants belong to three major families Asteraceae, Lamiaceae, and Rosaceae—which have been extensively studied for their bioactive compounds and therapeutic potential.

Asteraceae Family: Known for its wide range of medicinal plants, the Asteraceae family includes species like *Achillea millefolium* and *Artemisia absinthium*, which are traditionally used for gastrointestinal disorders, liver issues, and respiratory diseases. These plants have been well-documented in the literature for their antimicrobial, anti-inflammatory, and antioxidant properties [5, 7, 8].

Lamiaceae Family: Members of the Lamiaceae family, such as *Salvia officinalis* and *Lavandula angustifolia*, are renowned for their effects on the nervous system, as well as their antimicrobial and skin-healing properties. Studies have shown that these plants possess significant bioactive compounds that contribute to their therapeutic effects [5, 7].

Rosaceae Family: The Rosaceae family includes species like *Rosa canina* and *Rubus idaeus*, which are commonly used for treating gastrointestinal issues, skin diseases, and cardiovascular conditions. These plants are rich in polyphenols and other bioactive compounds, making them valuable in both traditional and modern medicine [10-12].

The selection of these specific plants was also influenced by the unique biodiversity of the Balkan region, particularly in Bosnia and Herzegovina, where these species have been historically used for medicinal purposes. Despite their long-standing use, there has been limited scientific validation of their efficacy, especially in the context of modern pharmacology. This study seeks to fill that gap by analysing their polyphenol and tannin content, antioxidant capacity, and antimicrobial activity, thereby providing a scientific basis for their traditional uses.

Table 1. Common uses of traditional medicinal plants from families Asteraceae, Lamiaceae and Rosaceae in Bosnia and Herzegovina (38-40; 15,16)

Plant species	Plant Family	Voucher No	Plant part used	Common use in traditional medicine
Achillea millefolium L.	Asteraceae	LBF 415	Aerial part	Injuries, gastrointestinal issues, liver issues
Arctium lappa L.	Asteraceae	LBF 416	Root	Gastrointestinal disorders, parasitic diseases, oral infections, hair growth, diabetes, skin eczema and acne
Artemisia absinthium L.	Asteraceae	LBF 417	Aerial part	Gastrointestinal disorders, respiratory diseases, cough
Artemisia vulgaris L.	Asteraceae	LBF 418	Aerial part	Liver and gallbladder disorders, nervous system disorders
Calendula officinalis L.	Asteraceae	LBF 419	Aerial part	Lung cancer, skin cancer, liver cancer
Carlina acaulis L.	Asteraceae	LBF 420	Root	parasitic diseases, skin diseases
Cichorium intybus L.	Asteraceae	LBF 421	Root	Gastrointestinal disorders (liver and gallbladder disorders), lung cancer, beneficial for prostate and the reproductive system
Helichrysum italicum (Roth) G. Don	Asteraceae	LBF 422	Root	Liver and gallbladder disorders, cough
Inula helenium L.	Asteraceae	LBF 423	Root	Lung diseases, skin diseases, regulation of the menstrual cycle
Matricaria discoidea DC.	Asteraceae	LBF 424	Flower and leaves	Stomach diseases, skin diseases, calming as eye pads
Petasites hybridus (L.) G. Gaertn. & al.	Asteraceae	LBF 425	Leaves and root	Liver and stomach diseases, pest
Taraxacum officinale F. H. Wigg.	Asteraceae	LBF 426	Flowers, leaves, root	Anaemia, haemorrhoids, icterus
Tussilago farfara L.	Asteraceae	LBF 427	Flower and leaves	Cough and other respiratory problems
Ajuga reptans L.	Lamiaceae	LBF 428	Aerial part	Diarrhoea, wounds
Glechoma hederacea L.	Lamiaceae	LBF 429	Leaves	Epilepsy, psychosis, tuberculosis, anaemia, gastrointestinal disorders, flu
Lamium maculatum (L.) L.	Lamiaceae	LBF 430	Flower and aerial parts	Rheumatism, arthritis
Lamium purpureum L.	Lamiaceae	LBF 431	Leaves and young sprouts	Gastrointestinal disorders, nervous system disorders

Lavandula angustifolia Mill.	Lamiaceae	LBF 432	Leaves	Skin diseases, rheumatism, dandruff
Melissa officinalis L.	Lamiaceae	LBF 433	Leaves	Neurosis, heart diseases
Mentha longifolia (L.) L.	Lamiaceae	LBF 434	Flower and leaves	Fibroids in the female reproductive system, gastrointestinal disorders, flu
Mentha spicata L.	Lamiaceae	LBF 435	Leaves	Gingivitis, liver diseases, rheumatism
Rosmarinus officinalis L.	Lamiaceae	LBF 436	Leaves	Disorders of the genitourinary and nervous system, anaemia
Salvia officinalis L.	Lamiaceae	LBF 437	Aerial part	Skin diseases, respiratory diseases
Thymus serpyllum L.	Lamiaceae	LBF 438	Aerial part	Neurosis, cough, stomach diseases
Crataegus germanica (L.) Kuntze	Rosaceae	LBF 439	Flower and leaves	Cardiovascular diseases
Crataegus monogyna Jacq.	Rosaceae	LBF 440	Flower, leaves, fruit	Cardiovascular diseases
Crataegus rhipidophylla Gand.	Rosaceae	LBF 441	Flower, leaves, fruit	Cardiovascular diseases
Cydonia oblonga Mill.	Rosaceae	LBF 442	Leaves	Gastrointestinal disorders (diarrhoea, stomach diseases)
Filipendula vulgaris Moench	Rosaceae	LBF 443	Flower	Flu, cold, respiratory diseases,
Fragaria vesca L.	Rosaceae	LBF 444	Leaves	Diarrhoea, immune system strengthening,
Malus pumila Mill.	Rosaceae	LBF 445	Fruit	Metabolism disorders, weight maintenance, against constipation, hyperlipidaemia, neurosis, flu and cold
Prunus armeniaca L.	Rosaceae	LBF 446	Leaves	Skin diseases
Prunus avium (L.) L.	Rosaceae	LBF 447	Leaves, fruit	Diarrhoea, flu, malarial fever
Prunus domestica L.	Rosaceae	LBF 448	Fruit	Gastrointestinal disorders, skin diseases, circulatory system diseases
Prunus persica (L.) Batsch	Rosaceae	LBF 449	Leaves	Metabolism disorders, skin diseases, immune system strengthening
Prunus spinosa L.	Rosaceae	LBF 450	Fruit	Hyperlipidaemia, anaemia, diarrhoea

Rosa canina L.	Rosaceae	LBF 451	Fruit	Diarrhoea, roundworm disease
Rubus idaeus L.	Rosaceae	LBF 452	Fruit	Bruising and injuries
Rubus plicatus Weihe & Nees	Rosaceae	LBF 453	Leaves	Diarrhoea, skin diseases

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2. RESULTS AND DISCUSSION

2.1. Phytochemicals

The total phenolic, flavonoid and tannin content was evaluated for the investigated species and the values are reported in Table 2. Plant extracts containing more than 4.5% (5mg/mL) of phenolics are considered to have antioxidant properties as well as other beneficial effects [1,24]. All analysed extracts contained high concentrations of phenolics with some exceeding 300 mg GAE/g DW such as *Melissa officinalis* leaf extract, *Filipendula vulgaris* flower extract and *Rubus plicatus* leaf extract. Only *Malus pumila* fruit extracts had low phenolic concentration, with just 8.89 mg GAE/g DW.

Flavonoids were present in all the investigated extracts, with varying concentrations ranging from 0.56 mg/mL in *Malus pumila* fruit extract to 55.67 mg/mL in *Filipendula vulgaris* flower extract (Table 2). The beneficial effects of flavonoids on human health and disease prevention have been confirmed through their anti-inflammatory, antimicrobial activity, and enzyme inhibition properties [1]. Overall, there were no significant differences in the average values of phenolics or flavonoid content recorded across the three families (Figure 1A&B).

Filipendula vulgaris has recently been recognized as a rich source of phenolic compounds [24] as well as a new source of ingredients for organic cosmetics [25]. The major phytochemical constituents of *F. vulgaris* flowers include kaempferol (molecular weight 286.23 g/mol), quercetin (molecular weight 302.236 g/mol), hyperoside (molecular weight 464.38 g/mol), astragalin (molecular weight 448.4 g/mol), spiraeoside (molecular weight 464.37 g/mol), myricetin (molecular weight 318.2351 g/mol), taxifolin (molecular weight 304.25 g/mol), avicularin (molecular weight 434.35 g/mol), various phenolic acids such as ellagic acid (molecular weight 302.197 g/mol), and many other components [26]. The high phenolic content in *Filipendula vulgaris*, can be explained by the use of hydroethanolic, and thus more polar, solvents, which allow effective extraction of polyphenols, particularly high molecular weight phenolics [27]. Tannins were detected in only 8 of the investigated plants/extracts, with high concentrations (above 100 mg/mL) found in the leaf extracts of *Petasites hybridus*, *Crataegus germanica*, *Crataegus rhipidophylla*, as well as in flower extract of *Crataegus rhipidophylla* and fruit extract of *Rosa canina* (Table 2). Tannins have been documented as effective agents against bacteria as well as *Candida albicans* [1]. In this study, tannins were not found in any of the analysed plants from the Lamiaceae family, while the members of the Rosaceae family had much higher tannin content than those of the Asteraceae family (Figure 2B).

2.2. Antioxidant capacity

The antioxidant capacity of analysed plants/extracts was determined using the DPPH and FRAP assays, with results expressed in mg of Trolox equivalent per gram of dry weight (mg TE/g DW). There were no significant differences in the DPPH values across the various families, nor in the FRAP values. However, the Rosaceae family exhibited the highest DPPH values, while the Lamiaceae family had the highest FRAP values (Figure 1B). Particularly high DPPH scavenging activity (> 300 mg TE/g DW) was recorded for the leaf extracts of *Fragaria vesca*, *Prunus armeniaca*, *Rubus plicatus* and *R. ideus*, as well as for the fruit extracts of *Rosa canina*. The flower extracts of *Filipendula vulgaris* exhibited the highest DPPH scavenging activity, reaching 702.39 mg TE/g DW. The high antioxidant activity of *F. vulgaris* flower extracts is likely due to presence of spiraeoside, myricetin, quercetin and kaempferol [26] as well as the nature of the extraction solvent, as ethanol has been noted as an effective medium for extracting antioxidant compounds from *Filipendula vulgaris* [27]. Additionally, some studies suggest that ursane-type compounds containing aldehyde groups (2*a*, 3*β*-dihydroxyurs-12-en-28-aldehyde) may contribute to the antioxidant capacity of *F. vulgaris* [27]. Furthermore, the antioxidant activity of *F. vulgaris* has been associated with a wide variety of biological activities including antifungal, anti-inflammatory, antidiabetic, and anticarcinogenic activity [26]. Results recorded of this study confirm the value of this plant as a potent antioxidant.

Table 2. Phenolic content and antioxidant capacity of selected medicinal plants from Asteraceae, Lamiaceae and Rosaceae families widely used in traditional medicine in Bosnia and Herzegovina

Plant species	Plant Family	Locality	Plant part	Flavonoids (mg QE/g DW)	Tannins (mg CA/g DW)	Total phenolics (mg GAE/g DW)	DPPH (mg TE/g DW)	FRAP (mg GAE/g DW)
Achillea millefolium L.	Asteraceae	Malešići	Aerial part	18.56 ± 0.38	nd	229.30^{de} ± 3.83	323.65e ± 6.20	129.69^{cd} ± 1.30
Arctium lappa L.	Asteraceae	Sjenina rijeka	Root	6.60 ± 1.48	nd	171.18^{bd} ± 5.62	213.58f ± 4.70	62.66^{e} ± 1.16
Artemisia absinthium L.	Asteraceae	Olovske Luke	Inflorescence	9.57 ± 0.23	nd	31.61^{f} ± 1.02	74.03 ± 4.70	32.90^{fg} ± 2.14
Artemisia vulgaris L.	Asteraceae	Olovske Luke	Inflorescence	15.10 ± 0.25	nd	221.27^{de} ± 1.27	339.93e ± 8.13	86.41^{e} ± 0.41
Calendula officinalis L.	Asteraceae	Trzanj	Flowers	12.12 ± 0.02	nd	50.11^{ef} ± 1.44	142.50 ± 0.08	17.60^{h} ± 1.90
Carlina acaulis L.	Asteraceae	Olovske Luke	Root	3.74 ± 0.20	nd	8.56^{h} ± 112.16	27.21 ± 1.96	13.35^{h} ± 0.38
Cichorium intybus L.	Asteraceae	Kobiljača	Root	1.86 ± 0.05	nd	27.43^{f} ± 0.47	22.84 ± 0.20	7.87^{h} ± 0.42
Helichrysum italicum (Roth) G. Don	Asteraceae	Vrapčići	Flowers	83.16 ± 2.83	nd	321.75^{b} ± 1.75	574.21b ± 25.80	100.50^{d} ± 1.21
Inula helenium L.	Asteraceae	Igman	Root	4.82 ± 0.17	nd	18.91^{f} ± 0.89	61.12 ± 1.44	5.30^{h} ± 0.51
Matricaria discoidea DC.	Asteraceae	Trzanj	Inflorescence	19.80 ± 0.15	nd	65.70^{ef} ± 1.72	215.36f ± 21.39	45.02^{fg} ± 0.27
Petasites hybridus (L.) G. Gaertn. & al.	Asteraceae	Bijambare	Leaves	12.58 ± 0.48	110.43 ± 2.97	191.00^{bd} ± 2.79	390.42de ± 10.42	92.07^{de} ± 5.96
Taraxacum officinale F. H. Wigg.	Asteraceae	Ponijeri	Flowers	19.50 ±0.04	nd	46.36ef ± 36.52	116.28 ± 5.38	20.37^{gh} ± 0.48
Taraxacum officinale F. H. Wigg.	Asteraceae	Sjenina rijeka	Leaves	24.83 ± 1.24	nd	101.67^{d} ± 1.49	140.58 ± 0.02	31.20^{gh} ± 0.15
Taraxacum officinale F. H. Wigg.	Asteraceae	Igman	Root	2.01 ± 0.02	nd	43.82ef ± 1.12	59.70 ± 2.70	7.86^{h} ± 0.20
Tussilago farfara L.	Asteraceae	Bijambare	Leaves	23.84 ± 0.69	nd	104.73^{d} ± 86.21	369.70de ± 3.82	119.35^{d} ± 2.17
Ajuga reptans L.	Lamiaceae	Trzanj	Leaves	5.87 ± 0.19	nd	42.62ef ± 0.32	72.20 ± 2.89	nd

Plant species	Plant Family	Locality	Plant part	Flavonoids (mg QE/g DW)	Tannins (mg CA/g DW)	Total phenolics (mg GAE/g DW)	DPPH (mg TE/g DW)	FRAP (mg GAE/g DW)
Glechoma hederacea L.	Lamiaceae	Sjenina rijeka	Leaves	34.91 ± 0.94	nd	125.44^{bd} ± 5.73	211.05^{f} ± 15.21	31.31^{fg} ± 1.07
Lamium maculatum (L.) L.	Lamiaceae	Sjenina rijeka	Inflorescence	34.85^{c} ± 1.31	nd	83.02^{ef} ± 0.38	294.24e^{fg}± 6.97	50.68^{g} ± 0.42
Lamium purpureum L.	Lamiaceae	Trzanj	Flowers	14.48^{e} ± 0.56	nd	56.47^{fg} ± 2.02	177.10^{g} ± 2.31	19.29^{h} ± 2.83
Lavandula angustifolia Mill.	Lamiaceae	Jablanica	Leaves	15.37^{e} ± 0.10	nd	34.29^{f} ± 0.67	21.94^{h} ± 0.06	11.35^{h} ± 1.39
Melissa officinalis L.	Lamiaceae	Sjenina rijeka	Leaves	32.94^{cd} ± 0	nd	380.92^{a} ± 10.12	357.68^{de} ± 52.25	251.64^{a} ± 0.98
Mentha longifolia (L.) L.	Lamiaceae	Ponijeri	Inflorescence	21.59^{d} ± 0.35	nd	226.50^{de} ± 8.18	379.23^{de} ± 5.56	155.78^{c} ± 1.13
Mentha spicata L.	Lamiaceae	Jablanica	Leaves	22.25^{d} ± 0.44	nd	223.62^{de} ± 0.47	324.36^{e} ± 8.26	113.50^{c} ± 0.02
Rosmarinus officinalis L.	Lamiaceae	Sedrenik	Leaves	9.15^{f} ± 0.09	nd	89.42^{ef} ± 0.60	74.22^{gh} ± 1.21	44.52^{g} ± 1.26
Salvia officinalis L.	Lamiaceae	Doboj	Leaves	24.56^{d} ± 0.25	nd	119.54^{e} ± 2.61	279.37^{fe} ± 19.03	71.39^{fg} ± 2.24
Thymus serpyllum L.	Lamiaceae	Ponijeri	inflorescence	33.64^{cd} ± 5.61	nd	261.66^{c} ± 11.77	391.69^{de} ± 2.81	125.52^{cd} ± 9.07
Crataegus germanica (L.) Kuntze	Rosaceae	Sjenina rijeka	Leaves	29.82^{d} ± 2.39	129.55^{c} ± 1.66	277.22^{c} ± 3.89	456.98^{c} ± 19.90	124.41^{cd} ± 1.04
Crataegus monogyna Jacq.	Rosaceae	Trzanj	fruits	11.06^{ef} ± 0.16	nd	91.43^{eg} ± 1.13	183.19^{f} ± 10.42	18.20^{g} ± 1.28
Crataegus rhipidophylla Gand.	Rosaceae	Ponijeri	Flowers	33.65^{cd} ± 1.40	113.53^{c} ± 14.65	238.43^{d} ± 4.57	395.65^{de} ± 9.69	51.01^{f} ± 3.53
Crataegus rhipidophylla Gand.	Rosaceae	Ponijeri	Leaves	23.74^{d} ± 0.73	152.86^{b} ± 6.72	283.32^{c} ± 47.67	294.06^{e} ± 0.61	193.89^{b} ± 1.00
Cydonia oblonga Mill.	Rosaceae	Sjenina rijeka	Leaves	25.10^{d} ± 0.05	nd	232.91^{de} ± 9.66	334.46^{de} ± 7.53	83.08^{e} ± 3.75
Filipendula vulgaris Moench	Rosaceae	Sjenina rijeka	Flowers	55.67^{a} ± 0.87	nd	311.24^{b} ± 6.60	702.39^{a} ± 8.01	172.43^{bd} ± 1.75
Fragaria vesca L.	Rosaceae	Sjenina rijeka	Leaves	23.99^{d} ±0.13	117.54^{c} ± 8.82	227.13^{de} ± 1.13	351.99^{d} ± 9.41	97.31^{de} ± 57.78
Malus pumila Mill.	Rosaceae	Sjenina rijeka	Fruit	0.56^{g} ± 0.02	nd	8.89^{h} ± 0.95	92.36^{g} ± 12.32	1.37^{f} ± 0.29

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Plant species	Plant Family	Locality	Plant part	Flavonoids (mg QE/g DW)	Tannins (mg CA/g DW)	Total phenolics (mg GAE/g DW)	DPPH (mg TE/g DW)	FRAP (mg GAE/g DW)
Prunus armeniaca L.	Rosaceae	Sjenina rijeka	Leaves	44.71^{b} ± 0.67	nd	206.08^{d} ± 2.68	313.18^{e} ± 1.76	100.15^{c} ± 10.00
Prunus avium (L.) L.	Rosaceae	Sjenina rijeka	Leaves	54.53^{a} ± 1.31	nd	136.65^{bd} ± 2.72	161.63^{f} ± 10.21	33.77^{fe} ± 0.96
Prunus domestica L.	Rosaceae	Ponijeri	Fruit	0.78^{g} ± 0.10	nd	12.77^{h} ± 0.41	37.52^{h} ±	2.29^{f} ±
Prunus persica (L.) Batsch	Rosaceae	Sjenina rijeka	Leaves	31.13^{c} ± 0.97	nd	65.44^{f} ± 1.85	52.24^{gh} ± 0.34	14.57^{f} ± 0.34
Prunus spinosa L.	Rosaceae	Trzanj	fruits	2.21^{g} ± 0.17	nd	34.14^{f} ± 1.63	71.24^{gh} ± 1.39	6.96^{f} ± 0.41
Rosa canina L.	Rosaceae	Sjenina rijeka	fruits	2.08^{g} ± 0.12	172.18^{a} ± 13.96	126.08^{de} ± 4.79	336.37^{de} ± 12.74	67.55^{e} ± 6.79
Rubus idaeus L.	Rosaceae	Trzanj	Leaves	40.41^{b} ± 1.03	61.81^{d} ± 4.89	227.45^{de} ± 9.01	393.62^{de} ± 6.84	139.42^{cd} ± 1.29
Rubus plicatus Weihe & Nees	Rosaceae	Sjenina rijeka	Leaves	25.03^{d} ± 0.61	64.57^{d} ± 13.60	363.86^{a} ± 5.24	435.77^{d} ± 27.25	125.00^{cd} ± 5.03

[a] nd – not detected. Plant extracts sharing the same letter in superscript following the average value within one parameter do not differ significantly following Newman Keuls ANOVA post hoc test at level of significance $p<0.01$.

The FRAP assay is dependent on pH values, and alkaline conditions can enhance proton dissociation of phenolic compounds increasing the reducing capacity of the extract. The FRAP assay is often correlated with the total phenolic content in the sample [29] but it is still considered a more robust method for measuring antioxidant capacity in plant extracts [30].

2.3. Antimicrobial activity

To evaluate antimicrobial potential of 38 commonly used traditional medicinal plants (42 different extracts from various plant organs) the disc diffusion method was used as a preselection tool prior to determining MIC and MBC. According to the results of the disc diffusion test, of the 42 tested extracts, only 7 plants showed no microbial activity, while some exhibited activity only against selected bacteria and/or fungi (Table 3). *Salmonella abony* showed the highest resistance to the evaluated extracts, with only five plants demonstrating antibacterial activity against this pathogen. The highest activity against *Salmonella* was recorded for *Rubus plicatus* leaf extracts. Leaf buds of *R. plicatus* have been found to be rich in polyphenolics, with high antioxidant capacity and high antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* [31]. High antimicrobial activity of *R. plicatus* was recorded in our study with larger inhibition zones against Methicillin-resistant *Staphylococcus aureus* (25 mm), *Pseudomonas aeruginosa* (25 mm), *Escherichia coli* (24 mm) and *Salmonella abony* (24 mm). Additionally, leaf extracts of *R. plicatus* have been efficient against *Candida albicans*, with inhibition zones of 11 mm (Table 3).

High antimicrobial activity was also recorded for *Filipendula vulgaris* flower extract against all tested bacteria with no activity against *Candida albicans* (Table 3). Interestingly, previous tests of *Filipendula vulgaris* flower essential oils, methanol, and water extracts against various microorganisms revealed no antibacterial activity [32]. The high antibacterial activity recorded in our study could be related to the extraction procedure and the solvent type, which may affect the type of polyphenols extracted, thereby improving antibacterial effect of collected extracts. Generally, ethanol is an efficient solvent for high molecular weight polyphenols, while methanol generally extracts lower molecular weight polyphenols [27].

Sixteen out of the 38 studied plants demonstrated considerable antimicrobial activity, with inhibition zones exceeding 20 mm. For these plants MIC and MBC were determined starting with extract concentrations of 20 mg/mL, and serial dilutions were performed until the minimal inhibitory/bactericidal concentration was reached. For *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus*, the most efficient extract was *Helichrysum italicum*, with MIC values lower than 0.07mg/mL and 0.15 respectively (Table 4). *Helichrysum italicum* was also the most efficient extract against *Candida albicans*, with MIC values of 0.31 mg/mL. For *Pseudomonas aeruginosa*, *Thymus serpyllum* inflorescence extract and *Filipendula vulgaris* flower extract showed the same efficiency, with MIC of 2.5 mg/mL. Extracts of *F. vulgaris* and *R. plitactus* were the only two plant extracts effective against *Escherichia coli* and *Salmonella abony*, respectively, both having MIC values of 10 mg/mL. An MBC value of 20 mg/mL or lower was observed for *Staphylococcus aureus* and *Methicillin resistant Staphylococcus aureus* in 9 and 13 of the 16 examined extracts, respectively (Table 5).

While no correlation was found between phenolic content and antifungal activity, a correlation was observed between phenolic content and antibacterial activity against all tested bacteria (Table 6). A strong correlation between flavonoid content and antifungal activity against *Candida albicans* has also been recorded. The potent antifungal effects of flavonoids against *Candida albicans* have been validated through *in vitro* testing of their biological activities over the past decade. High anti-*Candida* effects have been recorded for the flavonols quercetin, myricetin and kaempferol [33,34]. In this study, *Prunus persica* leaf extract showed significant activity against *Candida albicans* (15 mm inhibition zone), consistent with the high antimicrobial activity reported for leaf extract of this plant in other studies [35]. This activity can be attributed to the rich flavonoid content and the high number of flavonoid compounds found in *P. persica* [36].

Table 3. Antimicrobial potential assessed using agar well diffusion method of selected medicinal plants from Asteraceae, Lamiaceae and Rosaceae families widely used in traditional medicine in Bosnia and Herzegovina

Plant species	Plant Family	Locality	Plant part	*Staphylococcus aureus*	*Methicillin resistant Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella abony*	*Candida albicans*
Achillea millefolium L.	Asteraceae	Malešići	Aerial part	$18^{cd} \pm 2$	nd	$15^{c} \pm 1$	nd	nd	nd
Arctium lappa L.	Asteraceae	Sjenina rijeka	Root	$16^{de} \pm 0$	$19^{c} \pm 1$	nd	$12^{d} \pm 0$	nd	nd
Artemisia absinthium L.	Asteraceae	Olovske Luke	Inflorescence	$17^{d} \pm 0$	nd	nd	nd	nd	nd
Artemisia vulgaris L.	Asteraceae	Olovske Luke	Inflorescence	$14^{de} \pm 0$	$20^{c} \pm 0$	nd	nd	nd	nd
Calendula officinalis L.	Asteraceae	Trzanj	Flowers	$22^{cd} \pm 3$	$19^{c} \pm 0$	$18^{b} \pm 1$	nd	nd	nd
Carlina acaulis L.	Asteraceae	Olovske Luke	Root	nd	nd	nd	nd	nd	nd
Cichorium intybus L.	Asteraceae	Kobiljača	Root	nd	nd	nd	nd	nd	nd
Helichrysum italicum (Roth) G. Don	Asteraceae	Vrapčići	Flowers	$40^{a} \pm 0$	$40^{a} \pm 0$	$15^{c} \pm 0$	$11^{d} \pm 0$	nd	$39^{a} \pm 1$
Inula helenium L.	Asteraceae	Igman	Root	$20^{cd} \pm 0$	$18^{c} \pm 0$	nd	nd	nd	nd
Matricaria discoidea DC.	Asteraceae	Trzanj	Inflorescence	$19^{cd} \pm 2$	$22^{c} \pm 1$	$17^{bc} \pm 0$	nd	nd	nd
Petasites hybridus (L.) G. Gaertn. & al.	Asteraceae	Bijambare	Leaves	nd	$20^{c} \pm 0$	nd	nd	nd	nd
Taraxacum officinale F. H. Wigg.	Asteraceae	Ponijeri	Flowers	$5^{f} \pm 9$	$13^{e} \pm 0$	nd	nd	nd	$10^{c} \pm 0$
Taraxacum officinale F. H. Wigg.	Asteraceae	Sjenina rijeka	Leaves	nd	$23^{c} \pm 0$	nd	nd	nd	nd
Taraxacum officinale F. H. Wigg.	Asteraceae	Igman	Root	nd	nd	nd	nd	nd	nd
Tussilago farfara L.	Asteraceae	Bijambare	Leaves	$20^{cd} \pm 1$	nd	nd	nd	nd	nd
Ajuga reptans L.	Lamiaceae	Trzanj	Leaves	nd	nd	$13^{c} \pm 0$	nd	nd	nd
Glechoma hederacea L.	Lamiaceae	Sjenina rijeka	Leaves	nd	$18^{cd} \pm 0$	nd	nd	nd	nd
Lamium maculatum (L.) L.	Lamiaceae	Sjenina rijeka	Inflorescence	nd	nd	nd	nd	nd	nd
Lamium purpureum L.	Lamiaceae	Trzanj	Flowers	$12^{e} \pm 0$	$12^{e} \pm 1$	$13^{c} \pm 0$	nd	nd	$14^{ce} \pm 0$
Lavandula angustifolia Mill.	Lamiaceae	Jablanica	Leaves	nd	nd	nd	nd	nd	nd
Melissa officinalis L.	Lamiaceae	Sjenina rijeka	Leaves	$22^{cd} \pm 0$	$25^{bc} \pm 0$	$15^{c} \pm 0$	nd	nd	$13^{de} \pm 1$

Plant species	Plant Family	Locality	Plant part	*Staphylococcus aureus*	*Methicillin resistant Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella abony*	*Candida albicans*
Mentha longifolia (L.) L.	Lamiaceae	Ponijeri	inflorescence	$22^{cd} \pm 0$	$23^{c} \pm 0$	$12^{c} \pm 1$	nd	nd	nd
Mentha spicata L.	Lamiaceae	Jablanica	Leaves	$25^{c} \pm 1$	$25^{bc} \pm 1$	$15^{c} \pm 0$	nd	nd	nd
Rosmarinus officinalis L.	Lamiaceae	Sedrenik	Leaves	nd	nd	nd	nd	nd	nd
Salvia officinalis L.	Lamiaceae	Doboj	Leaves	$31^{b} \pm 1$	$21^{c} \pm 0$	$18^{b} \pm 1$	$11^{d} \pm 1$	$21^{ab} \pm 6$	$21^{b} \pm 4$
Thymus serpyllum L.	Lamiaceae	Ponijeri	inflorescence	$22^{cd} \pm 0$	$26^{b} \pm 1$	$13^{c} \pm 0$	nd	nd	nd
Crataegus germanica (L.) Kuntze	Rosaceae	Sjenina rijeka	Leaves	$14^{de} \pm 1$	$15^{e} \pm 1$	nd	nd	nd	$10^{g} \pm 0$
Crataegus monogyna Jacq.	Rosaceae	Trzanj	fruits	nd	nd	nd	nd	nd	$12^{df} \pm 1$
Crataegus rhipidophylla Gand.	Rosaceae	Ponijeri	Flowers	$12^{e} \pm 1$	$13^{e} \pm 1$	nd	nd	nd	nd
Crataegus rhipidophylla Gand.	Rosaceae	Ponijeri	Leaves	$15^{d} \pm 0$	$17^{de} \pm 1$	nd	nd	nd	nd
Cydonia oblonga Mill.	Rosaceae	Sjenina rijeka	Leaves	$13^{de} \pm 0$	$12^{e} \pm 0$	$13^{c} \pm 0$	nd	nd	nd
Filipendula vulgaris Moench	Rosaceae	Sjenina rijeka	Flowers	$23^{c} \pm 0$	$29^{b} \pm 1$	$26^{a} \pm 1$	$24^{a} \pm 1$	$15^{b} \pm 8$	nd
Fragaria vesca L.	Rosaceae	Sjenina rijeka	Leaves	$16^{de} \pm 1$	$20^{c} \pm 0$	$18^{b} \pm 1$	$14^{c} \pm 1$	$15^{b} \pm 1$	nd
Malus pumila Mill.	Rosaceae	Sjenina rijeka	Fruit	nd	nd	nd	nd	nd	nd
Prunus armeniaca L.	Rosaceae	Sjenina rijeka	Leaves	nd	$10^{e} \pm 0$	nd	nd	nd	nd
Prunus avium (L.) L.	Rosaceae	Sjenina rijeka	Leaves	nd	nd	nd	nd	nd	nd
Prunus domestica L.	Rosaceae	Ponijeri	Fruit	nd	nd	nd	nd	nd	nd
Prunus persica (L.) Batsch	Rosaceae	Sjenina rijeka	Leaves	$19^{cd} \pm 1$	$18^{cd} \pm 0$	nd	nd	nd	$15^{c} \pm 1$
Prunus spinosa L.	Rosaceae	Trzanj	fruits	nd	nd	nd	nd	nd	nd
Rosa canina L.	Rosaceae	Sjenina rijeka	fruits	nd	nd	$14^{c} \pm 0$	nd	nd	nd
Rubus idaeus L.	Rosaceae	Trzanj	Leaves	$19^{cd} \pm 1$	$21^{c} \pm 0$	$20^{b} \pm 1$	$17^{b} \pm 1$	$19^{ab} \pm 1$	nd
Rubus plicatus Weihe & Nees	Rosaceae	Sjenina rijeka	Leaves	$11^{e} \pm 1$	$25^{bc} \pm 0$	$25^{a} \pm 1$	$24^{a} \pm 0$	$24^{a} \pm 0$	$11^{g} \pm 0$

[a]nd – not detected. Plant extracts sharing the same letter in superscript following the average value within one parameter do not differ significantly following Newman Keuls ANOVA post hoc test at level of significance $p<0.01$.

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Table 4. Identified minimal inhibitory concentration of selected medicinal plants from Asteraceae, Lamiaceae and Rosaceae families widely used in traditional medicine in Bosnia and Herzegovina

Plant species	**Plant Family**	**Plant part**	***Staphylococcus aureus***	***Methicillin resistant Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Salmonella abony***	***Candida albicans***
			Identified MIC concentration (mg/mL)					
Artemisia vulgaris L.	Asteraceae	Inflorescence	nd	10	nd	nd	nd	nd
Calendula officinalis L.	Asteraceae	Flowers	0.31	nd	20	nd	nd	nd
Helichrysum italicum (Roth) G. Don	Asteraceae	Flowers	<0.07	0.15	nd	nd	nd	0.31
Inula helenium L.	Asteraceae	Root	2.5	nd	nd	nd	nd	nd
Matricaria discoidea DC.	Asteraceae	Inflorescence	nd	5	nd	nd	nd	nd
Petasites hybridus (L.) G. Gaertn. & al.	Asteraceae	Leaves	nd	10	nd	nd	nd	nd
Taraxacum officinale F. H. Wigg.	Asteraceae	Leaves	nd	10	nd	nd	nd	nd
Melissa officinalis L.	Lamiaceae	Leaves	5	5	nd	nd	nd	nd
Mentha longifolia (L.) L.	Lamiaceae	Inflorescence	2.5	10	nd	nd	nd	nd
Mentha spicata L.	Lamiaceae	Leaves	5	10	nd	nd	nd	nd
Salvia officinalis L.	Lamiaceae	Leaves	0.31	1.25	5	nd	20	2.5
Thymus serpyllum L.	Lamiaceae	Inflorescence	5	10	nd	nd	nd	nd
Filipendula vulgaris Moench	Rosaceae	Flowers	0.62	0.62	2.5	10	nd	nd
Fragaria vesca L.	Rosaceae	Leaves	nd	2.5	nd	nd	nd	nd
Rubus idaeus L.	Rosaceae	Leaves	nd	5	5	nd	nd	nd
Rubus plicatus Weihe & Nees	Rosaceae	Leaves	nd	1.25	2.5	10	10	nd
Ampicillin/Nystatin			<0.001	1	>1	0.004	0.0034	0.002

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Table 5. Identified minimal bactericidal concentration (MBC) of selected medicinal plants from Asteraceae, Lamiaceae and Rosaceae families widely used in traditional medicine in Bosnia and Herzegovina

Plant species	Plant Family	Plant part	*Staphylococcus aureus*	*Methicillin resistant Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella abony*	*Candida albicans*
			Identified MBC concentration (mg/mL)					
Artemisia vulgaris L.	Asteraceae	Inflorescence	nd	20	nd	nd	nd	nd
Calendula officinalis L.	Asteraceae	Flowers	1.25	nd	>20	nd	nd	nd
Helichrysum italicum (Roth) G. Don	Asteraceae	Flowers	0.62	0.62	nd	nd	nd	2.5
Inula helenium L.	Asteraceae	Root	10	nd	nd	nd	nd	nd
Matricaria discoidea DC.	Asteraceae	Inflorescence	nd	20	nd	nd	nd	nd
Petasites hybridus (L.) G. Gaertn. & al.	Asteraceae	Leaves	nd	20	nd	nd	nd	nd
Taraxacum officinale F. H. Wigg.	Asteraceae	Leaves	nd	20	nd	nd	nd	nd
Melissa officinalis L.	Lamiaceae	Leaves	10	20	nd	nd	nd	nd
Mentha longifolia (L.) L.	Lamiaceae	Inflorescence	20	20	nd	nd	nd	nd
Mentha spicata L.	Lamiaceae	Leaves	20	20	nd	nd	nd	nd
Salvia officinalis L.	Lamiaceae	Leaves	2.5	nd	nd	>20	>20	10
Thymus serpyllum L.	Lamiaceae	Inflorescence	20	>20	nd	nd	nd	nd
Filipendula vulgaris Moench	Rosaceae	Flowers	2.5	2.5	20	>20	nd	nd
Fragaria vesca L.	Rosaceae	Leaves	nd	10	nd	nd	nd	nd
Rubus idaeus L.	Rosaceae	Leaves	nd	20	>20	nd	nd	nd
Rubus plicatus Weihe & Nees	Rosaceae	Leaves	nd	5	20	>20	>20	nd
Ampicillin/Nystatin	-	-	0.016	>1		>1	0.031	0.031	0.016

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Table 6. Correlation between secondary metabolites, antioxidant, and antimicrobial activity of selected medicinal plants from Asteraceae, Lamiaceae and Rosaceae families widely used in traditional medicine in Bosnia and Herzegovina

	Flavonoids (mg QE/g DW)	Tannins (mg CA/g DW)	Phenolics (mg GAE/g DW)	DPPH (mg TE/g DW)	FRAP (mg GAE/g DW)
Staphylococcus aureus subsp. aureus	**0.436***	-0.130	**0.457***	**0.498***	**0.476***
Methicillin-resistant Staphylococcus aureus	**0.533***	-0.001	**0.596***	**0.571***	**0.472***
Pseudomonas aeruginosa	**0.312***	0.069	**0.493***	**0.552***	**0.477***
Escherichia coli	**0.388***	0.137	**0.481***	**0.537***	**0.360***
Salmonella abony	0.231	0.191	**0.357***	**0.393***	0.302
Candida albicans	**0.479***	-0.061	0.269	0.268	0.123

[a] *statistically significant Pearson correlation at level of $p<0.01$.

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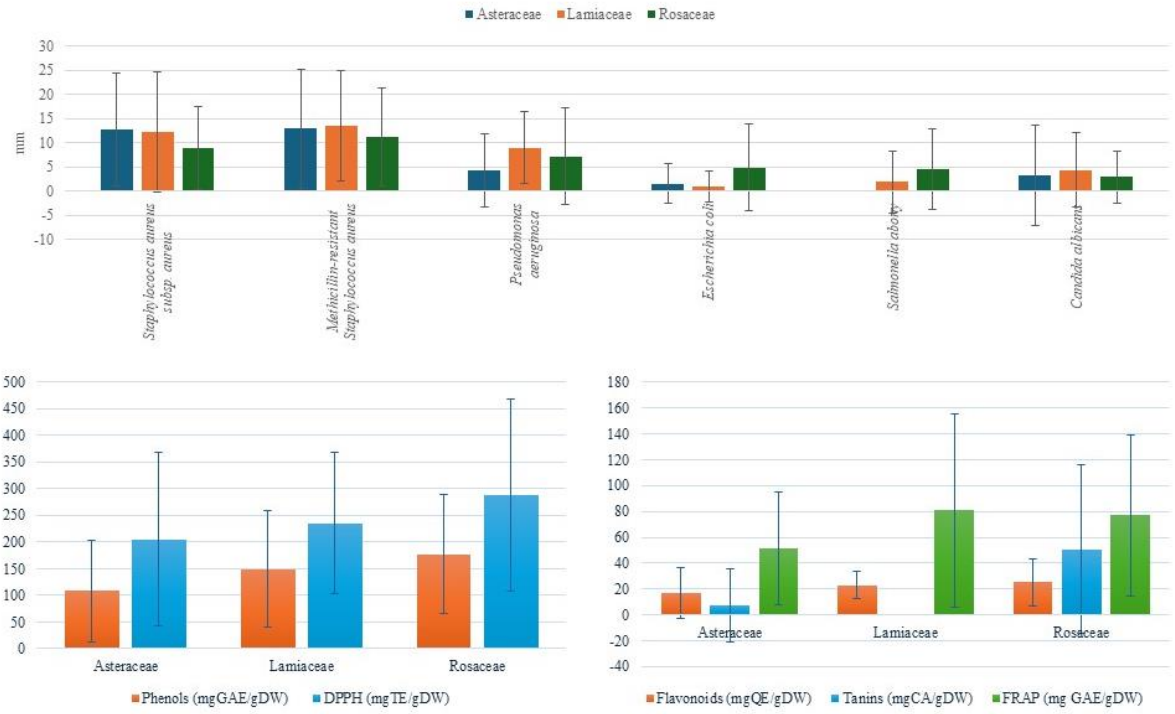

Figure 1. Comparison of phenolics, flavonoids, tannins, antimicrobial and antioxidant activity across three investigated plant families. Standard deviation indicates overall deviation across different organs and plant species within one plant Family.

3. CONCLUSION

The significant antimicrobial activity observed in this study can be attributed to the high content of bioactive compounds, particularly phenolics and flavonoids, in the tested plant extracts. The strong correlation between phenolic content and antibacterial activity, especially against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*, as well as the observed antifungal efficacy of flavonoids against *Candida albicans*, highlights the therapeutic potential of these plants. Notably, extracts from *Helichrysum italicum* and *Rubus plicatus* demonstrated the most potent antimicrobial effects, with MIC and MBC values as low as 0.07 mg/mL and 20 mg/mL, respectively. These findings validate the traditional use of these plants in treating infections and suggest that they could be valuable sources for developing natural antimicrobial agents. Further research into the specific bioactive compounds responsible for these effects could lead to the development of new, plant-based therapies for resistant microbial strains.

4. MATERIALS AND METHODS

4.1. Sample collection

The plant species and materials were selected based on ethnobotanical studies [9, 10; 14, 16] that describe their common usage in traditional medicine in Bosnia and Herzegovina. The locations from which the plant material was collected are summarised in Figure 2, while the specific localities for each plant are provided within the results section. The plant material was air-dried, stored and ground into powder prior to extraction. Each sample was assigned a voucher number, and voucher sample was stored in Laboratory for Plant physiology, Faculty of Science, University of Sarajevo.

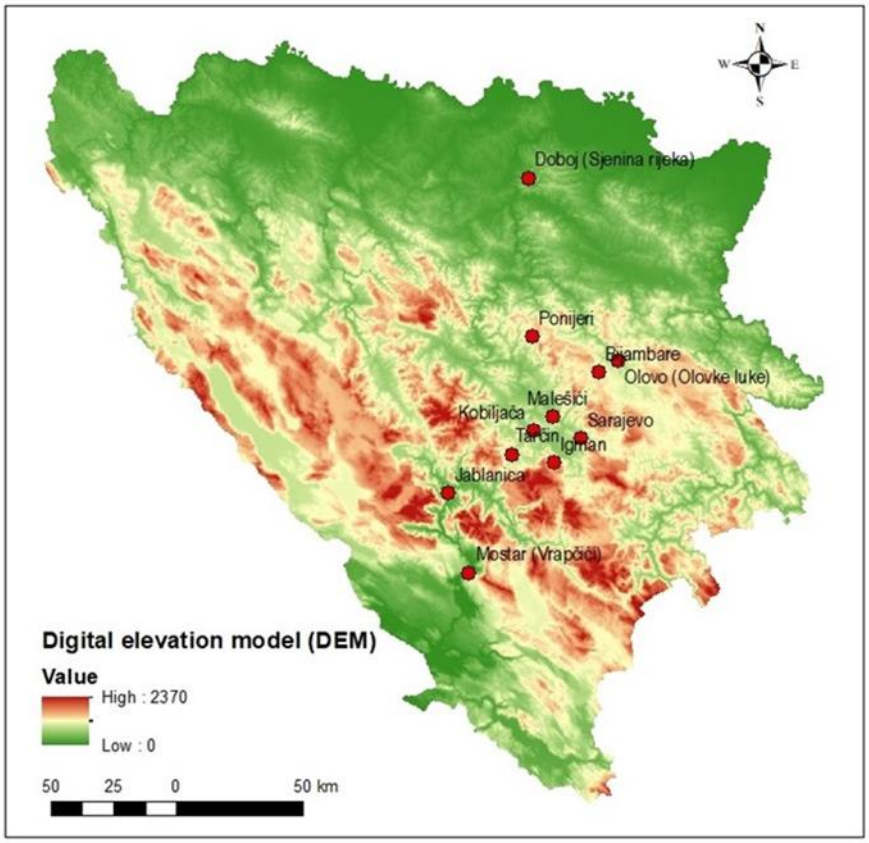

Figure 2. Sites where plant samples from the three studied plant families have been gathered.

4.2. Preparation of plant extracts

Powdered plant material (15 g) was extracted with 50 mL 70% ethanol for 20 min at 40°C and re-extracted with the same solvent volume, using an ultrasonic bath. The solvent was evaporated under reduced pressure. Prior to analyses, the crude extracts were redissolved in a mixture of ethanol and water (80:20) to achieve concentrations of 1-2 mg/mL.

Determination of total phenolics

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent. Briefly, 0.2 mL of the plant extract was added to the tubes containing 1.0 mL of a 1/10 water diluted Folin-Ciocalteu reagent. After 5 minutes, 0.8 mL of sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 30 min before measuring the absorbance at 743 nm. Results are expressed in gallic acid equivalents per gram of dry weight (mg GAE/g DW).

4.3. Determination of total flavonoids

The aluminium chloride colorimetric method [17] was used to analyse the flavonoid content, with a methanolic solution of quercetin (10–100 µg/mL) used to construct a standard calibration curve. The TFC is reported as quercetin equivalents per gram of dry weight (mg QE/g DW).

4.4. Determination of condensed tannins

The presence of tannins in all extracts was tested using a qualitative reaction with 5% $FeCl_3$, where a positive reaction is indicated by the production of a blue-green or dark green precipitate [18]. Samples with a positive reaction for tannins were further analysed to quantify the tannin content using a modified method [19]. To 0.25 mL of extract, 1.5 mL vanillin solution (4% in methanol) and 0.75 mL concentrated HCl were added. After 15 min of incubation at 20 °C in the dark, the absorbance was read at 500 nm. The condensed tannin was expressed as mg catechin equivalents per gram of dry weight (mg CE/g DW).

4.5. Antioxidant activity

The antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reducing ability of plasma) assay. The DPPH assay was performed following the method by [20]. To measure the antioxidant activity, 1 mL extract was mixed with 1 mL 0.16 mM DPPH solution in methanol. The mixture was then left to stand for 30 min in the dark, and absorbance was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a control. Results are expressed in milligrams of Trolox equivalents per gram of dry weight (mg TE/ g DW).
The FRAP analysis was performed following the method by [21]. The extract, standard, or blank (0.1 mL) was mixed with 3 mL of the FRAP reagent and 0.3 mL of distilled water, and the reaction was read at 593 nm after 6 min of incubation at 37 °C. The FRAP reagent was freshly prepared by mixing a 10 mM TPTZ solution in 40 mM HCl (37%), 20 mM $FeCl_3 \cdot 6H_2O$ solution, and 0.3 M acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v/v).

4.6. Antimicrobial activity

Antimicrobial activity was evaluated against five bacterial and one fungal strain. The bacterial strains used in the analysis included Gram-positive *Staphylococcus aureus* subsp. *aureus* ATCC 6538, Methicillin-resistant *Staphylococcus aureus* ATCC 33591, and Gram-negative *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Salmonella abony* ATCC 6017 and the yeast *Candida albicans* ATCC 10231.

4.6.1. Agar well diffusion method

The agar well diffusion method and microdilution method were used for the antimicrobial assay. Bacterial inoculum was obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar substrate and was standardized to 5×10^8 CFU/mL using McFarland standard for the agar well diffusion method. The agar well diffusion method was used to evaluate the antimicrobial activity of plant extracts and standards according to the National Committee for Clinical Laboratory Standards [22]. Each well contained 100 µL of extract (20 mg/mL). Ampicillin was used as a positive standard for bacterial strains and nystatin for *Candida albicans*. Ethanol was used as a negative control. The antimicrobial effect was expressed as the diameter of the inhibition zone in mm. Samples with a diameter zone > 20 mm were selected for further evaluation of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

4.6.2. Minimal inhibitory concentration

The MIC was determined by the broth microdilution method using 96-well microtiter plates according to NCCLS guidelines [23]. Bacterial inoculum was obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar substrate and was standardized to 5×10^5 CFU/mL for the microdilution method. A series of dilutions with concentrations ranging from 20 to 0.07 mg/mL of extracts were prepared for each microorganism. Two-fold dilutions of the extracts were prepared in Müller-Hinton broth (MHB). The 96-well plates were first filled with 100 µL MHB followed by 200 µL of plant extract, which was added to the first well and serially diluted up to the 9th well. The excess 100 µL was removed from the 9th well. Finally, 100 µL of MHB mixed with inoculum was added from the 1st to the 9th well. The 10th well contains broth with inoculum as fertility control, while the 11th well contained broth without intervention as sterility control and the 12th well contained plant extract. The final volume in each well was 200 µL. After incubation, the MIC was determined with resazurin. The boundary dilution where there was no color change of resazurin was defined as the MIC for the tested microorganism at the given concentration and expressed as mg/mL. Ampicillin was used as a positive control for bacterial growth inhibition, and nystatin for fungi.

4.6.3. Minimal bactericidal/fungicidal concentration

Minimum bactericidal/fungicidal concentration (MBC/MFC) was determined by re-culturing bacterial strains from the well plates that had an equal to or greater concentration of extracts than the MIC. The concentration of extracts where the first visual signs of bacterial growth were visible in the plates was considered as MBC/MFC and expressed as mg/mL.

4.7. Statistical analysis

All data were analysed using the STATISTICA 10.0 software (Statsoft Inc.). Experimental results were presented as the mean ± standard deviation of three independent replications. The obtained data were subjected to variance analysis (ANOVA) and the Newman-Keuls post hoc test was carried out to identify significant differences between the analysed plants and extracts. Mean values with $p<0.01$ were considered

statistically significant. Pearson correlations were performed to observe the possible correlation between the phenolic profile, antioxidant capacity, and detected antimicrobial activity at the level of significance $p<0.01$.

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Author contributions: Conceptualization, methodology, supervision - S.I; plant material identification, analysis -S. D.; Microbial testing - R. B.G.; Collection of plant material, plant identification, plant analysis - S. D.; Collection of plant material, analysis – H. G.; Writing - original draft, review and editing, formal analysis – E. K. All authors read and revised the manuscript.

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