applied sciences

MDPI

Article

Polyphenol Content, Mineral Compounds Composition, Antimicrobial and Antioxidant Activities of Selected Medicinal Herbs from Slovak Republic

Eva Ivanišová [1,*], Marek Horňák [1], Matej Čech [1], Ľuboš Harangozo [1], Miroslava Kačániová [2,3], Olga Grygorieva [4] and Przemysław Łukasz Kowalczewski [5,*]

1 Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovakia
2 Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovakia
3 Department of Bioenergy, Food Technology and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, 35-601 Rzeszów, Poland
4 M.M. Gryshko National Botanical Garden of Ukraine of National Academy of Sciences, 01014 Kyiv, Ukraine
5 Department of Food Technology of Plant Origin, Poznań University of Life Sciences, 60-624 Poznań, Poland
* Correspondence: eva.ivanisova@uniag.sk (E.I.); przemyslaw.kowalczewski@up.poznan.pl (P.Ł.K.)

Abstract: The aim of this study was to determine antioxidant activity (DPPH and phosphomolybdenum method), polyphenols content (total polyphenols, flavonoids, and phenolic acids), mineral compounds composition (Cu, Zn, Mn, Fe, Cr, Ni, Co, Pb and Cd) and antimicrobial activity (with disc diffusion method) of medicinal herbs traditionally used in the Slovak republic. The tested plants belonged to the Primulaceae, Urticaceae, Grossulariaceae, Rosaceae, Lamiaceae, Asteraceae, Equisetaceae, Tropaeolaceae, and Plantaginaceae families. The highest antioxidant activities were found in samples of *Rosa canina* L. (DPPH—29.43 ± 0.11 mg TE/g; TE—Trolox equivalent) and *Fragaria vesca* L. (phosphomolybdenum method—679.56 ± 3.06 mg TE/g), both from the *Rosaceae* family. Total polyphenols (determined using the Folin–Ciocâlteu-reagent) were most abundant in a sample of *Fragaria vesca* L.—124.51 ± 5.05 mg GAE/g (GAE—gallic acid equivalent), total flavonoids (determined using the aluminum chloride method)—in a sample of *Primula veris* L.—48.35 ± 3.77 mg QE/g (QE—quercetin equivalent), and total phenolic acids (determined using Arnova reagent)—in a sample of *Thymus serpyllum* L.—102.31 ± 2.89 mg CAE/g (CAE—caffeic acid equivalent). Regarding mineral compounds composition, samples of *Fragaria vesca* L. and *Thymus serpyllum* L. showed the highest levels of iron. In samples of *Calendula officinalis* L. and *Trapaeolum majus* L., the highest amounts of zinc were determined, while copper was the most abundant in samples of *Urtica dioica* L. and *Melissa officinalis* L. The amounts of heavy metals were within legally acceptable limits. The extract of *Equisetum arvense* L. showed the strongest inhibitory activity towards *Clostridium perfringens* CCM 4991 (6 mm), while the one from *Mentha piperita* L.—towards *Candida glabrata* CCM 8270 (4.83 mm) and *Candida tropicalis* CCM 8223 (4.33 mm).

Keywords: plants; bioactive compounds; antibacterial activity; microelements; heavy metals

Citation: Ivanišová, E.; Horňák, M.; Čech, M.; Harangozo, Ľ.; Kačániová, M.; Grygorieva, O.; Kowalczewski, P.Ł. Polyphenol Content, Mineral Compounds Composition, Antimicrobial and Antioxidant Activities of Selected Medicinal Herbs from Slovak Republic. *Appl. Sci.* **2023**, *13*, 1918. https://doi.org/10.3390/app13031918

Academic Editor: Monika Gibis

Received: 11 January 2023
Revised: 30 January 2023
Accepted: 1 February 2023
Published: 2 February 2023

1. Introduction

The use of medicinal herbs in disease treatment has been known since the beginning of civilization. These plants are also basic sources of nutritionally important chemical compounds. In general, medicinal plants are plants that, depending on their specific properties, are used in the treatment or as preventive measures for many diseases. The modern, busy lifestyle increases the risk of the development of many diseases caused by free radicals [1]. Plants used in traditional medicine are rich in various antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid, and enzymes

with antioxidant activity, which help the body in the fight against dangerous oxidative damage [2–6]. Approximately 500 medicinal plants that exert pharmacological effects are known in Europe. The annual increase in the consumption of these plants ranges from 8 to 9%, with Europe being the biggest consumer. The food, cosmetic, pharmaceutical, and chemical industries are the main contributors to global consumption growth. The range of specific applications of medicinal plants is broad [7–9].

The recently observed trend to use medicinal plants not only in traditional medicine but also in the food, pharmaceutical and cosmetic industries as well as modern gastronomy is a result of their sensory and biological value. This value comes from polyphenols that are found only in the plant kingdom. A number of substances contained in plants have proven effects on mental and physical conditions [10–12]. The most widespread way of administration is in the form of infusions [13]. In medicine, herbs find unlimited use because of their application in the treatment of many diseases, such as diseases of the digestive system, respiratory and urinary tracts, colds, skin problems, etc. They also have a positive effect on metabolism and are characterized by excellent detoxifying, anti-inflammatory and antibacterial properties, and many other therapeutic effects [14]. Considering their antioxidant and antimicrobial effects, such plants can be used in food preservation and for increasing the biological value of food. The increase in synthetically produced drugs and the associated increased occurrence of adverse side effects and intoxications has led to more and more therapists reaching for original plant drugs [2]. Plants contain a large number of natural chemicals or phytochemicals that interact with the active ingredient and help prevent side effects [15,16]. Treatment with synthetic drugs often involves the use of one concentrated component, which may result in the need for other pharmaceuticals that work against its adverse effects [17].

In the Slovak Republic, medicinal plants are paid outstanding attention. Collection, evaluation, reproduction and preservation of the gene pool of medicinal plants are of high priority, with 156 samples of medicinal plants deposited in the Gene Bank of the Slovak Republic in Piešťany. Of this number, 115 are in the active collection, 30 in the basic collection, and 11 in the field collection [18].

The aim of this study was to determine the antioxidant activity (DPPH and phosphomolybdenum method), polyphenols content (total polyphenols, flavonoids, and phenolic acids), mineral compounds composition (Cu, Zn, Mn, Fe, Cr, Ni, Co, Pb and Cd), and antimicrobial activity (with disc diffusion method) of medicinal herbs traditionally used in the Slovak Republic. The tested species and their specific parts were: *Primula veris* L.—flowers, *Urtica dioica* L.—leaves, *Ribes nigrum* L.—leaves, *Fragaria vesca* L.—leaves, *Mentha piperita* L.—leaves, *Thymus serpyllum* L.—flowering herbs, *Salvia officinalis* L.—leaves, *Potentilla anserina* L.—leaves, *Equisetum arvense* L.—leaves, *Melissa officinalis* L.—leaves, *Rosa canina* L.—fruits, *Calendula officinalis* L.—flowers, *Matricaria chamomilla* L.—flowers, *Achillea millefolium* L.—flowering herbs, *Tropaeolum majus* L.—seeds, *Plantago lanceolata* L.—leaves.

2. Materials and Methods

2.1. Materials

The following parts of medicinal plants obtained, collected in the locality of the village of Vrbovce—341 m.a.s.l. (Myjava district), were analyzed: *Primula veris* L.—flowers, *Urtica dioica* L.—leaves, *Ribes nigrum* L.—leaves, *Fragaria vesca* L.—leaves, *Mentha piperita* L.– leaves, *Thymus serpyllum* L.—flowering herbs, *Salvia officinalis* L.—leaves, *Potentilla anserina* L.—leaves, *Equisetum arvense* L.—leaves, *Melissa officinalis* L.—leaves, *Rosa canina* L.—fruits, *Calendula officinalis* L.—flowers, *Matricaria chamomilla* L.—flowers, *Achillea millefolium* L.– flowering herbs, *Tropaeolum majus* L.—seeds, *Plantago lanceolata* L.—leaves. All plants came from free harvest and were collected in the period recommended by the pharmacopoeia. The plant parts were botanically identified at the Institute of Food Sciences of the Faculty of Biotechnology and Food Sciences. They were dried at room temperature without access to sunlight and were stored in dark glass containers without

exposure to light. Before the analysis, all parts—flowers, leaves, fruits, seeds, and flowering herbs—were crushed to powder using a mortar.

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Bratislava, Slovakia).

2.2. Sample Preparation

A 0.2 g sample was extracted with 20 mL of 80% ethanol for 2 h. The supernatant, obtained by centrifugation at 4000× *g* (Rotofix 32 A, Hettich, Germany) for 10 min, was used for measurements (antioxidant activity, polyphenols, flavonoids, phenolic acids and antimicrobial activity). The extraction was carried out in triplicate.

2.3. Radical Scavenging Activity—DPPH Method

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [19]. The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the reaction mixture was determined using a spectrophotometer (Jenway 6405 UV/Vis, Nottingham, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; R^2 = 0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

2.4. Phosphomolybdenum Method

The reducing power of extracts was determined by the phosphomolybdenum method of Prieto et al. [20] with slight modifications. The mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled. Then, absorbance was measured at 700 nm using a spectrophotometer. Trolox (10–1000 mg/L; R^2 = 0.998) was used as the standard and the results were expressed in mg/g Trolox equivalents.

2.5. Total Polyphenol Content

Total polyphenol content was measured using Folin–Ciocâlteu-reagent in accordance with Singleton and Rossi [21]. A 0.1 mL sample was mixed with 0.1 mL of the Folin–Ciocâlteu-reagent, 1 mL of 20% (*w*/*v*) sodium carbonate, and 8.8 mL of distilled water, and left in darkness for 30 min. The absorbance at 700 nm was measured using a spectrophotometer. Gallic acid (25–300 mg/g; R^2 = 0.998) was used as a standard and the results were expressed in mg/g of gallic acid equivalent. The analysis was carried out in triplicate.

2.6. Total Flavonoid Content

Total flavonoids were determined using a modified method of Willett [22]. The sample (0.5 mL) was mixed with 0.1 mL of 10% (*w*/*v*) ethanolic solution of aluminum chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using a spectrophotometer. Quercetin (0.5–20 mg/L; R^2 = 0.989) was used as the standard and the results were expressed in mg/g quercetin equivalents.

2.7. Total Phenolic Acid Content

Total phenolic acids content was determined using the method of Jain et al. [23]. A 0.5 mL sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (*w*/*v*), and 0.5 mL of water. Absorbance at 490 nm was measured using a spectrophotometer. Caffeic acid (1–200 mg/L, R^2 = 0.999) was used as the standard and the results were expressed in mg/g caffeic acid equivalents.

2.8. Determination of the Content of Mineral Compounds

Samples were prepared by digesting 1 g of the material with a mixture of HNO3: redistilled water (1:1) for 55 min in a closed vessel high-pressure microwave digester (MARS X-press, New Zeland, Australia). After cooling to room temperature, the suspension was filtered through Munktell filter paper (grade 390.84 g/m^2, Bärenstein, Germany) and diluted to 50 mL with distilled water. Then, the sample digestates were subsequently analyzed for Cd, Pb, Cu, Zn, Co, Cr, Ni, Mn, and Fe using an atomic absorption spectrometer (Varian AA 240 FS, New York, NY, USA). The instrument was equipped with a D2 lamp background correction system, and an air–acetylene flame was used (air 13.5 L/min, acetylene 2.0 L/min, Varian, Ltd., Mulgrave, Australia). The wavelengths at which the heavy metals were tested following the calibration process were: Cd—228.8 nm, Pb—217.0 nm, Cu—324.8 nm, Zn—213.9 nm, Co—240.7 nm, Cr—357.9 nm, Ni—232.0 nm, Mn—279.5 nm, Fe—241.8 nm. The quantitative results were obtained using a multielemental standard for GF AAS (CertiPUR®, Merck, Darmstadt, Germany). The analysis was carried out in triplicate.

2.9. Antimicrobial Activity

Antimicrobial activity was tested with the disc diffusion method. Altogether, eight strains of microorganisms were used, including three yeasts (*Candida glabrata* CCM 8270, *Candida albicans* CCM 8186, and *Candida tropicalis* CCM 8223), one Gram-negative bacteria (*Haemophilus influenzae* CCM 4454) and three Gram-positive bacteria (*Staphylococcus aureus* CCM 2461, *Clostridium perfringens* CCM 4991, and *Enterococcus faecalis* CCM 4224). All the tested strains were from the Czech Collection of Microorganisms. The bacterial and yeast suspensions were cultured in the nutrient broth (Imuna, Bratislava, Slovakia) at 37 °C for 24 h before testing. A 0.1 mL aliquot of the suspension of the tested microorganism (10^5 cfu/mL) was spread on Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK). Filter paper discs of 6 mm in diameter were impregnated with 15 µL of the extract and placed on the inoculated agar plates. The plates were left at 4 °C for 2 h and then incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured after incubation. The analysis was carried out in triplicate.

2.10. Statistical Analysis

All the experiments were carried out in triplicate and the mean of replications with standard deviations were reported. The experimental data were subjected to analysis of variance (Duncan's test) at the significance level of 0.05 using SAS v9.2 software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Antioxidant Activity

The antioxidant activity determined in medicinal plants using the DPPH method ranged from 3.16 ± 0.51 mg TE/g to 29.43 ± 0.10 mg TE/g. All the samples demonstrated the ability to inhibit DPPH radicals and be ranked in the following order based on the results of this measurement: *Rosa canina* L. > *Urtica dioica* L. > *Fragaria vesca* L. > *Equisetum arvense* L. > *Ribes nigrum* L. > *Tropaeolum majus* L. > *Potentilla anserina* L. > *Matricaria chamomilla* L. > *Mentha piperita* L. > *Primula veris* L. > *Achillea millefolium* L. > *Melissa officinalis* L. > *Plantago lanceolata* L. > *Thymus serpyllum* L. > *Salvia officinalis* L. > *Calendula officinalis* L. (Table 1). Taneva et al. [24], analyzed the antioxidant activity of rosehip fruit extracts (*Rosa canina* L.) obtained using three different methods (distilled water, 50% ethanol, 70% ethanol), and found the following values: 211.5 ± 5.5 mM TE/g (distilled water), 295.0 ± 1.0 mM TE/g (50% ethanol) and 286.4 ± 2.9 mM TE/g (70% ethanol). Rovná et al. [25] also confirmed the high antioxidant activity of *R. canina*. It is known that the fruits of rosehip have the highest content of vitamin C (30–1300 mg/g) among fruits and vegetables. In addition, rosehip also contains other vitamins, mineral compounds, carotenoids, tocopherols, flavonoids, organic

acids, tannins, pectin, sugars, amino acids, and essential oils, which are all responsible for antioxidant activity [26].

Table 1. Antioxidant activity of tested medicinal herbs.

Sample	DPPH (mg TE/g DM)	Reducing Power (mg TE/g DM)
Primula veris L.	10.39 ± 0.11 ^{f}	322.01 ± 3.74 ^{g}
Urtica dioica L.	24.09 ± 0.36 ^{b}	186.53 ± 15.29 ^{jk}
Ribes nigrum L.	16.72 ± 0.31 ^{d}	417.24 ± 11.89 ^{e}
Fragaria vesca L.	22.24 ± 1.22 ^{bc}	679.65 ± 3.06 ^{a}
Mentha piperita L.	10.48 ± 3.62 ^{f}	328.61 ± 11.89 ^{g}
Thymus serpyllum L.	9.77 ± 1.89 ^{fg}	492.69 ± 4.08 ^{d}
Salvia officinalis L.	7.57 ± 0.71 ^{g}	356.81 ± 17.67 ^{f}
Potentilla anserina L.	13.39 ± 1.48 ^{e}	206.99 ± 4.76 ^{ij}
Equisetum arvense L.	21.17 ± 0.31 ^{c}	171.64 ± 13.25 ^{k}
Melissa officinalis L.	10.26 ± 0.21 ^{f}	472.09 ± 30.24 ^{d}
Rosa canina L.	29.43 ± 0.11 ^{a}	542.39 ± 35.68 ^{c}
Calendula officinalis L.	3.16 ± 0.51 ^{h}	185.82 ± 5.09 ^{jk}
Matricaria chamomilla L.	12.86 ± 0.87 ^{e}	223.64 ± 2.72 ^{hi}
Achillea millefolium L.	10.28 ± 0.05 ^{f}	197.01 ± 12.23 ^{jk}
Tropaeolum majus L.	14.85 ± 0.82 ^{de}	237.26 ± 2.04 ^{h}
Plantago lanceolata L.	10.21 ± 2.39 ^{f}	585.43 ± 5.87 ^{b}

Mean ± standard deviation; DPPH—2,2-diphenyl-1-picrylhydrazyl; mean ± standard deviation; TEAC—Trolox equivalent; different letters in a column denote mean values that statistically differ one from another; dry matter.

The antioxidant activity of an aqueous extract from nettle (*Urtica dioica* L.) measured with the DPPH method by Ebrahimzadeh et al. [27] was reported as (IC_{50}) 159.88 ± 1.57 µg/mL. These authors also noted that nettle leaves are rich in antioxidant compounds like chlorophylls, vitamins, mineral compounds, and polyphenols. Medicinal plants can be used as natural preservatives in the food industry, and have been used since ancient times for shelf-life prolongation and to improve sensory properties. In a study by Ahamdi et al. [28], hydroalcoholic and water extracts of nettle extended the shelf life of minced kilka from 2 to 8 days.

Based on the reducing power (determined using the phosphomolybdenum method), it is possible to rank the tested medicinal plants in the following order: *Fragaria vesca* L. > *Plantago lanceolata* L. > *Rosa canina* L. > *Thymus serpyllum* L. > *Melissa officinalis* L. > *Ribes nigrum* L. > *Salvia officinalis* L. > *Mentha piperita* L. > *Primula veris* L. > *Tropaeolum majus* L. > *Matricaria chamomilla* L. > *Potentilla anserina* L. > *Achillea millefolium* L. > *Urtica dioica* L. > *Calendula officinalis* L. > *Equisetum arvense* L. (Table 1). The results ranged from 171.64 ± 13.25 mg TE/g to 679.56 ± 3.06 mg TE/g.

The observed discrepancies between the parameters reflecting antioxidant activity obtained using both methods are mainly a result of methodological differences. As each method is based on a different principle, each captures a different range of biologically active substances. $DPPH^{\cdot}$ is a stable free radical, with a dark purple color and strong absorbance around 515 nm. Antioxidant compounds contained in the medium convert the $DPPH^{\cdot}$ radical to a more stable $DPPH^{\cdot}$ molecular product by donating an electron or a hydrogen atom. The resulting change in the color of the reaction solution from dark purple to pale yellow enables spectrophotometric determination of the antioxidant activity [29]. In the phosphomolybdenum method, the reducing power is generally associated with the presence of reductones (mainly polyphenols), which act by their antioxidant effect in a way such that they break the free radical chain by providing a hydrogen atom [30]. The reducing capacity of compounds can serve as an important indicator of their potential antioxidant activity [31]. Taking the above into consideration, in order to achieve the most accurate results, it is necessary to use several methods based on different principles when measuring antioxidant activity.

Mudnic et al. [32] similarly determined the total antioxidant activity, and content of total phenols and flavonoids in water extracts from the leaves of *Fragaria vesca* L. The total contents of phenols and flavonoids were reported as 9297.53 ± 24.57 mg/L GAE (gallic acid equivalent) and 7908.27 ± 106.30 mg/L QE (quercetin equivalent), respectively. The total antioxidant activity was measured using the FRAP (ferric-reducing antioxidant power) method, which works on a principle similar to the phosphomolybdenum method, only Fe is reduced instead of Mo. The reported value of the total antioxidant capacity was 43.29 ± 0.29 mmol/L TE (Trolox equivalent). Additionally, significant amounts of the following antioxidant compounds were found in the extract: catechin, epicatechin and epigallocatechin. Using the FRAP method, Katalinic et al. [33] determined the antioxidant activity of 1727 µmol Fe^{II}/L in an aqueous extract from *Plantago lanceolata* L. Gálvez et al. [34] identified luteolin-7-*O*-ß-glucoside as the main flavonoid responsible for antioxidant activity in most *Plantago* species.

3.2. Total Polyphenol, Flavonoid and Phenolic Acid Content

The total content of polyphenols in the tested medicinal herbs ranged from 14.54 ± 2.19 mg GAE/g to 124.51 ± 5.05 mg GAE/g. Based on the obtained results, the tested samples can be listed in the following order: *Fragaria vesca* L. > *Thymus serpyllum* L. > *Melissa officinalis* L. > *Rosa canina* L. > *Mentha piperita* L. > *Ribes nigrum* L. > *Plantago lanceolata* L. > *Primula veris* L. > *Salvia officinalis* L. > *Urtica dioica* L. > *Potentilla anserina* L. > *Matricaria chamomilla* L. > *Tropaeolum majus* L. > *Achillea millefolium* L. > *Equisetum arvense* L. > *Calendula officinalis* L. (Table 2). Peñarrieta et al. [35] determined the total content of polyphenols to range between 9.7 and 21 µmol GAE/g of fresh matter in *Fragaria vesca* L. leaves collected in Bolivia at altitudes between 2650 and 3300 m.a.s.l. Analysis with RP-HPLC demonstrated the presence of seven major constituents, identified as ellagic acid, cyanidin, pelargonidin, quercetin, kaempferol, gallic acid derivatives, and catechin. No apparent difference was found between strawberries growing at lower altitudes.

Table 2. Total polyphenol, flavonoid and phenolic acid contents of tested medicinal herbs.

Sample	TPC (mg GAE/g DM)	TFC (mg QE/g DM)	TPAC (mg CAE/g DM)
Primula veris L.	49.27 ± 2.51 ^{e}	48.35 ± 3.77 ^{a}	22.77 ± 1.44 ^{f}
Urtica dioica L.	38.67 ± 1.88 ^{fg}	10.11 ± 1.07 ^{h}	18.04 ± 2.11 ^{fg}
Ribes nigrum L.	70.12 ± 3.77 ^{d}	7.08 ± 2.29 ^{i}	22.18 ± 0.99 ^{f}
Fragaria vesca L.	124.52 ± 5.05 ^{a}	39.04 ± 0.28 ^{b}	34.85 ± 0.99 ^{e}
Mentha piperita L.	71.67 ± 6.29 ^{cd}	21.22 ± 0.01 ^{f}	84.61 ± 4.71 ^{b}
Thymus serpyllum L.	119.52 ± 1.89 ^{a}	40.89 ± 0.94 ^{b}	102.31 ± 2.89 ^{a}
Salvia officinalis L.	48.51 ± 7.86 ^{ef}	28.44 ± 0.81 ^{d}	31.67 ± 0.22 ^{e}
Potentilla anserina L.	37.13 ± 8.79 ^{gh}	32.31 ± 3.23 ^{c}	12.73 ± 2.72 ^{ghi}
Equisetum arvense L.	19.58 ± 0.63 ^{ij}	10.90 ± 0.13 ^{gh}	13.06 ± 0.83 ^{ghi}
Melissa officinalis L.	85.42 ± 13.19 ^{b}	28.65 ± 1.62 ^{d}	77.47 ± 12.98 ^{c}
Rosa canina L.	80.96 ± 8.17 ^{bc}	3.21 ± 1.62 ^{j}	61.78 ± 0.33 ^{d}
Calendula officinalis L.	14.54 ± 2.19 ^{j}	13.61 ± 0.54 ^{g}	5.49 ± 0.56 ^{i}
Matricaria chamomilla L.	32.02 ± 0.63 ^{hg}	12.71 ± 0.41 ^{gh}	9.81 ± 0.22 ^{hi}
Achillea millefolium L.	19.65 ± 0.32 ^{ij}	9.69 ± 0.40 ^{hi}	14.67 ± 0.28 ^{gh}
Tropaeolum majus L.	26.99 ± 7.29 ^{hi}	24.13 ± 2.96 ^{e}	13.39 ± 0.11 ^{gh}
Plantago lanceolata L.	66.27 ± 5.39 ^{d}	25.04 ± 0.69 ^{e}	76.89 ± 8.15 ^{c}

Mean ± standard deviation; TPC—Total polyphenol content; TFC—Total flavonoid content; TPAC—Total phenolic acid content; GAE—gallic acid equivalent; QE—quercetin equivalent; CAE—caffeic acid equivalent; different letters in a column denote mean values that statistically differ one from another; dry matter.

Polyphenols are secondary plant metabolites and are generally involved in the protection against ultraviolet radiation and various pathogens. Epidemiological studies and related meta-analyses suggest that long-term consumption of polyphenols in a plant-rich diet provides protection against cancer, cardiovascular diseases, diabetes, osteoporosis,

and various other neurodegenerative diseases. Accumulation of polyphenols in tissues is the most important phase of polyphenol metabolism as it enables the concentration of polyphenolic substances necessary for the biological effects to manifest [36]. Polyphenols also help induce antioxidant enzymes, such as glutathione peroxidase, catalase and superoxide dismutase, which break down hydroperoxides, peroxide hydrogen and superoxide anions, respectively. Additionally, they help to inhibit the expression of enzymes such as xanthine oxidase [37]. A statistically significant ($P < 0.05$) regression coefficient was observed in our study between the total polyphenol content and the antioxidant activity determined with the phosphomolybdenum method ($r = 0.892$).

The total content of flavonoids in the tested medicinal plants ranged from 3.21 ± 1.62 mg QE/g to 48.35 ± 3.77 mg QE/g. Based on the obtained results, the tested samples can be listed in the following order: *Primula veris* L. > *Thymus serpyllum* L. > *Fragaria vesca* L. > *Potentilla anserina* L. > *Melissa officinalis* L. > *Salvia officinalis* L. > *Plantago lanceolata* L. > *Tropaeolum majus* L. > *Mentha piperita* L. > *Calendula officinalis* L. > *Matricaria chamomilla* L. > *Equisetum arvense* L. > *Urtica dioica* L. > *Achillea millefolium* L. > *Ribes nigrum* L. > *Rosa canina* L. (Table 2). The highest content of flavonoids of 48.35 ±3.77 mg QE/g was determined in *Primula veris* L. The second highest content was determined in *Thymus serpyllum* L.—40.89 ± 0.94 mg QE/g and third was *Fragaria vesca* L.—39.05 ± 0.27 mg QE/g.

Jurca et al. [38] determined total flavonoids in the amount of 15.31 ± 7.61 mg QE/100 g in a 10% ethanol extract of *Primula* flowers and 17.65 ± 8.72 mg QE/100 g in a 20% extract. The results of their work suggest that *Primula* extracts may play a significant role in reducing oxidative stress but more detailed analyses are still necessary. Petrisor et al. [39] reported that, in *Mellisa,* quercetin (0.15 mg/g) and rutin (1.46 mg/g) are dominant flavonoids. In study of Hamad [40] it was determined that quercetin and kaempferol glycosides are present in the *Calendula* flower, while myricetin glycosides are absent. The flowers in Hamad study were extracted with ethanol 70% [40].

Flavonoids occur as aglycones, glycosides and methylated derivatives. The basic structure of flavonoids is an aglycone. The bioavailability, metabolism, and biological activity of flavonoids depend on the configuration, the total number of hydroxyl groups, and the ability of the functional groups to replace their nuclear structure. It has been proven that many flavonoids exhibit antioxidant activity and free radical scavenging capacity, decrease the risk of coronary heart disease, and induce hepatoprotective, anti-inflammatory and anticancer effects, while others exhibit potential antiviral effects [36]. Apel et al. [41] analyzed the flowers of three *Primula* species. The results showed a wide spectrum of flavonoids and their glycosylated and methylated derivatives, such as: (+)-catechin, kaempferol-3-*O*-galactoside-rhamnoside-7-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, and primulic acid. *Primula* is one of the most important medicinal plants that has been used in the treatment of gout and headaches since the Middle Ages. Triterpenoid saponins from roots and flowers can treat bronchitis and colds by improving the dissolution of mucus and expectoration.

The total content of phenolic acids was in the range of 5.49 ± 0.56 mg CAE/g to 102.31 ± 2.89 mg CAE/g. Based on the obtained results, the tested samples can be listed in the following order: *Thymus serpyllum* L. > *Mentha piperita* L. > *Melissa officinalis* L. > *Plantago lanceolata* L. > *Rosa canina* L. > *Fragaria vesca* L. > *Salvia officinalis* L. > *Primula veris* L. > *Ribes nigrum* L. > *Urtica dioica* L. > *Achillea millefolium* L. > *Tropaeolum majus* L. > *Equisetum arvense* L. > *Potentilla anserina* L. > *Matricaria chamomilla* L. > *Calendula officinalis* L. (Table 2). Phenolic acids are aromatic secondary plant metabolites widespread throughout the plant kingdom. In general, the name "phenolic acids" describes phenols that have a single functional carboxyl group, but when describing plant metabolites it refers to a different group of organic acids. Phenolic acids are derivatives of benzoic and cinnamic acids. These acids exhibit high antioxidant activity in vitro and thus are beneficial for human health. Because they are contained in all plant foods, people consume them daily. The estimated daily intake is 25 mg–1 g, depending on the diet. Caffeic acid, one of the most important naturally occurring acids, is known for selectively blocking the biosynthesis of leukotrienes

(components involved in immunoregulatory diseases, asthma and allergic reactions). Other studies report that caffeic acid and some of its esters may exhibit antitumor activity in colon carcinogenesis [42]. Sonmezdag et al. [43] investigated volatile aromatic substances and phenolic compounds in *Thymus serpyllum* L. In total, they discovered 24 volatile substances. While terpenes were the main chemical group of volatile substances, alcohols and acids were present in smaller amounts. They further discovered that the main aroma of the active substance is thymol, which is an example of biocides. Finally, they identified 18 phenolic compounds (expressed on a dry basis), of which 8 were phenolic acids: rosmarinic acid—21.72 mg/g, chlorogenic acid—7.88 mg/g, caffeic acid—1.73 mg/g, gallic acid—0.63 mg/g, and 10 flavanols: luteolin-7-*O*-glucoside—51.84 mg/g, and kaempferol *O*-glucuronide —15.21 mg/g. Bahadori et al. [44] determined in *Mentha* species the presence of gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, caffeic, syringic, *p*-coumaric, ferulic, sinapic and rosmarinic acids. Caffeic and rosmarinic acids are dominant from phenolic acid in *Salvia* species [45]. A closer examination of the occurrence, structures and activities of monocyclic phenolic acids may not only provide ecological insights but also lead to the development of natural and derivative pharmaceutical or agricultural chemicals, which may have significant benefits for human health and nutrition [46].

3.3. The Content of Mineral Compounds

The determined results of mineral compounds content were compared to the requirements of the following regulations: Ministry of Agriculture and Ministry of Health of Slovak Republic dated March 15, 2004 no. 608/3/2004-100, which issues the title of the Food Codex of the Slovak Republic regulating contaminants in food, Ministry of Agriculture and Ministry of Health of Slovak Republic from 11 September 2006 no. 18558/2006-SL, which issues the title of the Slovak Food Code of the Republic regulating contaminants in foodstuffs.

Among all the microelements quantified in individual medicinal plants, iron (Fe) was found in the highest concentrations. The highest iron content was determined in *Thymus serpyllum* L. (Table 3). Additionally, *Mentha piperita* L., Fragaria *vesca* L., *Equisetum arvense* L., *Salvia officinalis* L., and *Melissa officinalis* L. were the other plants with the most abundant iron concentration. Iron is an essential element in almost all living organisms. It is involved in a wide range of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. However, the concentration in body tissues must be regulated because iron can generate free radicals and its high concentration can lead to tissue damage [47]. Iron deficiency is a very common problem in humans, usually caused by insufficient intake of this element with food or excessive menstrual bleeding. Anemia is a common disease [48]. Using the AAS method, Mihaljev et al. [49] determined the content of iron in *Thymus serpyllum* L. to be 445.78 mg/kg, in *Mentha piperita* L.—443.90 mg/kg, and in *Equisetum arvense* L.—617.46 mg/kg. In our study, the highest contents of zinc (Zn), copper (Cu), and manganese (Mn) were found in in *Tropaeolum majus* L., *Urtica dioica* L., and *Achillea millefolium* L., respectively (Table 3). Mihaljev et al. [49] determined similar concentrations of zinc (Zn) in *Urtica dioica* L. (31.15 mg/kg) and of copper (Cu) in *Thymus serpyllum* L. (8.94 mg/kg). In our study, the value obtained for zinc (Zn) in *Urtica dioica* L. was 30.10 mg/kg and for copper (Cu) in *Thymus serpyllum* L.—9.20 mg/kg.

Zinc (Zn) is relatively non-toxic and necessary for the normal growth and reproduction of cells (enzymes responsible for DNA and RNA synthesis), metabolism, and normal functioning of taste and vision. Zinc deficiency is manifested in insufficient immunity, poor growth, weight loss, skin problems, and loss of appetite. The recommended daily dose for an adult is 15 mg, and 20–25 mg per day is recommended for pregnant and lactating women. Manganese (Mn) is an element necessary for several enzymatic processes. It helps in removing fatigue and reduces nervous irritability [48]. Under normal physiological conditions, people have developed antioxidant systems, including endogenous (enzymatic—SOD, catalase, etc.; non-enzymatic—glutathione, proteins transferrin, and

ferritin, binding to Fe, etc.) and exogenous antioxidants for protection against free radicals. Macro- and microelements, e.g., Fe, Cu, Mn, Co, Se, etc., are important for these systems, and the lack of even one of them can disrupt the function of the entire system [50].

Table 3. The content of minerals in tested medicinal herbs (mg/kg dry matter).

Sample	Copper (mg/kg)	Zinc (mg/kg)	Manganese (mg/kg)	Iron (mg/kg)	Chrome (mg/kg)	Nickel (mg/kg)	Cobalt (mg/kg)	Lead (mg/kg)	Cadmium (mg/kg)
Primula veris L.	7.51 ± 0.88 ^{g}	19.70 ± 0.87 ^{i}	22.20 ± 0.77 ^{k}	90.00 ± 0.76 ^{j}	0.70 ± 0.09 ^{ef}	2.80 ± 0.11 ^{c}	1.00 ± 0.08 ^{g}	1.00 ± 0.02 ^{g}	0.11 ± 0.02 ^{fg}
Urtica dioica L.	14.82 ± 1.32 ^{a}	30.10 ± 1.11 ^{de}	27.81 ± 0.98 ^{f}	77.40 ± 0.78 ^{k}	1.30 ± 0.19 ^{b}	3.20 ± 0.09 ^{b}	1.90 ± 0.09 ^{a}	1.40 ± 0.06 ^{cd}	0.30 ± 0.01 ^{bc}
Ribes nigrum L.	5.87 ± 0.99 ^{h}	19.70 ± 0.56 ^{i}	19.70 ± 0.73 ^{i}	91.90 ± 0.23 ^{i}	0.80 ± 0.09 ^{def}	2.40 ± 0.08 ^{ef}	1.60 ± 0.08 ^{cd}	1.50 ± 0.11 ^{c}	0.10 ± 0.02 ^{gh}
Fragaria vesca L.	4.83 ± 0.87 ^{h}	30.50 ± 0.65 ^{cd}	30.50 ± 0.21 ^{cd}	191.60 ± 0.75 ^{c}	1.10 ± 0.08 ^{bc}	1.90 ± 0.23 ^{g}	1.30 ± 0.11 ^{f}	1.40 ± 0.12 ^{cd}	0.15 ± 0.01 ^{efg}
Mentha piperita L.	12.63 ± 0.67 ^{b}	31.60 ± 0.32 ^{c}	31.60 ± 0.33 ^{c}	201.10 ± 0.88 ^{b}	1.00 ± 0.32 ^{cd}	2.70 ± 0.11 ^{cd}	1.60 ± 0.06 ^{cd}	1.30 ± 0.09 ^{de}	0.22 ± 0.09 ^{cd}
Thymus serpyllum L.	9.23 ± 0.45 ^{ef}	35.20 ± 0.22 ^{b}	35.20 ± 0.11 ^{b}	385.20 ± 0.09 ^{a}	1.60 ± 0.12 ^{a}	3.30 ± 0.13 ^{b}	1.50 ± 0.07 ^{de}	1.30 ± 0.09 ^{de}	0.16 ± 0.03 ^{ef}
Salvia officinalis L.	9.46 ± 0.11 ^{ef}	29.60 ± 0.54 ^{de}	29.60 ± 0.09 ^{de}	167.20 ± 0.07 ^{e}	0.90 ± 0.11 ^{cde}	2.30 ± 0.12 ^{f}	1.40 ± 0.11 ^{ef}	0.60 ± 0.07 ^{i}	0.19 ± 0.01 ^{de}
Potentilla anserina L.	8.95 ± 0.43 ^{ef}	34.90 ± 0.76 ^{b}	34.90 ± 0.87 ^{b}	116.70 ± 0.33 ^{h}	1.00 ± 0.07 ^{cd}	2.61 ± 0.05 ^{cde}	1.40 ± 0.12 ^{ef}	1.40 ± 0.08 ^{cd}	0.12 ± 0.02 ^{fg}
Equisetum arvense L.	10.76 ± 0.78 ^{cd}	16.40 ± 0.72 ^{jk}	16.40 ± 0.84 ^{jk}	172.00 ± 0.21 ^{d}	1.80 ± 0.09 ^{a}	3.30 ± 0.09 ^{b}	1.90 ± 0.12 ^{a}	2.60 ± 0.09 ^{a}	0.43 ± 0.02 ^{a}
Melissa officinalis L.	14.87 ± 0.32 ^{a}	23.71 ± 0.11 ^{g}	23.70 ± 0.98 ^{g}	152.10 ± 0.11 ^{f}	0.90 ± 0.04 ^{cde}	3.61 ± 0.06 ^{a}	1.70 ± 0.05 ^{bc}	1.80 ± 0.11 ^{b}	0.24 ± 0.01 ^{c}
Rosa canina L.	2.67 ± 0.32 ^{i}	13.20 ± 0.23 ^{l}	13.20 ± 0.34 ^{l}	7.30 ± 0.23 ^{o}	0.60 ± 0.04 ^{f}	1.30 ± 0.11 ^{h}	1.10 ± 0.09 ^{g}	0.60 ± 0.01 ^{i}	0.04 ± 0.01 ^{ij}
Calendula officinalis L.	13.87 ± 0.65 ^{a}	17.01 ± 0.55 ^{j}	17.00 ± 0.52 ^{j}	122.50 ± 0.15 ^{g}	0.80 ± 0.02 ^{def}	2.52 ± 0.13 ^{def}	1.40 ± 0.06 ^{ef}	0.60 ± 0.02 ^{i}	0.26 ± 0.02 ^{bc}
Matricaria chamomilla L.	9.45 ± 0.87 ^{ef}	28.80 ± 0.76 ^{ef}	28.81 ± 0.18 ^{ef}	61.00 ± 0.19 ^{m}	0.66 ± 0.09 ^{f}	2.25 ± 0.07 ^{def}	1.30 ± 0.03 ^{f}	n.d.	0.01 ± 0.01 ^{j}
Achillea millefolium L.	11.52 ± 0.76 ^{bc}	39.10 ± 0.76 ^{a}	39.10 ± 0.65 ^{a}	62.60 ± 0.43 ^{l}	0.90 ± 0.03 ^{cde}	3.80 ± 0.09 ^{a}	1.30 ± 0.02 ^{f}	0.80 ± 0.02 ^{h}	0.06 ± 0.01 ^{hi}
Tropaeolum majus L.	8.23 ± 0.55 ^{fg}	30.50 ± 0.52 ^{cd}	30.50 ± 0.88 ^{cd}	77.10 ± 0.76 ^{k}	0.90 ± 0.02 ^{cde}	2.50 ± 0.11 ^{def}	1.80 ± 0.12 ^{ab}	1.20 ± 0.06 ^{ef}	0.12 ± 0.03 ^{fg}
Plantago lanceolata L.	9.75 ± 0.45 ^{de}	15.60 ± 0.66 ^{k}	15.60 ± 0.42 ^{k}	37.90 ± 0.88 ^{n}	0.80 ± 0.07 ^{def}	2.80 ± 0.23 ^{c}	1.70 ± 0.13 ^{bc}	1.10 ± 0.03 ^{fg}	0.26 ± 0.02 ^{bc}

Mean ± standard deviation; n.d.—not detected; different letters in a column denote mean values that statistically differ one from another.

The highest content of chrome was determined in *Equisetum arvense* L. and the highest amount of cobalt in *Urtica dioica* L. and *Equisetum arvense* L. (Table 3). Chrome (Cr) is essential for carbohydrate metabolism and the synthesis of proteins and cholesterol. It plays an important role in the treatment of diabetes. Being directly linked to the function of insulin, it plays a role in maintaining proper glucose metabolism. Present in the pancreas, chrome is involved in insulin production. It also acts as an activator of several enzymes; its deficiency reduces the effectiveness of insulin and increases the concentrations of sugar and cholesterol in the blood. Chrome deficiency can cause growth disorders, insulin resistance, and impaired glucose tolerance, and can be a risk factor in atherosclerotic disease.

Cobalt (Co) is widely distributed in the body, with the highest concentrations found in the liver, kidneys, and bones. Vitamin B_{12}, found in food of animal origin, is the main source of cobalt. Considering that only 50% of vitamin B_{12} is absorbed in the intestine, its recommended daily intake is 3 mg. Vitamin B_{12} is necessary for the maturation of erythrocytes and the normal function of all cells [48].

Potential health risks are associated with food contamination with elements such as lead, cadmium, and nickel. The content of lead in the tested samples ranged from 0.00 mg/kg to 2.60 mg/kg, while the highest amount was found in *Equisetum arvense* L. (Table 3). According to the Slovak regulation from September 11, 2006, the highest permissible amount of this mineral in the category "tea for preparation the drink" is 10 mg/kg. Thus, the lead content in all tested samples met this limit. The highest amount of cadmium was detected in *Equisetum arvense* L., with a value of 0.43 mg/kg. According to the Slovak regulation from 11 September 2006, the maximum cadmium content in the category "soft drinks" is 0.05 mg/kg. In our study, mineral compounds were analyzed in dry matter, not in water extracts. No definite conclusion can thus be made regarding the amount of cadmium in aqueous solution in association with legal requirements. Sembratowicz and Rusinek-Prystupa [51] found that, after 5 min of extraction, 46.67% of cadmium was extracted into herbal tea samples. Prolongation of the extraction to 10 min resulted in a 53.10% extraction yield. Therefore, the extension of the extracting time caused a significant increase in the extraction yield. The authors also stated that the extraction efficiency of lead or cadmium is positively affected by acidification with lemon juice or citric acid. Cadmium (Cd) is released into the environment through natural activities (e.g., volcanic eruptions), weather conditions, and some human activities (e.g., mining, smoking, production of fertilizers, etc.). Cadmium is a highly toxic element for the kidneys, as it accumulates in

the proximal tubular cells. Inhaling excessive amounts of this element can lead to serious lung damage. Oral intake of higher amounts of this element leads to stomach irritation, vomiting, and diarrhea. Prolonged exposure to lower concentrations can lead to cadmium deposition in the kidneys, a condition that can lead to kidney diseases, bone thinning, and lung damage [52].

According to the Slovak standard n. 608/3/2004-100 from 15 March 2004, the maximum permissible amount of nickel in the category "legumes, nutritional supplements, snacks, honey, dehydrated and instant soups" is 6.0 mg/kg. Among our samples, the highest nickel concentration of 3.80 mg/kg was found in *Achillea millefolium* L. All the samples thus met the limit set by the standard for this category (Table 3).

It is necessary to consider the fact that all our samples were analyzed in the form of dry matter. The extractability of specific elements into an aqueous solution will make their concentrations in the liquid lower than that observed in dry matter.

3.4. Antimicrobial Activity

The first tested bacterial strain tested was *Haemophilus influenzae* CCM 4454 (Table 4). The size of the inhibition zones of the tested samples ranged from 1.00 to 13.33 mm, while the smallest zone was determined in *Thymus serpyllum* L. and the largest in *Equisetum arvense* L. The second tested bacterial strain was *Staphylococcus aureus* CCM 2461. The size of the inhibition zones ranged from 0 (*Calendula officinalis* L.) to 6 (*Fragaria vesca* L.) mm. The third tested strain of bacteria was *Clostridium perfringens* CCM 4991 (Table 4). The size of the inhibition zones ranged from 0.5 (*Salvia officinalis* L. and *Rosa canina* L.) to 3.5 (*Equisetum arvense* L.) mm. The fourth tested strain was *Enterococcus faecalis* CCM 4224 (Table 4). The size of the inhibition zones ranged from 0 (*Rosa canina* L.) to 5.83 mm (*Fragaria vesca* L.).

Table 4. Antimicrobial activity of tested medicinal herbs.

Sample	*Haemophilus influenzae* CCM 4454 (mm)	*Staphylococcus aureus* CCM 2461 (mm)	*Clostridium perfringens* CCM 4991 (mm)	*Enterococcus faecalis* CCM 4224 (mm)	*Candida glabrata* CCM 8270 (mm)	*Candida albicans* CCM 8186 (mm)	*Candida tropicalis* CCM 8223 (mm)
Primula veris L.	2.00 ± 0.00^{fghi}	1.00 ± 0.00^{de}	1.00 ± 0.00^{de}	1.17 ± 0.29^{ef}	0.00 ± 0.00^{g}	1.50 ± 0.00^{bc}	1.33 ± 0.29^{gh}
Urtica dioica L.	9.00 ± 1.73^{b}	1.50 ± 0.50^{cde}	1.33 ± 0.58^{bcd}	1.50 ± 0.50^{def}	2.33 ± 0.29^{bc}	1.00 ± 0.00^{cd}	3.17 ± 0.76^{cd}
Ribes nigrum L.	4.17 ± 0.77^{de}	2.17 ± 0.29^{bc}	1.67 ± 0.58^{bc}	1.50 ± 0.50^{def}	1.67 ± 0.29^{cde}	1.83 ± 0.29^{b}	3.17 ± 0.29^{cd}
Fragaria vesca L.	3.17 ± 0.29^{efg}	6.00 ± 1.00^{a}	1.00 ± 0.00^{de}	5.83 ± 0.76^{a}	1.67 ± 0.58^{cde}	2.67 ± 0.58^{a}	3.50 ± 0.50^{c}
Mentha piperita L.	5.67 ± 0.58^{cd}	2.67 ± 0.58^{b}	1.33 ± 0.29^{bcd}	2.67 ± 0.58^{c}	4.83 ± 0.76^{a}	3.17 ± 0.29^{a}	4.33 ± 0.58^{b}
Thymus serpyllum L.	1.00 ± 0.00^{i}	1.17 ± 0.29^{de}	1.17 ± 0.29^{cd}	1.67 ± 0.29^{def}	0.67 ± 0.29^{b}	1.00 ± 0.00^{cd}	0.00 ± 0.00^{j}
Salvia officinalis L.	2.83 ± 0.29^{efghi}	0.90 ± 0.11^{cde}	0.50 ± 0.00^{e}	5.33 ± 0.58^{a}	2.67 ± 0.29^{b}	2.67 ± 0.58^{a}	2.00 ± 0.00^{efg}
Potentilla anserina L.	1.67 ± 3.01^{ghi}	1.00 ± 0.00^{de}	1.00 ± 0.05^{de}	1.33 ± 0.29^{ef}	1.17 ± 0.29^{def}	1.50 ± 0.00^{bc}	0.00 ± 0.00^{j}
Equisetum arvense L.	13.33 ± 0.77^{a}	1.17 ± 0.29^{de}	3.50 ± 0.29^{a}	3.33 ± 0.29^{b}	4.50 ± 0.50^{a}	1.00 ± 0.00^{cd}	5.67 ± 0.58^{a}
Melissa officinalis L.	2.83 ± 0.58^{efgh}	1.17 ± 0.29^{de}	1.33 ± 0.00^{bcd}	2.17 ± 0.29^{cd}	1.67 ± 0.29^{cde}	1.00 ± 0.00^{cd}	0.50 ± 0.00^{ij}
Rosa canina L.	3.67 ± 0.29^{ef}	1.00 ± 0.00^{de}	0.50 ± 0.29^{e}	0.00 ± 0.00^{g}	0.50 ± 0.00^{fg}	1.33 ± 0.58^{bcd}	1.00 ± 0.00^{hi}
Calendula officinalis L.	1.17 ± 0.29^{hi}	0.00 ± 0.00^{f}	0.83 ± 0.00^{de}	1.17 ± 0.29^{ef}	1.00 ± 0.00^{ef}	1.00 ± 0.00^{cd}	0.50 ± 0.00^{ij}
Matricaria chamomilla L.	2.83 ± 0.00^{efgh}	1.33 ± 0.29^{de}	0.50 ± 0.29^{e}	1.00 ± 0.00^{f}	0.00 ± 0.00^{g}	1.83 ± 0.29^{b}	2.00 ± 0.00^{efg}
Achillea millefolium L.	3.00 ± 0.22^{efgh}	1.00 ± 0.00^{de}	1.17 ± 0.00^{cd}	1.00 ± 0.00^{f}	1.83 ± 0.29^{cd}	1.00 ± 0.00^{cd}	2.33 ± 0.58^{ef}
Tropaeolum majus L.	3.33 ± 0.58^{efg}	1.00 ± 0.00^{de}	1.00 ± 0.29^{de}	1.50 ± 0.00^{def}	1.17 ± 0.29^{def}	1.50 ± 0.50^{bc}	2.67 ± 0.58^{de}
Plantago lanceolata L.	6.00 ± 0.50^{c}	1.67 ± 0.58^{cd}	1.83 ± 0.23^{b}	1.83 ± 0.29^{de}	3.00 ± 1.00^{b}	0.83 ± 0.29^{d}	1.67 ± 0.59^{fgh}

Mean ± standard deviation; mm—millimeters; different letters in a column denote mean values that statistically differ one from another.

Sinha [53] analyzed the ethanolic extract of *Equisetum arvense* L. against two types of G+ bacteria (*Bacillus subtilis* and *Micrococcus luteus*) and four G^- bacteria (*Vibrio cholerae*, *Escherichia coli*, *Shigella flexneri* and *Shigella dysenteriae*) using the disk diffusion method. The highest average zone of inhibition (32 mm) was determined against the species *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae*. Nonetheless, other G+ and G^- bacteria showed sensitivity, indicating the broad-spectrum activity of the extract. Preliminary screening tests showed the presence of polyphenols, tannins, glycosides, alkaloids, saponins, terpenoids, and flavonoids to be responsible for the observed antimicrobial activity.

Growing antibiotic resistance of bacteria is considered a global problem in current medicine. This makes results such as the ones obtained in our study novel alternatives to antibiotics.

The first tested yeast strain was *Candida glabrata* CCM 8270 (Table 4). The size of the inhibition zones ranged from 0 (*Primula veris* L.) to 4.83 mm (*Mentha piperita* L.). The second tested species was *Candida albicans* CCM 8186. The size of the inhibition zones ranged from 0.83 (*Plantago lanceolata* L.) to 3.17 mm (*Mentha piperita* L.) The third tested yeast strain was *Candida tropicalis* CCM 8223. The size of the inhibition zones ranged from 0 (*Potentilla anserina* L.) to 4.33 mm (*Mentha piperita* L.).

Doddanna et al. [54] detected antimicrobial activity of *Mentha piperita* L. ethanolic extract against *Candida albicans* by the disk diffusion method. The reported diameter of the inhibition zone was 12.95 ± 0.07 mm after 24 h, and 13 ± 0.0 mm after 48 h. *Mentha piperita* L. leaves contain menthol, menthone, methyl esters, and monoterpene derivatives. The antimicrobial activity of terpenoids consists mainly of the disruption of microbial membranes. *Candida albicans* is the most common species of the genus *Candida*. Found in the oral cavity, it is responsible for most oral diseases. The use of natural products as alternative agents against fungal diseases is considered an interesting alternative to synthetic fungicides.

Plant extracts and phytochemicals with known antibacterial effects can be of great importance in the treatment of various diseases. The synergistic effect of antibiotics and plant extracts against resistant bacteria may lead to new treatment options for infectious diseases [55].

4. Conclusions

Medicinal herbs are an important part of human life. They are rich in bioactive compounds with many health benefits. In our study, the highest antioxidant activities were determined in *Rosa canina* L. (DPPH method), and in *Fragaria vesca* L. (phosphomolybdenum method). The highest content of polyphenols was found in *Fragaria vesca* L. and *Thymus serpyllum* L., while *Primula veris* L. was found to be the richest in flavonoids. Samples of *Thymus serpyllum* L. and *Mentha piperita* L. contained the highest content of total phenolic acids. Results of antimicrobial activity showed that *Equisetum arvense* L., *Mentha piperita* L., and *Fragaria vesca* L. had an inhibitory effect on *Candida* strains. The sample of *Equisetum arvense* L. showed a strong inhibitory effect on *Haemophilus influenzae*. Determination of the content of selected microelements showed that they are present in different concentrations in medicinal plants. These microelements are necessary for the human body, but in larger concentrations they can have a harmful effect. Among all the tested microelements in individual medicinal plants, iron (Fe) was found to be dominant. The highest content of Fe was found in *Thymus serpyllum* L. Noteworthy, significant levels of zinc (Zn) were also found. It was most abundant in *Tropaeolum majus* L.

Medicinal plants are an excellent source of flavonoids, carotenoids, and phenolic acids, which are potent antioxidants. The obtained results are proof of the antioxidant and antimicrobial potential of medicinal plants and indicate that the wide application of such plants in the food, pharmaceutical, and cosmetic industries is justified. The possibility of using natural raw materials as alternatives to synthetically produced products should be paid more attention.

Author Contributions: Conceptualization, E.I. and M.H.; methodology, E.I. and M.H.; software, M.Č; validation, E.I., M.H. and P.Ł.K.; formal analysis, E.I., M.H., Ľ.H., O.G., M.K. and P.Ł.K.; investigation, E.I., M.H. and P.Ł.K.; resources E.I., M.H., Ľ.H., O.G., M.K., M.Č. and P.Ł.K.; data curation, E.I., M.Č., M.H., M.K. and Ľ.H.; writing—original draft preparation, E.I., M.H. and P.Ł.K.; writing—review and editing, E.I., M.H., Ľ.H., O.G., M.K., M.Č. and P.Ł.K.; visualization, E.I. and P.Ł.K.; supervision, E.I. and P.Ł.K.; project administration, P.Ł.K.; funding acquisition, P.Ł.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the projects: 06-GASPU-2021 (50%) and Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund (50%).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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

References

1. Karpavičienė, B. Traditional Uses of Medicinal Plants in South-Western Part of Lithuania. *Plants* **2022**, *11*, 2093. [CrossRef]
2. Obakiro, S.B.; Kiprop, A.; Kowino, I.; Kigondu, E.; Odero, M.P.; Omara, T.; Bunalema, L. Ethnobotany, ethnopharmacology, and phytochemistry of traditional medicinal plants used in the management of symptoms of tuberculosis in East Africa: A systematic review. *Trop. Med. Health* **2020**, *48*, 68. [CrossRef] [PubMed]
3. Balogun, F.; Ashafa, A. A Review of Plants Used in South African Traditional Medicine for the Management and Treatment of Hypertension. *Planta Med.* **2019**, *85*, 312–334. [CrossRef] [PubMed]
4. Kachmar, M.R.; Naceiri Mrabti, H.; Bellahmar, M.; Ouahbi, A.; Haloui, Z.; El Badaoui, K.; Bouyahya, A.; Chakir, S. Traditional Knowledge of Medicinal Plants Used in the Northeastern Part of Morocco. *Evid.-Based Complement. Altern. Med.* **2021**, *2021*, 6002949. [CrossRef]
5. Kowalczewski, P.Ł.; Olejnik, A.; Świtek, S.; Bzducha-Wróbel, A.; Kubiak, P.; Kujawska, M.; Lewandowicz, G. Bioactive compounds of potato (*Solanum tuberosum* L.) juice: From industry waste to food and medical applications. *CRC Crit. Rev. Plant Sci.* **2022**, *41*, 52–89. [CrossRef]
6. Jafri, S.A.A.; Khalid, Z.M.; Khan, M.Z.; Jogezai, N. Evaluation of phytochemical and antioxidant potential of various extracts from traditionally used medicinal plants of Pakistan. *Open Chem.* **2022**, *20*, 1337–1356. [CrossRef]
7. Bilia, A.R.; Costa, M.d.C. Medicinal plants and their preparations in the European market: Why has the harmonization failed? The cases of St. John's wort, valerian, ginkgo, ginseng, and green tea. *Phytomedicine* **2021**, *81*, 153421. [CrossRef]
8. Kledecka, A.; Siejak, P.; Pratap-Singh, A.; Kowalczewski, P.Ł.; Fathordoobady, F.; Jarzębski, M.; Smułek, W. Extracts from *Frangula alnus* Mill. and Their Effects on Environmental and Probiotic Bacteria. *Plants* **2022**, *11*, 2719. [CrossRef]
9. Tuasha, N.; Petros, B.; Asfaw, Z. Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District, Sidama Zone, Ethiopia. *J. Ethnobiol. Ethnomed.* **2018**, *14*, 15. [CrossRef]
10. Hall, C.; Knuth, M. An Update of the Literature Supporting the Well-Being Benefits of Plants: A Review of the Emotional and Mental Health Benefits of Plants. *J. Environ. Hortic.* **2019**, *37*, 30–38. [CrossRef]
11. Medawar, E.; Huhn, S.; Villringer, A.; Veronica Witte, A. The effects of plant-based diets on the body and the brain: A systematic review. *Transl. Psychiatry* **2019**, *9*, 226. [CrossRef] [PubMed]
12. Fehér, A.; Gazdecki, M.; Véha, M.; Szakály, M.; Szakály, Z. A Comprehensive Review of the Benefits of and the Barriers to the Switch to a Plant-Based Diet. *Sustainability* **2020**, *12*, 4136. [CrossRef]
13. Adeniye, S.O.; Olufemi-Adeniyi, A.O.; Erinoso, S.M. Traditional/alternative medicine: An investigation into identification, knowledge, and consumption practices of herbal medicine among students with hearing impairment in Ibadan, south-western Nigeria. *J. Educ. Pract.* **2015**, *6*, 143–149.
14. Godswill, A.C. Medicinal plants: The medical food, and nutritional biochemistry and uses. *Int. J. Adv. Acad. Res.* **2019**, *5*, 220–240.
15. Khalid, W.; Arshad, M.S.; Ranjha, M.M.A.N.; Różańska, M.B.; Irfan, S.; Shafique, B.; Rahim, M.A.; Khalid, M.Z.; Abdi, G.; Kowalczewski, P.Ł. Functional constituents of plant-based foods boost immunity against acute and chronic disorders. *Open Life Sci.* **2022**, *17*, 1075–1093. [CrossRef] [PubMed]
16. Malik, F.; Iqbal, A.; Zia, S.; Ranjha, M.M.A.N.; Khalid, W.; Nadeem, M.; Selim, S.; Hadidi, M.; Moreno, A.; Manzoor, M.F.; et al. Role and mechanism of fruit waste polyphenols in diabetes management. *Open Chem.* **2023**, *21*, 20220272. [CrossRef]
17. Kalle, R.; Pieroni, A.; Svanberg, I.; Sõukand, R. Early Citizen Science Action in Ethnobotany: The Case of the Folk Medicine Collection of Dr. Mihkel Ostrov in the Territory of Present-Day Estonia, 1891–1893. *Plants* **2022**, *11*, 274. [CrossRef]
18. Benediková, D. Collection, Evaluation, and Preservation of Genetic of Plant Resources for Nutrition and Agriculture: Methodological Sheets. 2010. Available online: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://www.slpk.sk/eldo/2013/9788089417131.pdf (accessed on 10 January 2023).
19. Sánchez-Moreno, C.; Larrauri, J.A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270–276. [CrossRef]
20. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. [CrossRef]
21. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1964**, *16*, 144–158.
22. Willett, W.C. Balancing Life-Style and Genomics Research for Disease Prevention. *Science* **2002**, *296*, 695–698. [CrossRef] [PubMed]
23. Jain, R.; Rao, B.; Tare, A.B. Comparative analysis of the spectrophotometry based total phenolic acid estimation methods. *J. Anal. Chem.* **2017**, *72*, 972–976. [CrossRef]
24. Taneva, I.; Petkova, N.; Dimov, I.; Ivanov, I.; Denev, P. Characterization of rose hip (*Rosa canina* L.) fruits extracts and evaluation of their in vitro antioxidant activity. *J. Pharmacogn. Phytochem.* **2016**, *5*, 35–38.

25. Rovná, K.; Ivanišová, E.; Žiarovská, J.; Ferus, P.; Terentjeva, M.; Kowalczewski, P.Ł.; Kačániová, M. Characterization of *Rosa canina* Fruits Collected in Urban Areas of Slovakia. Genome Size, iPBS Profiles and Antioxidant and Antimicrobial Activities. *Molecules* **2020**, *25*, 1888. [CrossRef] [PubMed]
26. Roman, I.; Stănilă, A.; Stănilă, S. Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania. *Chem. Cent. J.* **2013**, *7*, 73. [CrossRef] [PubMed]
27. Ebrahimzadeh, M.; Gharekhani, M.; Ghorbani, M.; Dargany, P. Effect of Extract of Aerial Parts of *Urtica dioica* (Urticaceae) on the Stability of Soybean Oil. *Trop. J. Pharm. Res.* **2015**, *14*, 125. [CrossRef]
28. Ahmadi, M.; Razavilar, V.; Motallebi, A.; Kenari, R.; Khanipour, A.A. Effects of hydroalcoholic and water extracts of nettle leaf (*Urtica dioica* L.) on chemical properties of superchilled minced meat of common kilka (*Clupeonella cultriventris caspia*). *J. Food Qual. Hazards Control* **2014**, *1*, 85–88.
29. Akar, Z.; Küçük, M.; Doğan, H. A new colorimetric DPPH scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 640–647. [CrossRef]
30. Nagendra Prasad, K.; Yang, B.; Yang, S.; Chen, Y.; Zhao, M.; Ashraf, M.; Jiang, Y. Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinensis* Sonn.) seeds. *Food Chem.* **2009**, *116*, 1–7. [CrossRef]
31. El-Hajaji, H.; Lachkar, N.; Alaoui, K.; Yahya, C.; Farah, A.; Ennabili, A.; El Bali, B.; Lachkar, M. Antioxidant Properties and Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco. *Rec. Nat. Prod.* **2010**, *4*, 193–204.
32. Mudnic, I.; Modun, D.; Brizic, I.; Vukovic, J.; Generalic, I.; Katalinic, V.; Bilusic, T.; Ljubenkov, I.; Boban, M. Cardiovascular effects in vitro of aqueous extract of wild strawberry (*Fragaria vesca* L.) leaves. *Phytomedicine* **2009**, *16*, 462–469. [CrossRef] [PubMed]
33. Katalinic, V.; Milos, M.; Kulisic, T.; Jukic, M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* **2006**, *94*, 550–557. [CrossRef]
34. Gálvez, M.; Martín-Cordero, C.; López-Lázaro, M.; Cortés, F.; Ayuso, M.J. Cytotoxic effect of *Plantago* spp. on cancer cell lines. *J. Ethnopharmacol.* **2003**, *88*, 125–130. [CrossRef]
35. Peñarrieta, J.M.; Alvarado, J.A.; Bergenståhl, B.; Ákesson, B. Total Antioxidant Capacity and Content of Phenolic Compounds in Wild Strawberries (*Fragaria vesca*) Collected in Bolivia. *Int. J. Fruit Sci.* **2009**, *9*, 344–359. [CrossRef]
36. Pandey, K.B.; Rizvi, S.I. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxid. Med. Cell. Longev.* **2009**, *2*, 270–278. [CrossRef]
37. Tsao, R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients* **2010**, *2*, 1231–1246. [CrossRef] [PubMed]
38. Jurca, T.; Marian, E.; Vicas, L.; Oana, N.; Pallag, A. Bioactive compouds and antioxidant capacity of *Primula veris* L. flower extract. *An. Univ. Oradea Fasc. Ecotoxicologie Zooteh. Tehnol. Ind. Alimantară* **2015**, *14*, 235–241.
39. Petrisor, G.; Motelica, L.; Craciun, L.N.; Oprea, O.C.; Ficai, D.; Ficai, A. *Melissa officinalis*: Composition, Pharmacological Effects and Derived Release Systems—A Review. *Int. J. Mol. Sci.* **2022**, *23*, 3591. [CrossRef]
40. Hamad, M.N. Detection and isolation of flavonoids from *Calendula officinalis* (F.Asteraceae) cultivated in Iraq. *Iraqi J. Pharm. Sci.* **2017**, *25*, 1–6. [CrossRef]
41. Apel, L.; Kammerer, D.; Stintzing, F.; Spring, O. Comparative Metabolite Profiling of Triterpenoid Saponins and Flavonoids in Flower Color Mutations of *Primula veris* L. *Int. J. Mol. Sci.* **2017**, *18*, 153. [CrossRef]
42. Robbins, R.J. Phenolic Acids in Foods: An Overview of Analytical Methodology. *J. Agric. Food Chem.* **2003**, *51*, 2866–2887. [CrossRef] [PubMed]
43. Sonmezdag, A.S.; Kelebek, H.; Selli, S. Characterization of aroma-active and phenolic profiles of wild thyme (*Thymus serpyllum*) by GC-MS-Olfactometry and LC-ESI-MS/MS. *J. Food Sci. Technol.* **2016**, *53*, 1957–1965. [CrossRef] [PubMed]
44. Bahadori, M.B.; Zengin, G.; Bahadori, S.; Dinparast, L.; Movahhedin, N. Phenolic composition and functional properties of wild mint (*Mentha longifolia* var. calliantha (Stapf) Briq.). *Int. J. Food Prop.* **2018**, *21*, 183–193. [CrossRef]
45. Paje, L.A.; Choi, J.; Lee, H.-D.; Kim, J.; Yu, A.R.; Bae, M.-J.; Geraldino, P.J.L.; Lee, S. Phenolic acids and flavonoids from *Salvia plebeia* and HPLC-UV profiling of four *Salvia* species. *Heliyon* **2022**, *8*, e09046. [CrossRef]
46. Khadem, S.; Marles, R.J. Monocyclic Phenolic Acids; Hydroxy- and Polyhydroxybenzoic Acids: Occurrence and Recent Bioactivity Studies. *Molecules* **2010**, *15*, 7985–8005. [CrossRef]
47. Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. *J. Res. Med. Sci.* **2014**, *19*, 164–174.
48. Lokhande, R.; Singare, P.; Andhale, M. Study on mineral content of some ayurvedic indian medicinal plants by instrumental neutron activation analysis and AAS techniques. *Heal. Sci. J.* **2010**, *4*, 157–168.
49. Mihaljev, Z.; Zivkov-Balos, M.; Cupić, Z.; Jaksić, S. Levels of some microelements and essential heavy metals in herbal teas in Serbia. *Acta Pol. Pharm.* **2014**, *71*, 385–391.
50. Lemberkovics, É.; Czinner, E.; Szentmihályi, K.; Balázs, A.; Szőke, É. Comparative evaluation of Helichrysi flos herbal extracts as dietary sources of plant polyphenols, and macro- and microelements. *Food Chem.* **2002**, *78*, 119–127. [CrossRef]
51. Sembratowicz, I.; Rusinek-Prystupa, E. Effects of Brewing Time on the Content of Minerals in Infusions of Medicinal Herbs. *Pol. J. Environ. Stud.* **2014**, *23*, 177–186.
52. Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B.B.; Beeregowda, K.N. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* **2014**, *7*, 60–72. [CrossRef] [PubMed]
53. Sinha, N.S. In vitro antibacterial activity of ethanolic extract of *Equisetum arvense* L. *Int. J. Pharm. Biol. Res.* **2012**, *3*, 19–21.
54. Doddanna, S.; Patel, S.; Sundarrao, M.; Veerabhadrappa, R. Antimicrobial activity of plant extracts on Candida albicans: An in vitro study. *Indian J. Dent. Res.* **2013**, *24*, 401. [CrossRef] [PubMed]

55. Nascimento, G.G.F.; Locatelli, J.; Freitas, P.C.; Silva, G.L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.* **2000**, *31*, 247–256. [CrossRef]