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Antioxidant and Antibacterial Activity of Extracts from Selected Plant Material

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Abstract: Plants are a valuable source of biologically active molecules, mainly phenolic compounds. In the present study, the total phenolic content (TPC), DPPH· and $ABTS^+$ scavenging activity as well as ferric reducing ability (FRAP) of aqueous ethanolic (70%) extracts of *Cistus incanus* L. and *Asarum europaeum* L. herb, *Geum urbanum* L. rhizome, *Angelica archangelica* L. root, white mulberry (*Morus alba* L.), lemon balm (*Melisa officinalis* L.), red raspberry (*Rubus idaeus* L.) and *Betula pendula* Roth. leaves were determined. In addition, the phenolic profiles of the studied plant extracts and antibacterial activity have been investigated. The extracts from *C. incanus* and *G. urbanum* demonstrated the highest TPC and antioxidant capacity, while the extracts from *A. archangelica* and white mulberry were characterized by the lowest values. A remarkable correlation was also found between the TPC and antioxidant activity of the examined extracts. HPLC analysis showed that the studied extracts were sources of both phenolic acids and flavonoids. More flavonoids than phenolic acids were identified in the extracts of *C. incanus, M. alba, R. idaeus* and *B. pendula* compared to the other extracts tested. Not all extracts showed a significant impact on the growth of the tested bacterial strains. *Escherichia coli* was the most sensitive strain to lemon balm extract (MIC, 0.125 mg/mL), whereas the strains of *Acinetobacter baumannii* and *Bordetella bronchiseptica* were sensitive to the *G. urbanum* extract (MIC, 0.125 mg/mL). Among Gram-positive bacteria, *Enterococcus faecalis* was the most sensitive to *G. urbanum* extract. In turn, *Staphylococcus aureus* and *Staphylococcus epidermidis* were sensitive to the extracts from *C. incanus* herb (MIC, 0.125 mg/mL), red raspberry (MIC, 0.125 mg/mL) and lemon balm leaves (MIC. 0.25 mg/mL). Based on the obtained results, the applicability of the studied plant extracts as additives to food and cosmetic products may be considered in the future.

Keywords: aqueous ethanolic extracts; total phenolics; HPLC analysis; DPPH; ABTS; FRAP

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1. Introduction

Plant material is a source of many value components, such as phenolic compounds, which may scavenge free radicals and thus reduce oxidative stress [1–3]. Phenolic compounds showing antioxidant properties include flavonoids, phenolic acids, lignans and stilbenes. The properties of the aforementioned compounds are used by plants as a defense mechanism against the adverse effects of UV radiation, temperature and mechanical damage. They also act as an important chemical defense against herbivores through their specific physiological action on insects [4]. In addition, by reacting directly with the oxidation products of fatty acids, phenolic compounds can prevent adverse changes from occurring in both living organisms and food. They prevent the deterioration of the organoleptic and sensory characteristics of food products [5,6]. Phenolic compounds also

exhibit antimicrobial activity, causing the inhibition of microbial growth by interfering with the transport of nutrients that are important to their function. The functional groups present in phenolic compounds enable their building into the lipid membranes of microorganisms, causing changes in their permeability and reducing resistance to abiotic factors [7]. This action may often be enhanced or weakened due to the possibility of both synergistic or antagonistic effects between phenolic compounds and their interaction with other components present in the plant material [8]. The content of phenolic compounds in plant material may be influenced by both the cultivation system of plants and the method of harvesting and obtaining raw material, as well as the method of drying and storing it. The way in which the biologically active compounds are extracted from a plant material and the part of the plant used and its belonging to a specific botanical family are also important.

Nowadays, consumers are increasingly choosing products of natural origin or those that contain natural substitutes for synthetic additives. When reading the label, they pay attention to information about the presence of plant extracts derived from commercially available plant material, which is often used in natural medicine or culinary applications. In the current study, plant material that is popular with consumers due to its availability and recommended biological properties was used. *Cistus incanus* L. belonging to the Cistaceae family has been used in folk medicine for the treatment of diarrhea and fever and as an anti-inflammatory agent in skin diseases, rheumatism and nephritis. The herb and leaves of *C. incanus* exhibit antimicrobial [9], antiviral [10] and antioxidant properties [11]. Their aqueous solutions are a source of phenolic compounds, particularly flavonoids, phenolic acids and ellagitannins [12]. *Asarum europaeum* L., known as European wild ginger (Aristolochiaceae), is cultivated in Poland as an ornamental and useful plant. The herbal raw materials are shoots and roots, which emit a characteristic spicy odor and are used in the production of medicines that are used to treat respiratory diseases and in veterinary medicine [13,14]. *Geum urbanum* L. (Rosaceae), on the other hand, is used in folk medicine for gastrointestinal and liver diseases and externally to reduce gingivitis [15–17]. Its roots and rhizomes are a source of tannins, mainly ellagitannins, essential oils, flavonoids and triterpenes [18]. *Angelica archangelica* L. (Apiaceae), a valuable medicinal plant that has been partially protected in Poland since 2014, is characterized by its peculiar and pleasant fragrance. The stems and seeds are used in confectionery and in the preparation of liqueurs, and the leaves and roots for medicinal purposes, especially in digestive problems, anorexia, migraine or menstrual and obstetric complaints [19]. The roots are a source of coumarins, a flavonoid called archangelenone, palmitic acid and sugar [20]. White mulberry (*Morus alba* L., Moraceae family) grows as a shrub; the leaves are mainly used in China to feed silkworms [21], whereas in Poland, after drying, they are packaged and sold as herbal teas. They are recommended as preparations for decreasing blood glucose and reducing obesity, and they show antibacterial, anti-inflammatory and antioxidant activities. Flavonoids and phenolic acids play a key role in antioxidant activity [22]. In contrast, extracts of lemon balm (*Mellisa officinalis* L.) are a source of rosmarinic acid, which has documented antioxidant activity, as well as flavonoids and essential oils [23]. Lemon balm belongs to the Lamiaceae family, which in traditional medicine is used in the treatment of many diseases in different cultures, for example, to alleviate gastrointestinal and hepatic problems. It has sedative properties, so drinking an infusion of lemon balm leaves before bed accelerates sleep and is recommended for people with irritable bowel syndrome. Fruits and leaves of *Rubus idaeus* L. (Rosaceae) are valuable medicinal raw materials with nutritional and dietary values. They are used as a cold remedy, rich in mucilaginous compounds, pectin, macro- and micronutrients and ellagic acid [24]. Leaves can be included in herbal mixtures with diuretic and choleretic effects. On the other hand, *Betula pendula* leaves belonging to the Betulaceae family are purchased for their diuretic and diaphoretic properties. They are excellent for urinary tract problems and strengthening the body after an infection. Phenolic compounds, mainly flavonoids, usually predominate in the chemical composition of extracts prepared from birch leaves using a 20% ethanol solution [25] and methanol [26].

Obtaining extracts from the plant material in question with the use of ethanol (70%) and a preliminary assessment of their biological activity may be the basis for their future use, e.g., in food, to protect it against the harmful effects of external factors and thus have a positive effect on the human body, reducing the risk of certain diseases. The main purpose of this study was to obtain aqueous ethanolic extracts from the selected plant material available on the Polish market belonging to seven botanical families and to determine their antioxidants and antimicrobials. The content of phenolic compounds and their types were also determined using HPLC, especially with regard to phenolic acids and flavonoids.

2. Materials and Methods

2.1. Materials and Reagents

The dried herb of *Cistus incanus* L. and *Asarum europaeum* L., rhizome of *Geum urbanum* L., root of *Angelica archangelica* L., leaves of white mulberry (*Morus alba* L.), lemon balm (*Melisa officinalis* L.), red raspberry (*Rubus idaeus* L.) and *Betula pendula* Roth. bought from an herbal shop in Warsaw, Poland, were used as the plant material. Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were bought from Sigma-Aldrich (Poznań, Poland). High performance liquid chromatography (HPLC) standards were purchased from Sigma Life Science (Merck, Darmstadt, Germany) and ChromaDex® (Irvine, CA, USA), respectively. Other chemicals and solvents were of analytical grade and were used as received without further purification. They were obtained from Avantor Performance Materials (Gliwice, Poland).

The microorganisms used in this study were obtained from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw (Warsaw, Poland). They belonged to Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057, *Bacillus subtilis* ATCC 6633, *Geobacillus stearothermophilis* ATCC 7953 and Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 12453, *Listeria monocytogenes* 1043S, *Serratia marcescens* ATTC 13880, *Enterobacter cloacae* DSM 6234, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* ATCC 12714, *Bordetella bronchiseptica* ATCC 4617, *Acinetobacter baumannii* ATCC 19606.

2.2. Extract Preparation

The aqueous ethanolic extracts from the investigated plant material were performed with 70% ethanol, as described previously [27], with a minor alteration. For this purpose, 20 g of each plant material and 250 mL of aqueous ethanol were placed in a flask and stirred using a water bath for 10 h at 45 °C. The plant residues were then separated by filtration through a Whatman No. 1 paper filter and the ethanol was evaporated under vacuum on a rotary evaporator at 40 °C (Rotavapor R-200, Büchi Labortechnik, Flavil, Switzerland). The resulting extracts were lyophilized (Alpha 1-4 LSCplus, Osterode am Harz, Germany) and stored at −20 °C until further analysis. The extraction yield was evaluated on the basis of the mass balance.

2.3. Total Phenolics Content (TPC) Determination

The total amount of phenolic compounds was determined in the plant extracts using the Folin–Ciocalteu reagent according to Singleton and Rossi [28], with little modification. 1 mg of each extract was dissolved in 2 mL of 70% ethanol. Then, to 1 mL of the plant extract solution thus prepared, 9 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent were added. After 3 min, 20% Na_2CO_3 solution (5 mL) was added, and the total volume was made up to 50 mL with distilled water. The solution was incubated at 21 °C for 1 h and then the absorbance at 765 nm was measured using a Shimadzu UV-1650 PC spectrophotometer (Kyoto, Japan). TPC was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g of extract) using gallic acid as a reference standard (0.2–5 mg/mL).

2.4. HPLC Analysis

The phenolic compound determination in the plant extracts was performed by HPLC-DAD using the Shimadzu Prominence system equipped with two pumps LC-20AD, an auto-sampler SIL-20AC HT, a column oven CTO-10AS VP, and a diode-array UV/VIS detector SPD-M20A controlled by LC solution 1.21 SP1 software (Shimadzu, Kyoto, Japan). Compound separation was carried out on a C18 reversed-phase column filled with 2.6 µm particles with a solid core and porous outer layer, 100 mm × 4.60 mm (Kinetex™, Phenomenex®, Torrance, CA, USA). The mobile phase was composed of deionized water adjusted to pH 2 with phosphoric acid and filtered with 0.20 µm nylon membrane filter (Phenex™, Phenomenex®, Torrance, CA, USA) and MeCN with gradient elution at a flow rate of 1.5 mL/min. The gradient was used as follows: 0 min—12.5% B; 4.0 min—23% B; 6.0 min—50% B; 6.01 min—12.5% B; and 8 min—stop. The column temperature was set at 40 °C. The plant extracts (2 mg/mL) were dissolved in ethanol (70%) and filtered with Iso-Disc™ Filters PTFE-25-2, diameter 25 mm, and pore size 0.20 µm (Supelco Analytical™, Bellefonte, USA). The injection volume was 1 µL. The retention times of the eluted compounds and their UV-spectra were compared with the corresponding standards. The standard stock solutions were prepared by separately dissolving with MeOH in a 25 mL volumetric flask according to ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution and used as standard stock solutions [29]. The working standard solutions were made by diluting 0.01 mL and 0.1 mL of standard stock solutions with methanol in 10 mL volumetric flasks, 0.5 mL and 1 mL in 5 mL volumetric flasks, as well as 1 mL in 2 mL volumetric flasks. The working solutions and undiluted stock solutions were injected (1 µL) in six replicates (n = 6). The precision intra- and inter-day, linearity, range, LOD and LOQ tests were done on the basis of ICH guidelines (Table S1).

2.5. DPPH Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was conducted as described by Gow-Chin and Hui-Yin [30], with a slight modification. One milliliter of freshly prepared DPPH-methanol solution (0.3 mmol/L) was mixed with 3.8 mL of methanol and 0.2 mL of the plant extract solution (70% ethanol) at different concentrations. Then, the samples were incubated at room temperature in the dark. After 10 min, the absorbance was measured at 517 nm using a Shimadzu UV-1650 PC spectrophotometer (Kyoto, Japan). The results were expressed as mmol Trolox equivalents (TE) per gram of extract using the Trolox standard in the range 8–40 µmol/L. Antioxidant capacity was also calculated as the IC_{50} parameter. The sample concentration providing 50% inhibition was obtained by plotting the percentage of inhibition versus the amount of extracts.

2.6. ABTS Assay

The ABTS radical scavenging activity of the plant extracts was performed according to the procedure developed by Re et al. [31]. Initially, ABTS radical cations were produced by mixing 14 mmol/L of ABTS solution in phosphate-buffered saline (PBS, pH 7.4) with 4.9 mmol/L of potassium persulfate in equivalent amounts and kept for 12–16 h in the dark at room temperature. Then, 4 mL of $ABTS^{\bullet+}$ working solution (before the analysis, it was diluted in water to an absorbance value of 0.7 ± 0.05 at 734 nm) was mixed with 40 µL of the plant extract prepared by dissolving in 70% ethanol at different concentrations and left at room temperature in the dark. After 6 min, the absorbance of the samples was measured at 734 nm using a Shimadzu UV-1650 PC spectrophotometer (Kyoto, Japan). The results were expressed as mmol Trolox equivalents (TE) per gram of extract using Trolox in the range of 0–20 µM. The antioxidant activity was also determined by IC_{50} value as mentions above in the DPPH assay.

2.7. Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric reducing antioxidant power assay was determined according to Benzie and Strain [32], with minor changes. Three milliliters of the FRAP reagent prepared by mixing

10 mmol/L TPTZ solution in 40 mmol/L HCl, 300 mmol/L acetate buffer (pH 3.6) and 20 mmol/L $FeCl_3$ solution in proportions of 1:10:1 ($v/v/v$) was added to the plant extract (3 mg dissolved in 2 mL 70% ethanol). The reaction was conducted for 10 min at room temperature and then the absorbance was measured at 593 nm using a Shimadzu UV-1650 PC spectrophotometer (Kyoto, Japan). Trolox was used as a standard compound in order to be able to compare the results with those obtained for DPPH and ABTS methods. Results were reported as mmol Trolox equivalents (TE) per gram of extract using Trolox in the range 80–500 μmol/L.

2.8. Antibacterial Activity

The antibacterial activity of the plant extracts was determined by the disc-diffusion method and the MIC method under standard conditions using Mueller-Hinton II agar medium (Beckton Dickinson) in accordance with the guidelines established by the CLSI [33,34]. In the disc-diffusion assay, the solutions of the plant extracts in ethanol (70%) were dripped on sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatographic paper) to load 2 mg of the given extracts per disc. Then, the filter paper discs were placed on agar plates uniformly inoculated with the test microorganisms and incubated at 35 °C ± 2.5 °C for 18 h. A paper disc with 70% ethanol was used as a negative control. In contrast, commercial 6-mm diameter discs containing 0.03 mg of nitrofurantoin were used as a positive control. The diameter of the clear zone surrounding the disc was used to measure the antimicrobial activity of the given extracts. For MIC (minimum inhibitory concentration) evaluation, the solutions containing the plant extracts in dimethyl sulfoxide (DMSO) were added to a liquid solution of the agar medium to form two-fold serial dilutions in the range of 31.3 to 2000 mg/L. Next, solidified agar plates were inoculated using 2 μL aliquots. The final inoculum of all studied organisms was 104 colony forming units CFU/mL, except for the final inoculum of *E. faecalis* ATCC 29212, which was 105 CFU/mL. A plate of agar medium with DMSO was used as a negative control.

2.9. Statistical Analysis

The results were analyzed using analysis of variance (ANOVA) with post-hoc Tukey's HSD test at the confidence level $p < 0.05$ (Statistica 13, Statsoft, Tulsa, OK, USA). Pearson's test was used to find the correlation between the total polyphenol content and antioxidant activity determined by DPPH, ABTS and FRAP assay in the studied plant extracts. All the analyses were performed at least in triplicate and the data were expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Extraction Yield and Total Phenolics Content (TPC)

The results of the extraction yield of the plant material used in the study are presented in Table 1. They ranged from 16.79 to 40.16%. The lowest extraction yields were found for extracts obtained from *A. europaeum* herb, whereas the highest were from red raspberry leaves. The extracts obtained from white mulberry leaves, *G. urbanum* rhizome and *A. archangelica* root had similar extraction efficiencies with no statistical difference. In contrast, the aqueous ethanolic extracts from *C. incanus* herb, *B. pendula* and lemon balm leaves were obtained with 1.54–2.12 times lower yields than those obtained from red raspberry leaves. Considering leaves as part of the plant used in the extraction process, it was found that extracts from red raspberry leaves were obtained with the highest yield and those from *B. pendula* leaves with the lowest yield. On the other hand, the preparation of extracts using the *C. incanus* herb gave higher values of extraction yields than the preparation of extracts from the *A. europaeum* herb. The differences in extracts' yields could reflect the effects of multiple factors, including the type of plant raw material used, its origin, the location where the plant material was collected, drying method, water content or the presence of many other accompanying substances frequently interacting with the extracted components.

Table 1. Extraction yield of the plant material and total phenolics content (TPC) of the plant extracts.

Latin Name of Plant	Common Names (English)	Family	Part of Plant	Extraction Yield(%)	TPC (mg GAE/g of Extract)
Cistus incanus L.	hairy rockrose	Cistaceae	herb	23.26 ± 1.29 ^{d}	363.61 ± 2.29 ^{a}
Morus alba L.	white mulberry, common mulberry, silkworm mulberry	Moraceae	leaves	32.16 ± 1.03 ^{b}	45.94 ± 0.24 ^{f}
Geum urbanum L.	St. Benedict's herb, herb Bennet, wood avens, colewort	Rosaceae	rhizome	29.94 ± 0.20 ^{b}	234.52 ± 1.16 ^{b}
Asarum europaeum L.	European wild ginger, hazelwort, wild spikenard, asarabacca	Aristolochiaceae	herb	16.79 ± 0.38 ^{e}	73.35 ± 1.37 ^{e}
Rubus idaeus L.	raspberry, red raspberry	Rosaceae	leaves	40.16 ± 0.59 ^{a}	143.60 ± 2.23 ^{c}
Angelica archangelica L.	garden angelica, wild celery, Norwegian angelica	Apiaceae	root	30.43 ± 1.15 ^{b}	20.35 ± 0.37 ^{g}
Betula pendula Roth.	silver birch, warty birch, European white birch, East Asian white birch	Betulaceae	leaves	26.03 ± 0.58 ^{c}	97.23 ± 1.67 ^{d}
Melissa officinalis L.	lemon balm, English balm, garden balm, balm mint, common balm, melissa, sweet balm	Lamiaceae	leaves	18.92 ± 0.55 ^{e}	139.71 ± 1.40 ^{c}

Means with different lowercase letters ($^{a–g}$) within the same column indicate a significant difference at the significance level of 0.05.

The content of phenolic compounds in the aqueous ethanolic extracts obtained from the plant material and determined using the Folin–Ciocalteu reagent are presented in Table 1. Our results indicate that the extract of *C. incanus* herb contained the highest content of phenolic compounds, reaching a value of 363.61 ± 2.29 mg GAE/g of extract and that the extract of *A. archangelica* root contained the lowest (20.35 ± 0.37 mg GAE/g of extract). In contrast to our study, Bernacka et al. [35] and Ziarno et al. [2] showed lower phenolic content in water infusions prepared from *C. incanus* leaves. The differences may be due to the different preparation of the extract, the part of the plant material used and its origin and time of harvest. *Angelica archangelica* is a member of the Apiaceae family and all parts of the plant can be used for pharmacological and food purposes, as well as in traditional and folk medicine as a remedy for fever, skin rashes or bronchitis [19,35]. However, in the root and fruit extracts of *A. purpurascens*, almost twice as high a TPC content as in extracts from the aerial part was observed [36]. In turn, these values were higher than those obtained in the present work for the aqueous ethanolic extract of *A. archangelica*. The extracts that were also characterized by the high content of phenolic compounds were those obtained from *G. urbanum* rhizome, red raspberry and lemon balm leaves. In the case of aqueous ethanolic extracts from red raspberry and lemon balm leaves, the TPC values were similar and amounted to 143.60 ± 2.23 and 139.71 ± 1.40 mg GAE/g of extract, respectively. In contrast, in the leaf infusions of *R. idaeus* Glen Ample, Laszka and Radziejowa varieties growing in south-eastern Poland, the content of these biologically active compounds was lower and ranged from 18.2–27.3 mg/g d.w. calculated as caffeic acid [37]. In turn, the content of TPC in methanolic extracts of wild raspberry leaves from the central Balkan region was recorded in the range of 59.68 to 96.83 mg GA/g. The highest values of TPC were determined in samples taken from sunny localities, while the lowest values showed samples from shadowy sites [38].

The content of phenolic compounds in lemon balm extracts was influenced by both the type of solvent and the part of the plant used in the preparation. The TPC in the leaf extract (32.76 mg GAE/g dry material) was higher than that in the extract from stems (8.4 mg GAE/g dry material) [39]. Maceration increased the TPC content to 90.1 mg GAE/g dry material and the use of hydroalcoholic solvents allowed the phenolic compounds in lemon balm extracts to be estimated at 227.6 mg GAE/g dry material. In addition, aqueous extraction of *Melissa officinalis* using a pulsed electric field followed by ultrasound increased the content of TPC [40]. In another study carried out for aqueous extract of lemon balm, TPC ranged from 5.55 to 49.19 mg GA/g depending on temperature and extraction time and was almost three times lower than the values obtained in our experiments [41]. In contrast, aqueous ethanolic extracts from white mulberry and *B. pendula* leaves, and *A. europaeum* herb showed statistically significant ($p < 0.05$) differences in total polyphenol content. In this group of extracts, the highest polyphenol content was found in the *B. pendula* leaf extract and the lowest in the mulberry leaf extract. The high polyphenol content in *B. pendula* dry leaf extracts was also observed by Penkov et al. [26]. However, it was higher compared to our study and significantly influenced the ability of these extracts to reduce free DPPH radicals. On the other hand, the total polyphenol content in the powdered extracts from white mulberry leaves from Poland was similar to the values obtained in our experiment and amounted to 42.6 mg GAE/g DM [42]. Similar TPC values were also determined for leaf extracts of mulberry belonging to one of the three cultivars from the South China studied [21]. On the other hand, the ethanol-water leaf extract of *M. alba* from Poland was three-fold higher than in our extract [43].

The content of phenolic compounds in extracts obtained from a plant material is influenced by many factors, including geographical origin, location, climate conditions, harvest time or the way the plant material is dried. The manner in which the extraction process is carried out, i.e., time, temperature, type of solvent used, its polarity and the part of the plant subjected to this process, also play an important role. Choosing a solvent that is safe, cheap, non-toxic and able to extract phenolic compounds with the highest efficiency is particularly important in the food industry. Often, the use of a mixture of

solvents may be more effective in the extraction of phenolic acids and flavonoids. Therefore, in this study, ethanol in combination with water was chosen. This type of mixture was also chosen for the extraction of phenolic compounds from *C. incanus* growing in Strandja Mountain [44]. The TPC expressed as gallic acid and tannic acid equivalents in the extracts varied between 36.26 and 115.32 mg GAE/g d.w. and 71.88 and 228.56 mg TAE/g d.w. as a function of time, respectively. The highest content of phenolic compounds was found when a 30% ethanol solution was used and the extraction process was carried out for 390 min. Similar to our research results for TPC were obtained for *C. incanus* extracts prepared using 60% methanol in Soxhlet apparatus (331.82–347.27 mg GAE gd.w.) [45] and for aqueous and hydromethanolic extracts of *C. salviifolius* (408.43 ± 1.09 and 336.51 ± 1.22 mg GAE/g of extract dry weight, respectively) [46]. Lower levels of TPC were reported for the aqueous and hydromethanolic extracts of *C. incanus* grown in Turkey. It was also observed that the aqueous extracts of *Cistus* species were richer in phenolic compounds than their hydromethanolic counterparts [47].

3.2. Phenolic Compound Profile

Among the phenolics identified in the aqueous ethanolic extracts studied by the HPLC method (Table 2, Figure S1), there were ten phenolic acids and eight flavonoids. Chlorogenic acid was identified in five studied extracts, whereas caffeic, ferrulic and ellagic acids were identified in three extracts. In turn, gallic, neochlorogenic, isochlorogenic B and cichoric acids were identified in only two of the extracts studied. In contrast, rosmarinic acid was present only in the lemon balm leaf extract, whereas *p*-coumaric acid was present in the *B. pendula* leaf extract. The *C. incanus* leaf extract had a higher gallic acid content than the red raspberry leaf extract but a lower content of ellagic acid than the aqueous ethanolic extract of *G. urbanum* rhizome. The results presented in [48] showed that methanolic extracts of underground organs of *G. urbanum* were richer in gallic and ellagic acids in comparison to the herb, in which chlorogenic acid was the predominant phenolic acid. On the other hand, in Al-Snafi et al. [49], the content of ellagic acid in the aerial part extracts (46.71 ± 0.51 mg/g) of *G. urbanum* was higher than in the underground part extracts (32.19 ± 0.50 mg/g). In our experiment, ellagic acid was also present in *G. urbanum* extract but in lower amounts than in the study presented in [49]. In addition, the methanolic extracts of defatted seeds of *G. urbanum* were characterized by the presence of ellagic acid [18]. Chlorogenic and neochlorogenic acids were present in both the aqueous ethanolic extract of mulberry leaves and *A. europaeum* herb, but only chlorogenic acid was identified in the extract of red raspberry and *B. pendula* leaves and *A. archangelica* root. In contrast, caffeic acid was present in the extract of *A. europaeum* herb, red raspberry leaves and lemon balm, and ferulic acid in the extract of *A. europaeum* herb, *A. archangelica* root and lemon balm leaves. The highest content of caffeic acid was determined in the lemon balm leaf extract and ferulic acid in the *A. europaeum* herb extract. The lemon balm leaf extract and the *A. archanangelica* root extract were also sources of isochlorogenic acid B and cichoric acid. Aqueous ethanolic extracts from lemon balm leaves, red raspberry, *A. archangelica* root and *A. europaeum* herb were characterized by the presence of at least four phenolic acids in their phenolic compound profile. Phenolcarboxylic acids such as chlorogenic, caffeic, gentisic, ferulic and *p*-coumaric acids were also identified in the extracts from *Rubi idaei folium* (red raspberry leaves) collected in Romania [50]. The rest of the aqueous ethanolic extracts tested contained at least two out of ten identified phenolic acids, with the exception of the rhizome extract of *G. urbanum*, which was dominated exclusively by ellagic acid. In addition to phenolic acids, the following flavonoids were also found in the studied extracts: catechin, rutoside, hyperoside, isoquercetin, astragalin, peltatoside, nicotiflorin and tiliroside.

Table 2. Phenolic compounds (mg/g of extract) identified in the plant extracts determined by HPLC.

Phenolic Compound	*C. incanus*	*M. alba*	*G. urbanum*	*A. europaeum*	*R. idaeus*	*A. archangelica*	*B. pendula*	*M. officinalis*
Gallic acid	0.46 ± 0.01 [a]	- [1]	-	-	0.24 ± 0.02 [b]	-	-	-
(+)-Catechin	-	-	-	-	-	-	-	0.99 ± 0.30
Neochlorogenic acid	-	1.55 ± 0.10 [a]	-	1.58 ± 0.02 [a]	-	-	-	-
Chlorogenic acid	-	2.93 ± 0.02 [b]	-	2.46 ± 0.17 [c]	1.00 ± 0.04 [d]	3.65 ± 0.26 [a]	0.97 ± 0.09 [d]	-
Caffeic acid	-	-	-	0.52 ± 0.02 [c]	0.80 ± 0.06 [b]	-	-	2.45 ± 0.17 [a]
p-Coumaric acid	-	-	-	-	-	-	0.58 ± 0.02	-
Ferulic acid	-	-	-	0.58 ± 0.07 [a]		0.20 ± 0.01 [b]	-	0.14 ± 0.01 [b]
Peltatoside	2.94 ± 0.18	-	-	-	-	-	-	-
Rutoside	1.65 ± 0.20 [b]	2.9 8± 0.14 [a]	-	0.7 8± 0.01 [d]	1.26 ± 0.13 [c]	-	3.02 ± 0.08 [a]	-
Ellagic acid	0.39 ± 0.01 [a]	-	3.29 ± 0.15 [b]	-	3.65 ± 0.44 [b]	-	-	-
Hyperoside	6.69 ± 0.47 [a]	-	-	-	1.02 ± 0.07 [b]	-	7.20 ± 0.49 [a]	-
Isoquercetin	1.85 ± 0.11 [d]	5.00 ± 0.11 [b]	-	0.39 ± 0.01 [e]	6.27 ± 0.45 [a]	-	3.29 ± 0.10 [c]	-
Cichoric acid	-	-	-	-	-	0.20 ± 0.01 [b]	-	0.34 ± 0.05 [a]
Isochlorogenic acid B	-	-	-	-	-	2.06 ± 0.07 [a]	-	0.99 ± 0.09 [b]
Nicotiflorin	-	1.21 ± 0.01	-	-	-	-	-	-
Astragalin	-	1.80 ±0.03 [b]	-	-	4.11 ± 0.81 [a]	-	-	-
Tiliroside	0.54 ± 0.02 [a]	-	-	-	-	0.18 ± 0.00 [b]		-
Rosmarinic acid	-	-	-	-	-	-	-	23.70 ± 2.20
Phenolic acids content	0.85 ± 0.01 [h]	4.48 ± 0.09 [e]	3.29 ± 0.15 [f]	5.14 ± 0.21 [d]	5.69 ± 0.26 [c]	6.11 ± 0.31 [b]	1.55 ± 0.08 [g]	27.62 ± 2.29 [a]
Flavonoids content	13.67 ± 0.09 [a]	10.99 ± 0.28 [c]	-	1.17 ± 0.08 [d]	12.66 ± 0.41 [b]	0.18 ± 0.01 [e]	13.51 ± 0.36 [a]	0.99 ± 0.37 [d]

[1] not detected. Means with different lowercase letters ([a–h]) within the same row indicate a significant difference at the significance level of 0.05.

(+) Catechin was found only in the aqueous ethanolic extract of lemon balm leaves, whereas peltatoside was found in the extract of *C. incanus*. Rutoside was identified in the extract from *C. incanus*, *A. europaeum*, white mulberry, red raspberry and *B. pendula*. Its content in these extracts ranged from 0.78 to 3.02 mg/g of extract, with the highest value observed in the *B. pendula* leaf extract and the lowest in the *A. europaeum* herb extract. Among the flavonoids, hyperoside appeared to be the dominant chemical compound in terms of the content found in the extract from *C. incanus* and *B. pendula*. This flavonoid was also identified in the red raspberry extract but in much lower amounts than in the two previously mentioned extracts. Isoquercetin was also detected in aqueous ethanolic extract from red raspberry leaves. This compound was not detected in the phenolic compound profile of the extract from *G. urbanum*, lemon balm and *A. archangelica*. The highest content of isoquercetin was determined in the extract of red raspberry leaves and the lowest in the extract of *A. europaeum* herb. There is also information in the literature that among biologically active substances, plants of the genus *Asarum*, especially *A. europaeum*, may contain flavonoids including quercetin, isoquercetin and kaempferol derivatives [14,51]. These flavonoids accumulate mainly in leaves, reaching the maximal content in spring, during which they can show the protective action of the assimilation apparatus of young leaves against UV radiation. Astragalin, on the other hand, was found in the red raspberry leaf extract and in 2.3 times lower amounts in the white mulberry leaf extract. Among the eight flavonoids identified, nicoflorins were also found in the white mulberry extract and tiliroside in extracts from *C. incanus* and *A. archangelica*. The methanolic extracts (80%) from the mulberry leaves of different varieties were characterized by the presence of six primary polyphenolic compounds. Similar to our results, chlorogenic acid was the predominant phenolic acid, ranging from 2.45 to 10.24 mg/g d.w.and isoquercetin was present in the highest level among flavonoid glycosides (0.70–4.83 mg/gd.w.) [52]. In addition to these two compounds, rutin and astragalin were also considered components that positively correlated with the antioxidant activity determined for the studied extracts.

The aqueous ethanolic extract from *C. incanus* was richer in flavonoids than in phenolic acids compared to the other plant extracts studied. Additionally, the ethyl acetate fraction from *C. incanus* leaves was characterized by a higher content of flavonoids than phenolic acids, especially myricetin and quercetin derivatives [53]. Hyperoside (quercetin 3-*O*-galactoside) predominated in the extracts from *C. incanus* and *B. pendula*, isoquercetin from the white mulberry and red raspberry, chlorogenic acid from *A. europaeum* and *A. archangelica*, ellagic acid from *G. urbanum* and rosmarinic acid from lemon balm. Rosmarinic acid is known as the main substance responsible for the healing activity of lemon balm extracts [54]. Moreover, it is commonly found in plants of the Lamiaceae family. Its content in the phenolic fractions of lemon balm was the highest when microwave-assisted extraction procedures and ethanol as solvent were used [55]. The most important constituents of hydromethanolic extracts of *C. incanus* identified by LC-MS appeared to be myricetin and its derivatives and catechin derivatives [45]. However, HPLC analysis revealed the presence of fifteen phenolic compounds in fifteen batches of *C. incanus* extracts, but the aqueous extracts did not contain ferulic, chlorogenic and syringic acids [47]. Gallic, ellagic and *p*-coumaric acids were found in most of the *C. incanus* samples analyzed, and among the flavonoids isoquercetin, rutin, 7-luteolin glucoside and kaempferol were present. In the case of our study, no *p*-coumaric acid was detected in the aqueous ethanolic extracts of *C. incanus* and the most abundant flavonoid was hyperoside (quercetin-3-*O*-galactoside). Using the HPLC-DAD method to identify phenolic compounds present in the aqueous infusions of the *C. incanus* species tested, it was found that *C. incanus* of Bulgarian origin summer and winter leaves infusions were richer in a number of polyphenols found than *C. incanus* of Greek origin summer leaves infusion and *Melissa officinalis* Bulgarian origin leaves and stems infusion [56]. In addition, in the Bulgarian *C. incanus* winter leaf infusion, the concentrations of some bioactive compounds were lower than those found in the summer leaves. The same method was also used to characterize the major polyphenolic compounds in a crude ethanolic leaf extract of *C. incanus* [11]. The compounds identified

were classified into gallic acid derivatives, condensed tannins and flavonol glycosides. Based on UPLC-MS/MS profiling of aqueous extracts of two *Cistus* species wild growing in Croatia, it was revealed that they were also a rich source of polyphenols with flavonol derivatives [57]. The differences in the profile and quantity of the identified polyphenolic compounds are influenced by their chemical nature, the type and origin of the raw material, the method of extraction and the method of identification. Methanol and ethanol solvents make it possible to extract mainly polar compounds from plant material, which include polyphenols, sugars and some organic acids. Synergistic interactions can occur between these compounds, which can affect their antimicrobial and antioxidant activity. In the water extract of *Betula papyrifera* Marshall, nine phenolics and eleven acids were identified, while in the methanolic extracts, ten phenolic compounds and seven acids were found. Both extracts contained hydrobenzoic, caffeic and coumaric acids, but these were present in a higher proportion in the water extract [58]. In aqueous ethanolic extracts of *B. pendula*, chlorogenic acid and three flavonoids were identified instead of caffeic acid. In the birch leaf extracts from Estonia, the content of hyperoside as the principal flavonoid depended on the birch species (*B. pendula*, *B. pubesans*, *B. nana*, *B. humilis*) used in the study and the period of leaf harvest (June, August, Oktober) [25]. The leaves of *B. pendula* collected in October were slightly richer in these polyphenols compared to those collected during the other months. In contrast, they were almost five times poorer in hyperosides than in *B. pubescens* collected in June.

3.3. Antioxidant Activity

Many flavonoids and phenolic acids can influence the overall antioxidant activity of plants [59], protecting them against oxidative damage caused by endogenous free radicals [44]. They neutralize lipid free radicals and prevent hydroperoxides from decomposing into free radicals. Gori et al. [11] indicated that the ethyl acetate fraction of *C. incanus*, enriched in phenolic compounds, especially in flavonols, exhibited higher DPPH radical scavenging activity compared to the tannin-enriched aqueous fractions. Table 3 presents the antioxidant activity of the studied aqueous ethanolic plant extracts as determined by DPPH, ABTS and FRAP tests. All tested extracts showed the ability to scavenge DPPH radicals and ABTS cation radicals and changes in values in the FRAP method. The extract with the highest antioxidant activity in all the methods used was the extract from *C. incanus*, while the extracts from white mulberry leaves and *A. archangelica* roots showed the lowest activity. The ability of methanolic *A. archangelica* extracts from whole plants to scavenge DPPH free radicals was observed in [59]. It was noted that this activity increased as the concentration of the extracts used increased (20–100 µg/mL) [59] or as an essential oil from *A. glauca* [60], whose ability to scavenge DPPH radicals was lower than synthetic BHT. The selection of appropriate extraction conditions is also an important factor. Maximum DPPH scavenging activity was obtained when dry roots of *A. archangelica* were subjected to methanol extraction at a temperature of 60 °C and extraction time of 36 h [61]. With regard to the antioxidant activity determined for extracts from mulberry leaves collected in three regions in China, the species of plant material used and its origin had a strong influence. Generally, this activity by region showed the following trend: Guangdong > Guangxi > Chongging [21]. The values obtained for the *C. incanus* extract were statistically significantly different from the values obtained for the other plant extracts tested. All extracts of different parts of two *Cistus* species growing in Eastern Morocco also showed a high scavenging ability of DPPH radicals and it was higher when compared to those reported for essential oils of the leaf of *C. libanotis* and *C. ladanifer* [62]. In addition, this activity was similar to that of ascorbic acid, which is used as an antioxidant and preservative in a wide range of food products. This may indicate that the *Cistus* species extracts owe their antioxidant activity mainly to the presence of phenolic compounds, especially when the leaves of *C. ladanifer* were extracted with methanol:water (50:50). *Geum urbanum* rhizome extract also showed high antioxidant activity. The antioxidant activity of the methanolic extracts of the roots and aerial parts of *G. urbanum* and their fractions obtained by subse-

quent extraction with petroleum ether, ethyl acetate, and n-butanol was also investigated by Dimitrova et al. [16] and Farzaneh et al. [63]. Among all tested extracts, the best scavenging activity was demonstrated by the roots and aerial parts of ethyl acetate fractions, which were also characterized by the highest total phenolic content. In contrast, extracts from *A. europaeum* herb, red raspberry leaves, *B. pendula* and lemon balm varied in order of antioxidant activity depending on the method used to assess it. In the DPPH test, the order of these extracts was as follows: lemon balm > red raspberry > *B. pendula* > *A. europaeum*, in the ABTS test: lemon balm > *B. pendula* > *A. europaeum* > red raspberry; and in the FRAP method, the extract from red raspberry was first followed by lemon balm, *B. pendula* and *A. europaeum*. The genus *Asarum* L. has about 100 plant species distributed mainly in Europe, East Asia and North America. *Asarum europaeum* is used in folk medicine for lung diseases, gastrointestinal tract disorders or for disorders of the central nervous system such as epilepsy or migraines [17]. Contrary to our research, Saeedi et al. [64] showed that ethyl acetate fractions of *A. europaeum* rhizome and BHA use as the reference drug showed better antioxidant activity in the DPPH assay than aqueous and hydroalcoholic extracts. In the case of methanolic extracts of wild raspberry from the central Balkan region, higher antioxidant activity was demonstrated by extracts prepared with the use of leaves rather than fruit [38]. The leaves of the Radziejow variety also exhibited the highest total antioxidant activity determined with the FRAP method [37].

Table 3. Antioxidant activity of the plant extracts determined by DPPH, ABTS and FRAP assays.

Plant Material	DPPH (mmol TE/g of Extract)	IC_{50} DPPH (μg/mL)	ABTS (mmol TE/g of Extract)	IC_{50} ABTS (μg/mL)	FRAP (mmol TE/g of Extract)
C. incanus	2.52 ± 0.02 ^{a}	9.24 ± 0.08 ^{h}	3.58 ± 0.10 ^{a}	10.59 ± 0.72 ^{g}	1.82 ± 0.05 ^{a}
M. alba	0.23 ± 0.01 ^{g}	43.85 ± 1.49 ^{b}	0.30 ± 0.01 ^{f}	75.62± 2.80 ^{b}	0.08 ± 0.01 ^{d}
G. urbanum	1.15 ± 0.02 ^{b}	20.27± 0.14 ^{g}	2.97 ± 0.05 ^{b}	14.60 ± 0.88 ^{f}	0.39 ± 0.02 ^{b}
A. europaeum	0.40 ± 0.01 ^{f}	38.52± 1.17 ^{c}	0.74 ± 0.04 ^{e}	43.6 ± 2.05 ^{c}	0.11 ± 0.01 ^{d}
R. idaeus	0.54 ± 0.01 ^{d}	31.26 ± 0.62 ^{e}	0.71 ± 0.03 ^{e}	45.3 ± 1.44 ^{c}	0.34 ± 0.02 ^{b}
A. archangelica	0.16 ± 0.01 ^{h}	58.91 ± 2.07 ^{a}	0.12 ± 0.01 ^{g}	86.54 ± 3.24 ^{a}	0.07 ± 0.01 ^{d}
B. pendula	0.48 ± 0.01 ^{e}	36.72 ± 1.04 ^{d}	0.97 ± 0.04 ^{d}	37.1 ± 0.95 ^{d}	0.21 ± 0.02 ^{c}
M. officinalis	0.58 ± 0.01 ^{c}	29.75 ± 0.80 ^{f}	1.11 ± 0.04 ^{c}	33.6 ± 1.27 ^{e}	0.26 ± 0.01 ^{c}

Means with different lowercase letters ($^{a–h}$) within the same column indicate a significant difference at the significance level of 0.05.

The high content of polyphenolic compounds in plant extracts is often correlated with their significant antioxidant activity [45]. Our study also showed a noticeable correlation between the antioxidant activity and the TPC of the aqueous ethanolic extracts obtained from the selected plant material (Table 4). The coefficient of Pearson correlation between total phenolic contents and DPPH and ABTS scavenging activity and FRAP were 0.966, 0.957 and 0.903, respectively. The results of all antioxidant activity assays correlated positively with each other (r = 0.819–0.972). A high linear correlation was achieved between the results of the DPPH, ABTS and FRAP assays. In contrast, a lower correlation coefficient was observed between the results obtained for the ABTS and FRAP methods. Correlation analysis showed that antioxidant activity of *C. incanus* was strongly correlated to TPC, total flavonoids and total phenolic acids content [47]. The correlation between phenolic content and antioxidant activity measured by the DPPH method was also reported for aqueous extracts obtained from two *Cistus* species growing in Croatia [57]. In contrast, the FRAP assay did not show a positive correlation between antioxidant activity and polyphenol content in this study. On the other hand, Chwil and Kostryco [37] found a high correlation (r = 0.93) between the antioxidant activity determined by FRAP and the content of polyphenolic compounds in *Rubus idaeus* extracts. In Yu et al. [52], the

correlation coefficient between phenolic compounds present in mulberry leaves using DPPH and FRAP assays was higher than phenolic content and ABTS scavenging ability. A negative correlation was observed between kaempferol-malonyl-glucoside content and the DPPH and FRAP tests. In addition, no significant correlations were found between the DPPH and FRAP values for all analyzed mulberry extracts [23]. In turn, a positive correlation was also observed between antioxidant activity determined by DPPH and FRAP methodologies and the TPC content in extracts from lemon balm cultivated in Mexico [41]. This was influenced by the concentration of phenolic compounds, especially phenolic acids, mainly derived from hydroxycinnamic acids, such as rosmarinic acid. This information is in accordance with our data and is presented in [39]. Rosmarinic acid was confirmed by HPLC as one of the main components present in the profile of phenolic compounds of *Melissa officinalis* extracts. Both rosmarinic acid and caffeic acid are responsible for most of the biological activities of this plant, particularly its antioxidant and antibacterial activities [65]. In our study, they accounted for 91% of the phenolic compounds identified in lemon balm extract. In turn, ellagic acid identified in *G. urbanum* extract is responsible for various biological properties of this plant, including antioxidant, antimicrobial and anticancer activity [66,67]. Both this acid and quercetin and its derivatives identified in red raspberry (rutin, hyperoside) could also determine the biological properties of the extract obtained from this plant material, including its antibacterial and antioxidant activity [24,68]. These components accounted for about 67% of the total phenolic compounds identified in the red raspberry extract. Flavonoids, i.e., quercetin and kaempferol derivatives (94%), were also predominant among the phenolic components identified in the studied extract from *C. incanus*. They may contribute significantly to the biological activity assessed in this study. However, the polyphenols with the highest content in the tested material did not always determine their antioxidant activity. This would require additional research.

Table 4. Correlation (Pearson) coefficients between TPC and antioxidant activity determined by DPPH, ABTS and FRAP method.

	TPC	DPPH	ABTS	FRAP
TPC				
DPPH	0.966			
ABTS	0.957	0.929		
FRAP	0.903	0.972	0.819	

3.4. Antibacterial Activity

The antibacterial activities of the tested aqueous ethanolic extracts from the plant material in terms of the minimum inhibitory concentrations (MIC) and the diameters of the inhibition zones (IZ) are presented in Tables 5 and 6, respectively. The plant extracts showed an inhibitory effect on 9 of 18 bacterial strains tested with a mean diameter zone of inhibition of their growth in the range of 11.00–25.50 mm (Table 5). In turn, the methanolic and aqueous extracts of *Cistus ladanifers* exhibited high antibacterial activities against 9 and 7 of 14 bacterial strains tested, respectively (IZ, 15.5–21.5 mm and 15–20 mm, respectively) [9]. Lowering the polarity of the solvents in the extraction of *C. ladanifers* resulted in lower antimicrobial activity and, thus, smaller diameters of inhibition zones. In our studies, the aqueous ethanolic extracts of *B. pendula*, *C. incanus* and *G. urbanum* showed an inhibitory effect against the largest number of bacterial strains, i.e., *B. pendula* extract against seven and *C. incanus* and *G. urbanum* against six bacterial strains. In contrast, the aqueous ethanolic extracts of *A. europaeum* and *B. pendula* showed predominantly activity against five Gram-positive bacteria. The diameter of the inhibition zones for the *A. europaeum* was higher than for the *B. pendula* extract and ranged from 15.00 to 25.50 mm. The structure of the cellular wall of Gram-positive bacteria appeared to be more sensitive to the plant extracts compared to the Gram-negative bacteria cellular wall, which is also composed of several layers of peptidoglycan but additionally surrounded by a membrane containing fatty substances and polysaccharides, giving it less permeable characteristics.

Table 5. Antimicrobial activity of the plant extracts against the tested bacterial strains.

Bacterial Strain	Diameter of Inhibition Zone (IZ) in Mm								
	C. incanus	***M. alba***	***G. urbanum***	***A. europaeum***	***R. idaeus***	***A. archangelica***	***B. pendula***	***M. officinalis***	**Nitrofurantoin [2]**
Gram-positive bacteria									
S. aureus ATCC 6538P	trace	- [1]	11.00 ± 1.00 ^{b}	22.50 ± 0.50 ^{g}	-	-	trace	-	24.17 ± 0.28 ^{h}
S. aureus ATCC 25923	14.17 ± 0.29 ^{c}	-	14.00 ± 0.00 ^{c}	25.50 ± 0.50 ^{h,i}	11.33 ± 0.57 ^{b}	-	11.67 ± 0.56 ^{b}	-	23.33 ± 0.57 ^{h}
S. epidermidis ATCC 12228	17.33 ± 0.57 ^{e}	-	17.50 ± 0.50 ^{e}	trace	13.33 ± 0.57 ^{c}	-	13.00 ± 0.00 ^{c}	-	29.67 ± 0.29 ^{j}
E. faecalis ATCC 29219	-	-	-	15.00 ± 0.03 ^{d}	-	-	11.50 ± 0.50 ^{b}	-	26,83 ± 0.28 ^{i}
E. faecium ATCC 6057	-	-	-	-	-	-	-	-	17.67 ± 0.56 ^{e}
B. subtilis ATCC 6633	-	trace	trace	17.50 ± 0.50 ^{e}	-	trace	13.00 ± 0.00 ^{c}	-	29.33 ± 0.57 ^{j}
G. stearothermophilis ATCC 7953	12.00 ± 0.00 ^{b}	12.00 ± 0.50 ^{b}	11.50 ± 0.50 ^{b}	20.33 ± 0.57 ^{f}	11.00 ± 0.00 ^{b}	11.83 ± 0.57 ^{b}	13.50 ± 0.50 ^{c}	-	27.33 ± 0.58 ^{i}
Gram-negative bacteria									
E. coli ATCC 25922	-	-	-	-	-	-	-	-	24.00 ± 0.00 ^{h}
K. pneumoniae ATCC 13883	15.3 3± 0.76 ^{d}	-	-	-	-	-	11.00 ± 0.00 ^{b}	-	23.33 ± 0.57 ^{h}
P. vulgaris ATCC 13315	-	-	-	-	-	-	-	-	11.00 ± 0.00 ^{b}
P. mirabilis ATCC 12453	-	-	-	-	-	-	-	-	11.00 ± 0.00 ^{b}
L. monocytogenes 1043 S	-	-	-	-	-	trace	-	-	17.83 ± 0.76 ^{e}
S. marcescens ATTC 13880	-	-	-		-	-	-	-	11.50 ± 0.50 ^{b}
E. cloacae DSM 6234	-	-	-	-	-	-	-	-	18.3 3± 0.29 ^{e}
P. aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	21.67 ± 0.57 ^{g}
A. baumannii ATCC 19606	-	-	-	-	-	-	-	-	10.00 ± 0.00 ^{a}
S. maltophilia ATCC 12714	14.17 ± 0.29 ^{c}	-	12.00 ± 0.00 ^{b}	-	-	-	-	-	21.67 ± 0.56 ^{g}
B. bronchiseptica ATCC 4617	17.33 ± 0.57 ^{e}	-	14.00 ± 0.00 ^{c}	-	12.16 ± 0.76 ^{b}	-	12.00 ± 0.00 ^{b}	14.33 ± 0.57 ^{c}	21.50 ± 0.50 ^{g}

[1] not detected; [2] References compound, the diameter of commercial disc containing 0.03 mg of Nitrofurantoin was 6 mm (Mast Diagnostics, Merseyside, UK). Means with different lowercase letters ($^{a–j}$) indicate significant difference at the significance level of 0.05.

Table 6. Minimum inhibitory concentration (MIC) of the plant extracts against selected bacterial strains.

Bacterial Strain	MIC (mg/mL)								
	C. incanus	***M. alba***	***G. urbanum***	***A. europaeum***	***R. idaeus***	***A. archangelica***	***B. pendula***	***M. officinalis***	**Nitrofurantoin [2]**
Gram-positive bacteria									
S. aureus ATCC 25923	0.125	1	0.125	2	0.125	>2	0.25	0.25	0.025
S. epidermidis ATCC 12228	0.125	>2	0.125	1	0.125	>2	2	0.25	0.0125
E. faecalis ATCC 29219	0.125	0.5	0.0625	2	0.25	>2	2	>2	0.0125
E. faecium ATCC 6057	0.5	>2	0.25	2	>2	>2	2	>2	nd
B. subtilis ATCC 6633	- [1]	-	-	2	-	>2	-	0.5	0.0125
*G. stearothermophilis*ATCC 7953	-	-	-	2	-	>2	-	-	0.0125
Gram-negative bacteria									
E. coli ATCC 25922	>1	>2	>2	>2	>2	>2	>2	0.0625	0.00625
K. pneumoniae ATCC 13883	>1	>2	>2	1	>2	>2	>2	0.5	0.025
A. baumannii ATCC 19606	>1	>2	0.125	2	2	>2	>2	>2	nd
P. aeruginosa ATCC 27853	>1	>2	>2	>2	>2	>2	>2	>2	>0.4
S. maltophilia ATCC 12714	1	>2	0.25	2	>2	>2	>2	1	>0.4
B. bronchiseptica ATCC 4617	>1	>2	0.125	1	2	>2	>2	0.5	>0.4

[1] not detected; [2] References compound, the MIC of Nitrofurantoin was determined according to the CLSI recommendations.

Considering the MIC values achieved for *A. europaeum* herb and *B. pendula* leaf extracts, it was observed that among Gram-positive bacteria, *S. epidermidis* ATCC 12228 was the most sensitive to *A. europaeum* herb extract (MIC, 1 mg/mL) and *S. aureus* ATCC 25923 (MIC, 0.25 mg/mL) to *B. pendula* leaf extract. In contrast, among Gram-negative bacteria, a more pronounced antimicrobial effect was observed for the *A. europaeum* herb extract than for the *B. pendula* leaf extract. The *A. europaeum* herb extract was more effective than *B. pendula* leaf extract, especially against *K. pneumoniae* ATCC 13883 and *B. bronchiseptica* ATCC 4617, with an MIC value of 1 mg/mL. Strong antibacterial activity was also observed for water, methanolic and ethanolic extracts of *A. europaeum* grown in Turkey, especially against *S. aureus* [69]. In contrast, *S. epidermidis* was more sensitive to the methanolic extract of *A. europaeum* than to *K. pneumoniae* and *E. coli* against both methanolic and ethanolic extracts. The ethanolic extract (80%) of *B. pendula* showed a good antibacterial effect against *B. cereus* [70] and, as in our study, a moderate effect against the other strains tested, with *S. aureus* being the most sensitive strain with an MIC of 0.25 mg/mL. The *C. incanus* extract was more effective against Gram-positive bacteria than against Gram-negative bacteria. In the study by Viapiana et al. [47], it was also observed that aqueous extracts of *C. incanus* exhibited better activity against Gram-positive bacteria, mainly *S. aureus* and *S. epidermidis,* with MIC ranges between 0.5–8 and 0.25–4 mg/mL, respectively. In turn, Kuchta et al. [71] confirmed the reasonable antimicrobial activity of aqueous *C. incanus* extracts against all Gram-positive bacteria tested, with an MIC value of 4 mg/mL and no activity against Gram-negative bacteria in the tested concentration range. Aqueous extracts from two *Cistus* species (*C. creticus* and *C. salviifolius*) of Croation origin showed very similar activity toward the same bacterial species, especially against *A. baumannii* FSST-20 clinical isolate (MIC 250 ug/mL), *A. baumannii* ATCC 19606 (MIC 500 ug/mL) and *S. aureus* MRSA-1 (MIC 500 ug/mL) [57]. However, slightly better activity of *C. creticus* was found against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. In our study, the MIC results against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. faecalis* ATCC29219 of aqueous ethanolic *C. incanus* extracts were lower and reached the value of 0.125 mg/mL but against *E. faecium* ATCC 6057 it was 0.5 mg/mL. These differences may be related to the use of different species of *Cistus* originating from different geographical regions and the use of different solvents in the extraction process. Similar MIC values were also obtained for *S. aureus* and *S. epidermidis* strains when extracts from *G. urbanum* and red raspberry were studied. In comparison, the study by Dimitrova et al. [16] showed that the ethyl acetate and *n*-butanol fractions of the roots and aerial parts of *G. urbanum* inhibited the growth of Gram-positive pathogenic and opportunistic bacteria of the genus *Staphylococcus* more strongly than methanolic extracts and other fractions. The MIC values for the methanolic extracts of *G. urbanum* were higher than those obtained in our experiment. In addition, ethyl acetate and butanol extracts of fresh and dried *G. urbanum* roots showed a marked inhibitory effect on *S. aureus* after placing 0.6 mg extract on the disc [72]. In turn, the *E. faecalis* strain was the most sensitive to the aqueous ethanolic extract of *G. urbanum,* with an MIC value of 0.0625 mg/mL. The sensitivity of this bacterial strain was also found when white mulberry and red raspberry extracts were used in the study. The MIC values were higher than those for the *G. urbanum* extract. These were 0.5 mg/mL when the white mulberry extract was tested and 0.25 mg/mL for the red raspberry extract. In contrast, the methanolic extract of wild raspberry leaves from the Balkan region and aqueous mulberry extracts from Mae Hong Son were the most effective against *E. coli* ATCC 8739 [38,73].

The *G. urbanum* rhizome extract was also effective against selected strains of Gram-negative bacteria, achieving MIC values ranging from 0.125 to above 2 mg/mL. The most sensitive strains to *G. urbanum* rhizome extract were *A. baumani* (MIC, 0.125 mg/mL), *B. bronchiseptica* (MIC, 0.125 mg/mL) and *S. maltophilia* (MIC, 0.25 mg/mL). These bacterial strains were also susceptible to lemon balm extract, which showed significantly stronger antibacterial activity against *B. bronchiseptica* and *S. maltophilia* than against *K. pneumonia.* It is worth noting that the lemon balm extract showed a higher MIC value against *E. coli* than the other aqueous ethanolic extracts tested. However, the aqueous ethanolic extract

from the root of *A. archangelica* did not affect the growth inhibition of any of the bacterial strains. No growth inhibition zones were observed for either Gram-positive or Gram-negative bacteria, and the MIC values were above 2 mg/mL. When the various fractions of *A. archangelica* extracts obtained by flash chromatography were subjected to antibacterial activity against four bacterial strains, it was shown that they were all active against the pathogens tested [74]. However, the most interesting results were obtained for all ethyl acetate fractions from methanol, methanol:water (1:1) and water. MIC values were in the range 125–500 μg/mL. Relatively better antimicrobial activity of *Angelica* species was assessed for essential oil (EO) than extracts. EO of *A. archangelica* root showed considerable antimicrobial activity against *E. faecalis* and *Candida albicans* and a weaker activity against the intestinal flora [75].

4. Conclusions

The extracts obtained using 70% ethanol commercially available natural medicine-used plant material from *C. incanus*, *G. urbanum*, *M. officinalis* and *R. idaeus* had the highest total polyphenol content and DPPH radical scavenging activity. Among these extracts, extracts from *C. incanus* and *G. urbanum* also showed a pronounced ability to scavenge ABTS cation radicals and antioxidant activity, as measured by the FRAP method. Antioxidant activity correlated with the content of total polyphenols in the tested extracts. The extracts from *C. incanus*, *M. alba*, *R. idaeus* and *B. pendula* demonstrated a higher content of flavonoids than phenolic acids compared to other tested extracts. Rosmarinic acid could affect the antioxidant activity of the lemon balm extract, whereas ellagic acid probably influenced the antioxidant activity of the *G. urbanum* extract. On the other hand, the identified flavonoids belonging to quercetin derivatives could be responsible for the antioxidant activity of *C. incanus* and red raspberry. The aqueous ethanolic extracts from *A. europaeum* herb displayed pronounced antibacterial activity against the tested Gram-positive bacteria when the diameter of the inhibition zones of the tested strain was determined, while the lowest MIC values were obtained for the extracts from *C. incanus*, *G. urbanum* and *R. idaeus*. These data provide the basis for further studies. The chemical composition of the extracts should be carefully analyzed, among other things, in terms of the contribution of individual polyphenols to the biological activity to be assessed. This will enable the application of some extracts, especially *C. incanus* herb and *G. urbanum* rhizome extracts, as natural antioxidant and antimicrobial agents in food and cosmetic products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12199871/s1, Figure S1. HPLC-DAD chromatograms of the studied plant extracts: (a) *Cistus incanus* L. herb; (b) *Morus alba* L. leaves; (c) *Geum urbanum* L. rhizome; (d) *Asarum europaeum* L. herb; (e) *Rubus idaeus* L. leaves; (f) *Angelica archangelica* L root.; (g) *Betula pendula* Roth. leaves; (h) *Melissa officinalis* L. leaves. Table S1. Characteristic parameters of the HPLC analysis.

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