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Antioxidant Properties of Alien Plant Species: Assessing Phenolic and Flavonoid Content, Antioxidant, Oxidant Capacities, and Antimicrobial Activity

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Abstract: Alien plant species pose significant threats to natural ecosystems due to their adaptability and invasive potential. In this study, the antioxidant activity of 13 alien and invasive plant species was investigated, regarding their total phenolic content, flavonoid content, total antioxidant capacity, total oxidant capacity, and antimicrobial activity. Plant tissues were macerated, evaporated to dryness, and the extracts were dissolved in water. Total phenolic content ranged from 6.55 mg GAE/g extract to 30.82 mg GAE/g extract. Flavonoid content spanned from 3.04 mg Quercetin/g extract to 40.81 mg Quercetin/g extract. The total antioxidant capacity ranged between 662 CEAC µM and 1540 CEAC µM. Total oxidative capacity can be correlated to the abiotic stress. *Rhus typhina* L. and *Impatiens balfourii* Hooker f., exhibited positive total oxidant capacity values of 122.56 ± 7.85 µM H_2O_2 Equiv./L and 141.38 ± 4.33 µM H_2O_2 Equiv./L, respectively. These two species also showed the highest total antioxidant capacity that were 1190,06 ± 137,36 CEAC µM for *Rhus typhina* L and 1540,34 ± 270,84 for CEAC *Impatiens balfourii* Hooker f. The results suggest that the extraction solvent significantly affects the antioxidant capacity, with ethanol generally providing higher extraction efficiency for many species. The antimicrobial activity was determined against *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Candida albicans*. *Rhus typhina* L was active against *E. coli* and *P. mirabilis*. This data indicates that plants increased antioxidant defense systems against abiotic stresses. Clearly, the correlation between total oxidant capacity and total antioxidant capacity values in plants is a valuable factor indicating the mechanism of the plant defense.

INTRODUCTION

Plants possess a robust antioxidant system that protects against oxidative stress, a condition induced by an imbalance between reactive oxygen species (ROS) production and detoxification. This system controls cellular homeostasis but may also be a biomarker for environmental stressors. Reactive oxygen species (ROS), produced in a different subcellular compartment, have dual roles—serving as signaling molecules in biological processes while potentially causing damage such as DNA alteration, membrane disruption, and cell death when their levels are uncontrolled (Apel et al., 2004; Mittler, 2002). The group of antioxidant enzymes, including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), are most important protectors against exacerbated ROS production forming a dynamic defense network to mitigate oxidative damage (Noctor et al. 1998, Foyer et al. 2005).

For example, in environments with elevated ozone (O_3) levels, plants face increased oxidative burdens, as O_3 exposure triggers increased ROS production, leading to metabolic disturbances. Both enzymatic and non-enzymatic mechanisms contribute to ROS generation, with their activity modulated by oxygen availability (Sharma et al. 2012, Veljovic-Jovanovic et al. 2006). This adaptability

underlines the complexity of the plant antioxidant system, highlighting its critical role in maintaining redox balance and its potential utility as an indicator of environmental stress (Ainsworth et al. 2016, Feng et al. 2019).

The measurement of flavonoids and phenolic content in plants plays a critical role in understanding their antioxidant capacity and their response to environmental stress. Flavonoids and phenolic compounds are secondary metabolites with potent antioxidative properties, acting as ROS scavengers and metal chelators, thus contributing to the mitigation of oxidative stress. These compounds can directly neutralize ROS, reduce oxidative damage, and enhance the overall resilience of plants under stress conditions such as drought, salinity, and high ozone levels (Heim et al. 2002, Rice et al. 1997).

The accumulation of flavonoids and phenolics is often triggered by environmental factors, making their quantification a valuable biomarker for assessing plant stress levels. For instance, under ultraviolet (UV) radiation or ozone exposure, plants tend to increase the synthesis of these compounds as part of their protective strategy. Moreover, flavonoids, particularly those localized in the epidermal tissues, can act as UV filters, reducing light-induced oxidative damage. Similarly, phenolic compounds play a role in strengthening the plant cell wall and modulating enzyme activities associated with stress response pathways (Agati et al. 2010, Nicholson et al. 1992).

Total antioxidant capacity (TAC) represents the overall ability of the plants to neutralize reactive oxygen species (ROS) and prevent oxidative damage. It encompasses the combined effects of all antioxidant molecules, including enzymatic antioxidants (e.g., superoxide dismutase, catalase, and peroxidase), non-enzymatic antioxidants (e.g., ascorbic acid, glutathione, and carotenoids), and secondary metabolites like flavonoids and phenolics (Apel et al. 2004, Rice-Evans et al. 1997). On the other hand, flavonoid and phenolic content specifically quantify the presence of these particular secondary metabolites, which are important but not the sole contributors to antioxidant defense (Heim et al. 2002, Sharma et al. 2012).

Flavonoids and phenolics play a significant role in scavenging free radicals and chelating metal ions, directly mitigating oxidative stress. However, enzymatic antioxidants react with specific ROS in distinct subcellular compartments, and non-phenolic compounds like ascorbate and tocopherols give specific antioxidant functions that flavonoids and phenolics cannot fully substitute (Foyer et al. 2005, Stalikas, 2018). However, during severe oxidative stress, enzymatic antioxidants may play a more dominant role by regenerating non-enzymatic antioxidants and repairing damaged molecules (Noctor et al. 1998).

Alien (non-native, introduced, exotic) plant species are the species that were introduced to an area in which they were never naturally distributed, by intentional or unintentional anthropogenic activities (Mitić et al., 2008). Many of them have adapted to the conditions of the new habitat and become a danger to natural ecosystems by invading the habitats of native species and suppressing their growth, or human health, by causing allergies. Most of these plants are resistant to herbicides, parasites and other plant pests, probably as the result of various antimicrobial compounds they contain. However, the research of the antimicrobial and antioxidant activity of alien species has only begun to attract the attention of researchers in the last decades (e.g. Ahmad et al., 2011; Albouchi et al., 2013; Rattanata et al., 2014; Morah t al., 2015; Szewczyk et al. al., 2016, Aissani et al., 2018).

The aim of this research was to evaluate the total oxidative stress antioxidant capacity, and the phenolic and flavonoid content of plant species alien to the region of Bosnia and Herzegovina. Selected plants with the highest antioxidative capacity were tested for antimicrobial activity. Alien plants, often characterized by their adaptability and robust secondary metabolite production, may offer novel antioxidant agents. This research not only contributes to understanding the biochemical potential of these species but also highlights their role as a possible resource for developing new pharmaceuticals with high antioxidant capacity.

MATERIALS AND METHODS

Samples

For the purpose of this research, the following alien plant species were selected: *Rhus typhina* L. (deciduous tree of the Anacardiaceae family, native to eastern North America), *Robinia pseudoacacia* L. (deciduous tree of the Fabaceae family, native to North America), *Acer negundo* L. (deciduous tree of the Aceraceae family, native to North America), *Ailanthus altissima* (Mill.) Swingle (deciduous tree of the Simaroubaceae family, native to Asia), *Reynoutria japonica* Houtt. (herbaceous perennial of the Polygonaceae family, native to Asia), *Impatiens balfourii* Hooker f. (herbaceous perennial of the Balsaminaceae family, native to Asia), *Amaranthus albus* L. (herbaceous annual of the Amaranthaceae family, native to the tropical Americas), *Galinsoga parviflora* Cav. (herbaceous annual of the Compositae family, native to South America), *Commelina communis* L. (herbaceous annual of the Commelinaceae family, native to Asia), *Panicum capillare* L. (herbaceous annual of the Poaceae family, native to North America), *Senecio inaequidens* DC (herbaceous perennial of the Compositae family, native to Africa), and *Erigeron annuus* (L.) Pers. and *E. canadensis* L. (herbaceous annuals of the Compositae family, native to North and Central America). The plant samples (leaves for trees, all aboveground parts for herbaceous plants) were collected within the administrative boundaries of the city of Sarajevo in Septemper and October 2023, air-dried in the laboratory, powdered in a blender and kept in marked, sealed glass jars at room temperature.

Chemicals

Following chemical were used: Gallic acid, p.a. ACS reagent ≥98 %, (Carl Roth), Folin-Ciocalteu's phenol reagent, Analytical CRM (CPAchem), Sodium carbonate, puriss. p.a. ACS reagent Ph. Eur anhydrous ≥99,8 % (Sigma-Aldrich), Aluminium chloride hexahydrate, p.a. >99 %, (Centrohem), Quercetin, HPLC ≥95 % (Sigma-Aldrich), Sodium chloride, puriss. p.a. ACS reagent Ph Eur. ≥99,5 % (Honeywell), Xylenol orange sodium salt, ACS reagent (Acros Organics), Sulfuric acid, 98 %, (Merck), o – Dianisidine dihydrochloride, 98 %, (Thermoscientific), Ammonium iron (II) sulfate

hexahydrate, analytical reagent grade ≥99 % (Fisher Scientific), Glycerol, analytical reagent grade ≥99 %, (Fisher Scientific), Ethanol, puriss. p.a. absolute ≥99,8 % (Honeywell). The chemicals were used as received from their original packaging without undergoing any additional purification.

Maceration extraction

Extracts of all 13 plants were prepared by weighing approximately 2.5 ± 0,01 g of dried plant material, which was then transferred to a 500 mL Erlenmeyer flask. Subsequently, 250 mL of distilled water was added, and the mixture was placed on a magnetic stirrer at 500 rpm and 60°C for 60 minutes. After the extraction, the mixture was filtered through Whatman filter paper. The filtrate was collected and evaporated to dryness in a water bath at 60°C. The extracts were weighed.

Soxhlet extraction

Extracts of all 13 plants were prepared using Soxhlet extraction. Dried plant material were weighed and transferred into an extraction thimble, which was then placed into the Soxhlet extractor. Subsequently, 150 mL of 96 % ethanol was added, and the Soxhlet extraction was performed for 3 hours. After extraction, the extracts were filtered through Whatman filter paper and evaporated to dryness in a water bath at 60°C. The extracts were then weighed.

Samples

The samples for analysis were prepared by weighing approximately 0.1 g ± 0,1 mg and dissolving it in 10 mL of 96 % ethanol and in deionized water. The following analyses were performed on these samples: total oxidant capacity, total antioxidant capacity, total phenolic content, total flavonoid content, and antimicrobial activity.

Total oxidant capacity

In this assay (Erel, 2005), oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion, a process that is enhanced by the presence of glycerol in the reaction mixture. The ferric ion then reacts with xylenol orange in an acidic medium, producing a colored complex. The intensity of the color, which can be measured using a spectrophotometer, reflects the total concentration of oxidants in the sample. The assay is calibrated using hydrogen peroxide, and results are reported in micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 Equiv./L) mean value ± SD in the Table 1. The composition of Reagents 1 and 2 is described below. For the measurement, 20 μL of the sample is mixed with 200 μL Reagent 1, and the absorbance is first measured at 560 nm. Afterward, 50 μL of Reagent 2 is added, and the absorbance is recorded again at the same wavelength after 45 minutes.

Reagent 1 contains 150 μM xylenol orange, 140 mM sodium chloride, 1.36 M glycerol, with the pH adjusted to 1.75. This solution is stable for at least 6 months when stored at 4°C. Reagent 2 includes 5 mM ferrous ammonium sulfate and 10 mM o-dianisidine dihydrochloride, and it also remains stable for a minimum of 6 months at 4°C. Both reagents are prepared in 25 mM sulfuric acid. The results are given in Table 1. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Total antioxidant capacity

Antioxidant capacity was tested by e-BQC lab instrument based on a redox potential of the tested compounds and its comparison with antioxidant activity of ascorbic acid. Measurements are expressed in charge units—micro Coulombs (μC). To compare the results (μC) to antioxidant capacity of Vitamin C Antioxidant Capacity Equivalents (CEAC) are used, and results are expressed as mean value ± SD in the Table 2. Calibration curve with a standard solution of ascorbic acid was ussed to measure charge values.

Determination of Total Flavonoid Content

The total flavonoid content was determined using the method described by Cao-Ngoc (2020), with slight modifications. A 150 μL aliquot of the sample was transferred to a microplate, followed by the addition of 150 μL of 2 % Aluminum Chloride solution. After a 10-minute incubation, the absorbance was measured. The assay was calibrated using a standard quercetin solution, and the results were expressed as mg Quercetin equivalents per gram of extract (mg QE/g), calculated as the mean value ± standard deviation (SD). The results are given in the Table 3. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Determination of Total Phenolic Content

The total phenolic content in the extracts was measured using the Folin-Ciocalteu method (Gonçalves et al., 2013), with slight modifications. A 500 μL aliquot of the extract was transferred to a 10 mL volumetric flask, followed by the addition of 500 μL of deionized water and 500 μL of Folin-Ciocalteu reagent. After 3 minutes, 1,5 mL of 20 % sodium carbonate was added, and the flasks were filled to volume with deionized water. The solutions were incubated in the dark for 45 minutes, after which the absorbance was recorded at 765 nm. Gallic acid was used as the standard, and total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). Results are presented as mean values ± standard deviation (SD) in the Table 4. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Determination of Antimicrobial Activity

Luria Bertani (LB) agar was prepared by dissolving 40 g of growth media (Carl Roth, Germany) in 1 L of distilled water. LB broth was prepared by dissolving 10 g of Tryptone, 5 g of Yeast extract, and 10 g of NaCl (all Sigma-Aldrich, USA) in 1 L of distilled water. Saburaud dextrose agar (Liofilchem, Italy) was prepared by dissolving 65 g of media in 1 L of distilled water. Saburaud dextrose broth (Merck, Germany) was prepared by dissolving 30 g of media in 1 L of distilled water. All growth media were autoclaved at 121°C for 20 minutes.

Antimicrobial activity screens

Antimicrobial activity was tested against the following bacteria and a fungus: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, and Candida albicans*.

The bacterial cultures were grown for 24 h at 37°C in LB broth and Candida albicans in Saburaud dextrose broth under the same conditions. Next, the cultures were diluted in 0.9% sodium chloride solution to match a 0.5 McFarland

turbidity standard. The uniform growth was obtained by streaking 4 mm thick LB agar plates three times with a Drigalski spatula (100 µL of initial inoculum), rotating the plate for 90° in-between streaks. To establish putative antimicrobial activity against Candida albicans, Saburaud dextrose agar plates were streaked. The plates were air-dried under sterile conditions for 5 minutes. Then, wells with a diameter of 8 mm were punched aseptically with a sterile cork borer. Next, ethanol and water extracts (100 µL) of plants selected for this study were introduced into the wells. The experiments were performed in triplicates. The plates were left at room temperature for 15 mins and incubated for 24 h at 37°C. Zones of inhibition were measured as the diameter of the halo around the well.

RESULTS

Total oxidant capacity

The total oxidant capacity (TOC) results (Table 1.) demonstrate that the activity of different plant species varies depending on the extraction solvent. *Rhus typhina* has similar TOC values for both water and ethanol extracts, with slightly higher activity in water, suggesting that both polar and semi-polar compounds contribute to its properties. *Impatiens balfourii* shows much higher activity in water extracts, indicating that water-soluble compounds play a key role. On the other hand, *Erigeron canadensis* displays high TOC only in ethanol extracts, indicating its active compounds are more soluble in ethanol.

Table 1. Total oxidant capacity

Species	Water extracts	Ethanol extracts
Rhus typhina	122,56 ± 7,85	111,31 ± 1,13
Erigeron annuus	/	/
Erigeron canadensis	/	164,75 ± 3,66
Impatiens balfourii	141,38 ± 4,33	70,98 ± 3,02
Amaranthus albus	/	78,85 ± 3,65
Robinia pseudoacacia	/	79,95 ± 2,00
Ailanthus altissima	/	96,20 ± 3,28
Galinsoga parviflora	/	92,08 ± 0,80
Acer negundo	/	109,77 ± 6,18
Reynoutria japonica	/	/
Commelina communis	/	88,84 ± 5,30
Panicum capillare	/	82,84 ± 3,96
Senecio inaequidens	/	185,95 ± 10,16

Other plants, such as *Amaranthus albus*, *Robinia pseudoacacia*, and *Ailanthus altissima*, exhibit moderate TOC in ethanol extracts, with *Acer negundo* having the highest among them. *Senecio inaequidens* shows the strongest overall activity in ethanol extracts. The lack of TOC values for water extracts in some species may be due to the poor solubility of their compounds in water.

Total antioxidant capacity

The results for antioxidant capacity ($\overline{X} \pm SD$ CEAC µM), given in the Table 2., show varying activities across different plant species and extraction solvents, with both water and ethanol extracts yielding distinct values. In general, ethanol extracts exhibit slightly higher antioxidant capacities than water extracts for many species.

Table 2. Total antioxidant capacity

Species	Water extracts	Ethanol extracts
Rhus typhina	1190,06 ± 137,36	797,21 ± 134,02
Erigeron annuus	1132,71 ± 22,72	1250,18 ± 88,09
Erigeron canadensis	937,21 ± 105,54	1084,31 ± 167,63
Impatiens balfourii	1540,34 ± 270,84	1250,90 ± 165,00
Amaranthus albus	662,85	1023,33 ± 108,89
Robinia pseudoacacia	820,88	1022,24 ± 70,27
Ailanthus altissima	993,58 ± 31,33	1326,40 ± 123,63
Galinsoga parviflora	730,63	1434,56 ± 166,93
Acer negundo	1003,03 ± 116,57	703,57 ± 65,23
Reynoutria japonica	1224,60 ± 230,01	1401,53 ± 32,74
Commelina communis	982,17	1131,85 ± 59,65
Panicum capillare	1097,19 ± 181,60	786,33 ± 65,48
Senecio inaequidens	971,75	1440,73 ± 79,67

Rhus typhina shows a significant difference between water (1190.06 ± 137.36 µM) and ethanol (797.21 ± 134.02 µM) extracts, with the water extract demonstrating stronger capacity. Similarly, *Erigeron annuus* displays higher activity in ethanol (1250.18 ± 88.09 µM) compared to water (1132.71 ± 22.72 µM). In contrast, *Impatiens balfourii* has a notably higher antioxidant capacity in water (1540.34 ± 270.84 µM) than in ethanol (1250.90 ± 165.00 µM).

Amaranthus albus and *Robinia pseudoacacia* show relatively low activity in water extracts (662.85 µM and 820.88 µM, respectively) compared to their ethanol counterparts (1023.33 ± 108.89 µM and 1022.24 ± 70.27 µM). This trend continues with *Ailanthus altissima*, which shows higher capacity in ethanol (1326.40 ± 123.63 µM) than in water (993.58 ± 31.33 µM). *Galinsoga parviflora* follows a similar pattern with a substantial increase in ethanol (1434.56 ± 166.93 µM) compared to water (730.63 µM).

Reynoutria japonica displays high antioxidant capacity in both extracts, with ethanol (1401.53 ± 32.74 µM) slightly exceeding water (1224.60 ± 230.01 µM). In contrast, *Acer negundo* and *Panicum capillare* show a reverse pattern, with higher activity in water (1003.03 ± 116.57 µM and 1097.19 ± 181.60 µM) than in ethanol (703.57 ± 65.23 µM and 786.33 ± 65.48 µM). Lastly, *Senecio inaequidens* shows its highest antioxidant capacity in ethanol (1440.73 ± 79.67 µM) compared to water (971.75 µM).

These results suggest that the extraction solvent significantly affects the antioxidant capacity, with ethanol generally providing higher extraction efficiency for many species, though there are notable exceptions where water extracts show superior activity.

The total phenolic content

The results for total phenolic content (TPC) (Table 3.) in different plant extracts show that water generally yields higher TPC compared to ethanol. For example, ***Erigeron canadensis*** has the highest TPC in water (30.82 ± 3.12 mg GAE/g), while ***Erigeron annuus*** also has high phenolic content in water (29.51 ± 2.65 mg GAE/g). Conversely, ***Galinsoga parviflora*** has the lowest TPC in water (6.55 ± 1.15 mg GAE/g), but its ethanol extract shows a higher value (11.75 ± 0.44 mg GAE/g).

Impatiens balfourii shows a significant difference between water (17.46 ± 0.38 mg GAE/g) and ethanol (7.33 ± 0.61 mg GAE/g), favoring water. However, ***Acer negundo*** is one of the few plants where ethanol (21.52 ± 1.54 mg GAE/g) outperforms water (18.77 ± 0.90 mg GAE/g).

Table 3. Total phenolic content

Species	Water extracts	Ethanol extracts
Rhus typhina	27,22 ± 0,97	20,93 ± 1,79
Erigeron annuus	29,51 ± 2,65	13,29 ± 0,82
Erigeron canadensis	30,82 ± 3,12	20,24 ± 2,62
Impatiens balfourii	17,46 ± 0,38	7,33 ± 0,61
Amaranthus albus	29,23 ± 2,45	12,78 ± 1,36
Robinia pseudoacacia	22,49 ± 1,62	13,05 ± 1,99
Ailanthus altissima	23,74 ± 1,11	19,69 ± 1,42
Galinsoga parviflora	6,55 ± 1,15	11,75 ± 0,44
Acer negundo	18,77 ± 0,90	21,52 ± 1,54
Reynoutria japonica	27,62 ± 0,98	16,69 ± 0,98
Commelina communis	14,07 ± 0,74	9,59 ± 2,99
Panicum capillare	23,74 ± 1,62	9,58 ± 0,74
Senecio inaequidens	17,88 ± 1,87	16,69 ± 1,03

Overall, these results suggest that water is generally a more effective solvent for phenolic extraction, but ethanol can be superior for certain species. This variability underscores the need to optimize extraction methods depending on the plant and the application, such as in antioxidant production or natural product research.

The Total Flavonoid Content

The results for total flavonoid content (TFC) (Table 4.) across water and ethanol extracts reveal distinct patterns in solvent efficiency for flavonoid extraction. In contrast to the results for phenolic content, ethanol extracts generally show higher flavonoid content compared to water extracts. For example, *Rhus typhina* shows a significant difference, with ethanol extracts yielding 65.14 ± 1.22 mg QE/g, far higher than the water extract (24.10 ± 0.84 mg QE/g). Similarly, *Impatiens balfourii* has an exceptionally high TFC in ethanol (69.41 ± 0.55 mg QE/g) compared to water (9.62 ± 2.28 mg QE/g).

Table 4. Flavonoid phenolic content

Species	Water extracts	Ethanol extracts
Rhus typhina	24,10±0,84	65,14 ± 1,22
Erigeron annuus	19,05± 2,59	18,18 ± 1,01
Erigeron canadensis	40,81±1,83	47,77 ± 2,68
Impatiens balfourii	9,62±2,28	69,41 ± 0,55
Amaranthus albus	32,02±2,88	67,24 ± 1,81
Robinia pseudoacacia	14,76±2,00	52,09 ± 5,20
Ailanthus altissima	19,78±2,64	82,18 ± 3,31
Galinsoga parviflora	4,66±0,03	57,64 ± 1,20
Acer negundo	3,04±0,03	71,80 ± 2,67
Reynoutria japonica	40,22±1,64	36,29 ± 0,78
Commelina communis	13,13±0,53	34,99 ± 1,17
Panicum capillare	22,27±1,19	57,32 ± 1,46
Senecio inaequidens	18,63±3,30	66,04 ± 2,41

Some species show more balanced results, like *Erigeron canadensis*, where both water (40.81 ± 1.83 mg QE/g) and ethanol (47.77 ± 2.68 mg QE/g) extracts contain similarly high levels of flavonoids. *Reynoutria japonica* is another outlier, where the water extract (40.22 ± 1.64 mg QE/g) slightly exceeds the ethanol extract (36.29 ± 0.78 mg QE/g), suggesting that water is more effective for flavonoid extraction in this species.

Species like *Ailanthus altissima* (82.18 ± 3.31 mg QE/g), *Acer negundo* (71.80 ± 2.67 mg QE/g), and *Amaranthus albus* (67.24 ± 1.81 mg QE/g) show significantly higher flavonoid content in ethanol, indicating that ethanol is generally the better solvent for extracting flavonoids from most of the species in this study. The variability between species and solvents highlights the importance of choosing the appropriate solvent depending on the plant and target compounds for specific applications.

Antimicrobial and fungicidal activity screens

Out of 26 plant extracts tested, only extracts corresponding to *Rhus typhina* showed antimicrobial activity. Water extracts of *Rhus typhina* inhibited the growth of *Escherichia coli* (average inhibition zone diameter 15 mm) and *Proteus mirabilis* (17 mm). Ethanol extracts of the same species inhibited the growth of *Staphylococcus aureus* (21 mm), *Proteus mirabilis* (20 mm), and *Pseudomonas aeruginosa* (19 mm). Antimicrobial activity against *Candida albicans* was not detected.

DISCUSSION

Total phenolic and total flavonoid content

The total phenolic content of *Rhus typhina* in the water extracts (27.22 ± 0.97 mg GAE/g) and ethanol extracts (20.93 ± 1.79 mg GAE/g) is consistent with values reported in the literature, such as Liu (2019), who reported a range of 25.96 ± 52.26 mg GAE/g. However, our values are notably higher than those for fruit extracts reported by Fu (2011), which ranged from 0.1188 ± 0.0011 to 5.85 ± 0.1859 mg GAE/g. Similarly, the total flavonoid content in water extracts (24.10 ± 0.84 mg QE/g) falls within the range of 15.71 to 62.53 mg QE/g reported by Wang (2015) for leaf extracts, while the ethanol extracts (65.14 ± 1.22 mg QE/g) are near the upper limit of this range. For fruit extracts, Wang (2015) found values between 11.31 to 71.46 mg QE/g. These variations may result from differences in extraction solvents, plant parts used, or environmental factors.

The total phenolic content of *Erigeron annuus* in our water extracts (29.51 ± 2.65 mg GAE/g) is lower than the range reported by Zhang (2020), which was 31.6 to 152.8 mg GAE/g, with water being the most effective solvent. Similarly, our ethanol extracts (13.29 ± 0.82 mg GAE/g) are considerably lower than the 92.6 ± 1.2 mg GAE/g reported by Zhang. This variation could be due to differences in extraction methods, geographic origin of the plant material, or environmental conditions.

For the total flavonoid content, our water extracts (19.05 ± 2.59 mg QE/g) and ethanol extracts (18.18 ± 1.01 mg QE/g) are also significantly lower than the range reported by Zhang, where flavonoid content ranged from 46.7 to 480.2 mg QE/g, with the ethanol extract reaching 26.8 ± 0.4 mg QE/g. The lower flavonoid content in our extracts could be attributed to different plant parts, solvent efficiency, or extraction techniques used.

The total phenolic content of *Erigeron canadensis* in our water extracts (30.82 ± 3.12 mg GAE/g) and ethanol extracts (20.24 ± 2.62 mg GAE/g) is significantly lower than the values reported by Barhoumi et al. (2024) and Polat et al. (2022). Barhoumi et al. reported much higher phenolic content (95.59 ± 0.4 mg GAE/g) from essential oils produced by hydrodistillation, while Polat et al. found 71.34 ± 0.53 mg GAE/g using different extraction methods. These differences highlight the impact of extraction techniques and plant material used on phenolic yield.

For total flavonoid content, water extracts (40.81 ± 1.83 mg QE/g) and ethanol extracts (47.77 ± 2.68 mg QE/g) are much lower than the 467.00 ± 0.53 mg QE/g reported by Barhoumi et al. (2024). However, our values exceed those reported by Polat et al. (2022), who found 18.91 ± 1.46 mg CA/g. The differences in results could be influenced by the use of different standards (QE vs. CA) and extraction methods, as Barhoumi et al. focused on essential oils, which may yield different concentrations of phenolics and flavonoids.

The total phenolic content of *Impatiens balfourii* in water extracts (17.46 ± 0.38 mg GAE/g) is similar to the flower extracts reported by Maleš et al. (2020), which were 18.6 ± 0.6 mg GAE/g. However, our ethanol extracts (7.33 ± 0.61 mg GAE/g) are closer to the values reported for leaf extracts (7.9 ± 0.2 mg GAE/g) by Maleš et al. This variation may be due to the different plant parts used, as we used stem extracts, which may yield different phenolic concentrations compared to leaves and flowers.

Regarding flavonoid content, our water extracts (9.62 ± 2.28 mg QE/g) and ethanol extracts (69.41 ± 0.55 mg QE/g) show higher values than the leaves and flowers reported by Maleš et al. (2020), where leaf extracts contained 4.6 ± 0.1 mg QE/g and flower extracts 20.5 ± 0.1 mg QE/g. The notably higher flavonoid content in our ethanol extracts suggests that solvent choice and plant part can significantly influence flavonoid yield.

The total phenolic content of *Amaranthus albus* is 29.23 ± 2.45 mg GAE/g in water extracts and 12.78 ± 1.36 mg GAE/g in ethanol extracts, indicating that water is a more effective solvent for extracting phenolics in this case. In contrast, the total flavonoid content is 32.02 ± 2.88 mg QE/g in water extracts and 67.24 ± 1.81 mg QE/g in ethanol extracts, suggesting that ethanol is more efficient for flavonoid extraction.

These results highlight differences in extraction efficiency depending on the solvent used. Comparing these findings with data from other *Amaranthus* species could provide a broader understanding of the variability in phytochemical content within the genus. Future research could focus on these comparisons to further elucidate the phytochemical profile and potential applications of *Amaranthus albus*.

The total phenolic content (TPC) of *Robinia pseudoacacia* in our study was measured at 22.49 ± 1.62 mg GAE/g in water extracts and 13.05 ± 1.99 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was found to be 14.76 ± 2.00 mg QE/g in water extracts and 52.09 ± 5.20 mg QE/g in ethanol extracts.

Comparatively, Stankov et al. (2018) reported a total phenolic content of 0.77 mg GAE/mL and a total flavonoid content of 0.17 mg QE/g of extract from flower extracts of *Robinia pseudoacacia.* Uzelac et al. (2023) found a significantly higher TPC of 123.38 mg GAE/g of dry weight and a TFC of 21.21 mg CE/g DW from leaf extracts.

These variations underscore the influence of the plant part utilized and the extraction method on phytochemical yield. While our findings for TPC are higher than those reported by Stankov et al., they remain lower than the values obtained by Uzelac et al. The observed higher flavonoid content in ethanol extracts suggests ethanol is more effective for flavonoid extraction in this species.

The total phenolic content (TPC) for *Ailanthus altissima* in our study was 23.74 ± 1.11 mg GAE/g in water extracts and 19.69 ± 1.42 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was measured at 19.78 ± 2.64 mg QE/g in water extracts and 82.18 ± 3.31 mg QE/g in ethanol extracts.

Comparing these findings to the literature, Luís et al. (2012) reported significantly higher TPC values in stems (44.95 ± 0.97 mg GAE/g), stalks (52.48 ± 1.69 mg GAE/g), and leaves (136.55 ± 1.31 mg GAE/g) of *Ailanthus altissima*. Similarly, the TFC values from Luís et al. were lower in stems (8.04 ± 0.005 mg QE/g) and stalks (17.30 ± 0.63 mg QE/g), but the leaf extracts had a notably higher TFC of 87.09 ± 0.94 mg QE/g, comparable to our ethanolic extract.

Further, Cocîrlea et al. (2024) reported that the TPC of leaf extracts using a polar to non-polar sequential extraction method was 72.56 ± 0.14 mg GAE/g, with a TFC of 91.97 ± 0.53 mg QE/g. Using non-polar to polar sequential extraction, they obtained TPC values of 37.91 ± 0.04 mg GAE/g and TFC values of 60.20 ± 0.35 mg QE/g.

The differences in phenolic and flavonoid content across studies may be attributed to the varying extraction techniques, solvents, and plant parts used. Our results for water and ethanol extracts of *Ailanthus altissima* demonstrate a moderate phenolic content compared to the higher values found in other studies, particularly for leaves. The notably high flavonoid content in our ethanolic extracts is consistent with the high TFC values reported by Luís et al. and Cocîrlea et al., suggesting that ethanol may be particularly effective in extracting flavonoids from this species. Further exploration into the effect of extraction methods and plant part selection could provide a deeper understanding of the phytochemical composition of *Ailanthus altissima*.

The total phenolic content (TPC) for *Galinsoga parviflora* in our study was measured at 6.55 ± 1.15 mg GAE/g in water extracts and 11.75 ± 0.44 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was found to be 4.66 ± 0.03 mg QE/g in water extracts and 57.64 ± 1.20 mg QE/g in ethanol extracts.

Comparing these values to previous studies, Studzińska-Sroka et al. (2018) reported a significantly higher TPC of 98.30 ± 0.14 mg CA/g DW and a TFC of 6.15 ± 0.41 mg QE/g in *Galinsoga parviflora*. Similarly, Al-Robai et al. (2023) reported a TPC of 19.71 ± 0.51 mg GAE/g, with a TFC of approximately 10 mg QE/g.

The differences observed in our study, particularly the lower TPC values, may be attributed to differences in the plant parts used, solvent types, or geographic and environmental factors. The notably higher flavonoid content in our ethanol extracts (57.64 ± 1.20 mg QE/g) compared to the literature suggests that ethanol might be a more effective solvent for flavonoid extraction in *Galinsoga parviflora* under the conditions employed in this study. These findings highlight the variability in phytochemical content based on extraction methods and plant sources, warranting further research into optimizing extraction techniques for higher yields of bioactive compounds.

The total phenolic content (TPC) of *Acer negundo* was 18.77 ± 0.90 mg GAE/g in water extracts and 21.52 ± 1.54 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was 3.04 ± 0.03 mg QE/g in water extracts and 71.80 ± 2.67 mg QE/g in ethanol extracts. Since no comparative data for *Acer negundo* are available in the literature, these findings provide new insights into the phytochemical composition of this species. The higher flavonoid content in ethanol extracts suggests ethanol is particularly effective for flavonoid extraction. Further studies are needed to explore the influence of solvent selection and plant part on the phytochemical yield of *Acer negundo*.

The total phenolic content (TPC) of *Reynoutria japonica* was found to be 27.62 ± 0.98 mg GAE/g in water extracts and 16.69 ± 0.98 mg GAE/g in ethanol extracts, while the total flavonoid content (TFC) was 40.22 ± 1.64 mg QE/g in water extracts and 36.29 ± 0.78 mg QE/g in ethanol extracts.

Comparatively, Bozin et al. (2017) reported significantly higher TPC values for methanolic extracts from various plant parts, with the inflorescence showing 203.03 ± 6.07 mg GAE/g, and the rhizome with 186.45 ± 1.32 mg GAE/g. Stefanowicz et al. (2021) reported TPC values in tannic acid equivalents (TAE) for different plant parts, with leaves having 46 ± 6 mg TAE/g and stems 18 ± 6 mg TAE/g. The TFC reported by Bozin et al. for methanolic extracts was notably lower in leaves (2.84 ± 0.03 mg QE/g) and inflorescences (2.40 ± 0.08 mg QE/g) but showed higher values in the rhizome (16.31 ± 0.09 mg QE/g) and stems (18.21 ± 0.41 mg QE/g).

The differences between these findings and the current study can be attributed to variations in extraction solvents, plant parts, and environmental conditions. The notably higher flavonoid content observed in both water and ethanol extracts suggests that *Reynoutria japonica* is a rich source of flavonoids, especially in comparison to the values reported in methanolic extracts by other researchers.

No results were found in the literature for plant extracts from *Commelina communis*, *Panicum capillare*, and *Senecio inaequidens*, indicating a gap in research on these species. The findings presented in this study provide novel data on the phenolic and flavonoid content of these plants, contributing valuable insights to the current knowledge of their phytochemical composition.

CONCLUSION

The total phenolic content (TPC) and total flavonoid content (TFC) of the analyzed plant species demonstrate notable variation depending on the extraction solvent and plant part used. In most cases, ethanol extracts exhibited higher flavonoid content, while water extracts often yielded higher phenolic content, underscoring the importance of solvent selection in optimizing the extraction of specific compounds. Comparison with existing literature highlights substantial differences in TPC and TFC values, which can be attributed to variations in extraction methods, environmental factors, and plant material. The correlation between total flavonoid content (TFC) and total phenol content (TPC) is often influenced by various environmental factors such as temperature, light intensity, soil composition, and water availability. The environmental factors such as temperature, light intensity, soil composition, and water availability or nutrient deficiency may also trigger increased accumulation of these secondary metabolites. Therefore, TFC and TPC often show a positive correlation under environmental stress, suggesting a shared regulatory response to external stimuli.For certain species, such as *Acer negundo*, *Amaranthus albus*, *Commelina communis*, *Panicum capillare*, and *Senecio inaequidens*, limited or no data were available in the literature, making these findings an important contribution to the understanding of their phytochemical profiles. Ethanol was particularly effective in extracting flavonoids, especially in *Acer negundo* and *Reynoutria japonica*, while species like *Rhus typhina* and *Ailanthus altissima* showed strong phenolic and flavonoid presence across both solvents.

These results offer valuable insights into the potential bioactivity of these plants and lay the foundation for further research into their antioxidant and medicinal properties.

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Summary/Sažetak

Strane biljne vrste predstavljaju značajnu prijetnju prirodnim ekosistemima zbog svoje prilagodljivosti i invazivnog potencijala. U ovom radu ispitivana je antioksidativna aktivnost 13 stranih i invazivnih biljnih vrsta, s fokusom na ukupni sadržaj fenola, sadržaj flavonoida, ukupni antioksidativni kapacitet, ukupni oksidativni kapacitet te antimikrobnu aktivnost. Biljna tkiva su macerirana, isparena do suhoće, a ekstrakti su rastvoreni u vodi. Ukupni sadržaj fenola varirao je od 6,55 mg GAE/g ekstrakta do 30,82 mg GAE/g ekstrakta. Sadržaj flavonoida kretao se od 3,04 mg kvercetina/g ekstrakta do 40,81 mg kvercetina/g ekstrakta. Ukupni antioksidativni kapacitet iznosio je između 662 CEAC µM i 1540 CEAC µM. Ukupni oksidativni kapacitet može se povezati s abiotskim stresom. *Rhus typhina L.* i *Impatiens balfourii Hooker f.* pokazale su pozitivne vrijednosti ukupnog oksidativnog kapaciteta: 122,56 ± 7,85 µM H_2O_2 ekv./L i 141,38 ± 4,33 µM H_2O_2 ekv./L, redom. Ove dvije vrste su također pokazale najviši ukupni antioksidativni kapacitet, koji je iznosio 1190,06 ± 137,36 CEAC µM za *Rhus typhina L.* i 1540,34 ± 270,84 CEAC µM za *Impatiens balfourii* Hooker f. Rezultati sugeriraju da rastvarač za ekstrakciju značajno utiče na antioksidativni kapacitet, pri čemu etanol općenito omogućava veću efikasnost ekstrakcije kod mnogih vrsta. Antimikrobna aktivnost određena je protiv *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* i *Candida albicans*. *Rhus typhina L.* je pokazala aktivnost protiv *E. coli* i *P. mirabilis*. Ovi podaci ukazuju na to da biljke pojačavaju antioksidativne odbrambene sisteme kao odgovor na abiotske stresove. Jasna povezanost između vrijednosti ukupnog oksidativnog kapaciteta i ukupnog antioksidativnog kapaciteta kod biljaka predstavlja vrijedan pokazatelj mehanizma biljne odbrane.