Antimicrobial and Antioxidant Activity of Black Elder, Stinging Nettle, Marigold and Ribwort Plantain

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Abstract
The aim of the study was to determine antimicrobial and antioxidant activity of plant extracts against pathogenic microorganisms *in vitro*. Extracts of black elder (*Sambucus nigra* L.), stinging nettle (*Urtica dioica* L.), marigold (*Calendula officinalis* L.) and ribwort plantain (*Plantago lanceolata* L.) were used in experiments and tested against gram-negative bacteria: *Pseudomonas aeruginosa* CCM 1959, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, gram-positive bacteria: *Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* subsp. *aureus* CCM 4223, *Streptococcus pneumoniae* CCM 4501, and against yeasts: *Candida albicans* CCM 8186, *Candida krusei* CCM 8271, *Candida tropicalis* CCM 8223. The evaluation of antimicrobial effects was performed with disc diffusion method and the minimum inhibitory concentration (MIC) method. Determination of antioxidant activity was performed using the spectrophotometric method. Some species of selected plants were extremely effective against individual pathogenic microorganisms. Using the disc diffusion method, the best antimicrobial activity was reported for the extract from black elder (*Sambucus nigra* L.) and stinging nettle (*Urtica dioica* L.). The best antimicrobial activity by the MIC method was found in the extract of ribwort plantain (*Plantago lanceolata* L.) which was effective against all gram-negative bacteria. The highest antioxidant activity was determined in the extract from black elder (*Sambucus nigra* L.).

Keywords: antimicrobial activity, antioxidant activity, plant extract, pathogenic microorganisms, disk diffusion method, MIC method, spectrophotometric method.

1. Introduction

Herbs are a diverse multi-purpose group of plants that have been used over time for their healing, culinary options [1]. Important properties of herbs include antioxidant effects, which protect the body from deterioration caused by oxidation. The antioxidant effects of herbs are responsible for their components - polyphenols, flavonoids, vitamins and dyes. They also act as antimicrobials, thereby maintaining microbial safety by suppressing the growth of pathogens. Furthermore, their organoleptic properties (smell, taste) are important in herbs, which influence the choice of food and various health effects (eg. diuretic, laxative). Thanks to their constituents, herbs are used not only in the pharmaceutical industry but also as antimicrobial and antioxidant

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substances for the preparation of medicines. Their medicinal effects are influenced by their composition and the content of active substances, furthermore their effect depends on the time of harvest, the soil type, the treatment of the harvested plants and the length of time of drying. They are also widely used in the food industry as important components of food additives and flavorings. They are also used in the cosmetic industry (cosmetics, oral hygiene products) and also in the chemical industry. The healing effects of herbs are used in home medicine as part of treatment, as well as a prevention against various diseases, as well as in phytotherapy and aromatherapy. The main therapeutic components of herbs include alkaloids, glycosides, bitters and essential oils [2].

The black elder is a shrub belonging to the family Adoxaceae. It is a shrub 2 to 8 meters high. It is most common in Central Europe, but can be found throughout Europe, Asia, Caucasus, western Siberia, and even North Africa [3]. *Sambucus nigra* L. is often used as a nutritional supplement in functional foods and has many pharmacological activities to prevent diseases such as colds and fevers, diabetes and cancer. Elder extract is used in home medicine to treat cold, gallbladder and stomach problems [4]. In recent years, it has also been found to have antibacterial, antiviral, antidepressant, antitumor and hypoglycemic properties and is used to reduce body fat and lipid levels. Due to its beneficial and sensory properties, black elder is mainly used in the food and pharmaceutical industries [5].

Urtica dioica L. is a perennial plant belonging to the family Urticaceae. Originally native in a wide range of climatic conditions and is widespread throughout Europe, North America, North Africa and parts of Asia. Plants belonging to the genus *Urtica* are herbaceous perennials and can grow to a height of 2 m [6].

Stinging nettle is used to treat many diseases around the world [7]. The herbal extract from *Urtica dioica* L. has several vital effects, including bacteriostatic effects against some bacteria, including methicillin-resistant *Staphylococcus*, vancomycin-resistant *Enterococcus, Escherichia coli, Klebsiella, Pseudomonas*, and Acinetobacter resistant to imipenem. It also exhibits some antifungal properties that can suppress the growth and reproduction of *Aspergillus, Mucor* and *Candida albicans*, which are dangerous human pathogens [8]. Quercetin, which is accumulated mainly in the leaves, is one of the most important antioxidants of *Urtica dioica* L. [9].

Marigold is a medicinal plant of the genus *Calendula* of the family Astraceae and is widely naturalized in Europe and elsewhere in warm temperate regions of the world [10]. The biological activity of each extract is due to its components, mostly plant secondary metabolites. The major classes of compounds found in *Calendula officinalis* L. are terpenoids, flavonoids, phenolic acids, carotenoids, coumarins, quinones, volatile oils, amino acids, and lipids [11]. Aqueous extracts from the leaves of *C. officinalis* L. have good antimicrobial effects against many microorganisms e.g. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Aspergillus niger* [12].

Plantago is a large genus of the Plantaginaceae family, comprising about 275 species. *Plantago lanceolata* L. is native to Eurasia and has been introduced to North America and other parts of the world. It is a common weed on cultivated land. *Plantago* plants are used worldwide as functional foods and traditional medicine to treat many diseases. Some of them have commercial value due to their use as pharmaceuticals and nutraceuticals [13]. The results of antimicrobial tests of *Plantago lanceolata* L. extracts showed significant inhibitory effects on various microorganisms. It should be noted that the extract showed higher activity against gram-positive than gram-negative microorganisms [14].

2. Materials and methods

The aim of study was antimicrobial and antioxidant activity of four medicinal plants. Four species of plants – black elder (*Sambucus nigra* L.), stinging nettle (*Urtica dioica* L.), marigold (*Calendula officinalis* L.), and ribwort plantain (*Plantago lanceolata* L.) were used in this study. Their leaves, which were dried on filter paper at room temperature in the dark were used. The plants were dried in sufficiently ventilated areas to avoid the negative effects of humid air.

Three gram-negative bacteria: *Pseudomonas aeruginosa* CCM 1959, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, three gram-positive bacteria: *Enterococcus faecalis* CCM 4224,

Staphylococcus aureus subsp. *aureus* CCM 4223, *Streptococcus pneumoniae* CCM 4501 and three yeasts: *Candida albicans* CCM 8186, *Candida krusei* CCM 8271, *Candida tropicalis* CCM 8223 were used for antimicrobial activity. The microorganisms were cultured in a thermostat for 24 hours at 37°C, bacteria in Muller - Hinton broth (MBH) and yeast at 25°C in Sabouraud dextrose broth (SDB).
For the antioxidant activity and antimicrobial activity disc diffusion method and microdilution broth method were described previously [15].

3. Results and discussion

The highest antioxidant activity was found in the elder extract, with the value 73.45% ± 0.50. The second highest antioxidant activity showed the marigold extract, with the value 71.3±0.26%. The ribwort plantain had an antioxidant activity of 68.94±0.12%, and the stinging nettle acquired 64.01±0.97% of the antioxidant activity. Each of our tested plant extracts can be classified as potent antioxidants, as they all have antioxidant activity higher than 60% (Table 1).

Table 1. Antioxidant activity of plants

Plant tested	Part of plant	% inhibition (mean ± SD)
Sambucus nigra L.	Leaves	73.45 ± 0.50
Urtica dioica L.	Leaves	64.01 ± 0.97
Plantago lanceolata L.	Leaves	68.94 ± 0.12
Calendula officinalis L.	Leaves	71.30 ± 0.26

Bahadori et al. [13], in their research work focused on testing the antioxidant activity of *Plantago lanceolata* L. where they report its antioxidant activity of 51±0.1%. Compared to our results, their antioxidant activity of *Plantago lanceolata* L. was lower. Joshi et al. [16], found antioxidant activity in *Urtica dioica* L. 78.99 ± 0.17%. Compared to our results, their antioxidant activity of *Urtica dioica* L. was higher than in our results. Ercetin et al. [17], in their research work found the antioxidant activity of *Calendula officinalis* L. 92.57±0.10%. Compared to our results, their antioxidant activity was higher. Dawidowicz et al. [18] found in their work the antioxidant activity of *Sambucus nigra* L. 48.52±0.34%. Compared to our results, our determined antioxidant activity of *Sambucus nigra* L. extract was higher. Domínguez et al. [19], in their research work focused on the antioxidant activity of *Sambucus nigra* L., in which they determined the content of antioxidants at 53.09 ± 0.33%. Compared to our results, they reached a lower value, our value was 73.45±0.50%. Abudunia et al. [20], in their research work focused on the determination of the antioxidant activity of *Calendula officinalis* L., in which they determined the antioxidant activity of 33.2±2.12%. However, their value was lower than in our extract of *Calendula officinalis* L. Abate et al. [21], in their scientific work focused on the determination of the antioxidant activity of the extract of *Plantago lanceolata* L. Their antioxidant activity was 90.6±1.60%. Compared to our results, they achieved a higher antioxidant activity in their work. Khare et al. [22] tested the antioxidant activity of the extract of *Urtica dioica* L., for which they determined the antioxidant activity of 88.33±2.88%. Compared to the results from our testing, they achieved higher results.
Plant extracts of *Urtica dioica* L. and *Plantago lanceolata* L. with an inhibition zone of 7.66±0.57 resp. 6.33±0.57 mm were the most effective against *Pseudomonas aeruginosa* CCM 1959. The most significant effect against *Salmonella enterica* subsp. *enterica* CCM 3807 showed *Sambucus nigra* L., where the inhibition zone was 5.66±0.57 mm. The extract of marigold (*Calendula officinalis* L.), with an inhibition zone of 8.33±0.57 mm, was the most effective against *Yersinia enterocolitica* CCM 5671. The growth of *Enterococcus faecalis* CCM 4224 was best inhibited by *Urtica dioica* L. with an inhibition zone of 7.33±0.57 mm. Against *Staphylococcus aureus* subsp. *aureus* CCM 4223 appeared to be the most effective *Urtica dioica* L. with the inhibition zone 7.66±0.57 mm. The bigger inhibition zone against *Streptococcus pneumoniae* CCM 4501 was with *Sambucus nigra* L. extract. *Sambucus nigra* L. and *Plantago lanceolata* L. were most effective against *Candida albicans* CCM 8186. Against *Candida krusei* CCM 8271 was the most effective marigold extract (*Calendula officinalis* L.) where an inhibition zone was 4.33±0.57 mm. The best effect against *Candida tropicalis* CCM 8223 showed *Sambucus nigra* L. with an inhibition zone 5.33±0.57 mm (tab. 2). Efstratiou et al. [12], in their research work focused on testing the antimicrobial activity of *Calendula officinalis* L. with disk diffusion method against selected bacteria: *Pseudomanas*

aeruginosa, Staphylococcus aureus, Enterococcus faecalis and yeasts: *Candida albicans* and *Candida krusei*. Ethanolic extract was most effective against *Staphylococcus aureus* and *Enterococcus faecalis*. Compared to our results, ethanolic extract of marigold (*Calendula officinalis* L.) against *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Candida albicans* and *Candida krusei* was more effective than our extracts. Gülçin et al. [23] used an extract prepared from *Urtica dioica* L. against *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae* and *Candida albicans*. for testing. The extract of *Urtica dioica* L. didn't inhibit *Pseudomonas aeruginosa*. Compared to our results, our extract from *Urtica dioica* L. was most effective against *Pseudomonas aeruginosa*. Fayera et al. [24], in their research work, tested the antimicrobial activity of the *Plantago lanceolata* L. extract against the gram-positive bacterium *Staphylococcus aureus* by the disk diffusion method.

Table 2. Antimicrobial activity of medicinal plants in mm

Microorganisms	Plant extract	Zone of inhibition
	Sambucus nigra L.	3.33±0.57
P. aeruginosa	*Urtica dioica* L.	7.66±0.57
	Plantago lanceolata L.	6.33±0.57
	Calendula officinalis L.	2.33±0.57
	Sambucus nigra L.	5.66±0.57
S. enterica subsp. *enterica*	*Urtica dioica* L.	4.66±0.57
	Plantago lanceolata L.	4.33±0.57
	Calendula officinalis L.	2.33±0.57
	Sambucus nigra L.	3.66±0.57
Y. enterocolitica	*Urtica dioica* L.	5.33±0.57
	Plantago lanceolata L.	5.66±0.57
	Calendula officinalis L.	8.33±0.57
	Sambucus nigra L.	5.66±0.57
E. faecalis	*Urtica dioica* L.	7.33±0.57
	Plantago lanceolata L.	5.66±0.57
	Calendula officinalis L.	2.33±0.57
	Sambucus nigra L.	4.66±0.57
S. aureus subsp. *aureus*	*Urtica dioica* L.	7.66±0.57
	Plantago lanceolata L.	5.66±0.57
	Calendula officinalis L.	6.66±0.57
	Sambucus nigra L.	6.33±0.57
S. pneumoniae	*Urtica dioica* L.	4.66±0.57
	Plantago lanceolata L.	4.33±2.31
	Calendula officinalis L.	5.33±0.57
	Sambucus nigra L.	3.33±0.57
C. albicans	*Urtica dioica* L.	2.33±0.57
	Plantago lanceolata L.	3.66±0.57
	Calendula officinalis L.	2.66±0.57
	Sambucus nigra L.	3.33±0.57
C. krusei	*Urtica dioica* L.	3.66±0.57
	Plantago lanceolata L.	3.33±0.57
	Calendula officinalis L.	4.33±0.57
	Sambucus nigra L.	5.33±0.57
C. tropicalis	*Urtica dioica* L.	2.66±0.57
	Plantago lanceolata L.	2.33±0.57
	Calendula officinalis L.	1.66±0.57

The antimicrobial activity of plant extracts was demonstrated against all gram-positive and gram-negative bacteria tested as well as against the tested yeast (tab. 3). The growth of *Pseudomonas auruginosa* was most effectively inhibited by *Plantago lanceolata* L. extract, even at concentrations of MIC 50: 8.44 µg/mL and MIC 90: 10.28 µg/mL. The *Urtica doica* L. extract was

similarly effective at MIC concentrations of 50: 9.56 µg/mL and MIC 90: 10.24 µg/mL. Against *Salmonella enterica* subsp. *enterica*, two extracts were effective: *Urtica dioica* L. and *Plantago lanceolata* L., at which the minimum inhibition concentration was at MIC 50: 7.42 µg/mL and at MIC 90: 8.82 µg/mL. Extracts from *Calendula officinalis* L. and *Plantago lanceolata* L. showed the highest inhibitory effect against gram-negative *Yersinia enterocolitica*, with the minimum inhibition concentration MIC 50: 3.36 µg/mL and MIC 90: 3.56 µg/mL. Against the gram-positive *Enterococcus faecalis*, the most effective extract was *Plantago lanceolata* L. with a minimum inhibition concentration of MIC 50: 4.26 µg/mL and MIC 90: 4.76 µg/mL. Growth of *Staphylococcus aureus* subsp. *aureus* was also best inhibited by *Plantago lanceolata* L. extract with a minimum inhibition concentration of MIC 50: 1.81 µg/mL and MIC 90: 2.83 µg/mL. Marigold extract was the best to inhibit *Streptococcus pneumoniae* with a MIC 50: 3.36 mg/ml and MIC 90: 3.56 µg/mL. Two extracts were most effective against the pathogenic yeast *Candida albicans*: *Sambucus nigra* L. and *Calendula officinalis* L., at which the minimum inhibitory concentration was MIC50: 23.56 µg/mL and MIC 90: 36.44 mg/mL. The most effective extracts against *Candida krusei* were *Urtica dioica* L. and *Plantago lanceolata* L. at MIC 50: 23.56 µg/mL and MIC 90: 36.44 µg/mL. Growth of *Candida tropicalis* was best inhibited with two extracts: *Sambucus nigra* L. and *Calendula officinalis* L. with a minimum inhibition concentration MIC 50: 23.56 µg/mL and MIC 90: 36,44 µg/mL. Hammami et al. [14] focused on testing the antimicrobial activity of *Plantago lanceolata* L. on selected pathogenic microorganisms *Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* with the method of minimum inhibition concentration. The *Plantago lanceolata* L. extract was the most effective against the gram-positive *Staphylococcus aureus* and *Candida albicans*. Compared to our results, ethanol extract of *Plantago lanceolata* L. was more effective than their extract. Zenão et al. [25] focused on the determination of the antimicrobial activity of *Urtica dioica* L. with the MIC method against *Staphylococcus aureus*. Compared to our results, our extract was less effective. Faustino et al. [26], in their research work focused on testing marigold extract against the pathogenic yeast *Candida albicans* with the method of minimal inhibition concentration. Compared to our results, our marigold extract was more effective against *Candida albicans*. Hwang et al. [27], in their scientific work, focused on determination of the antimicrobial activity of different extracts against *Candida albicans.* Quave et al. [28], focused their research on the antimicrobial activity of extracts from black elder, stinging nettle and ribwort plantain against *Staphylococcus aureus*. The elder extract proved to be the most effective at MIC of 50: 12.00 µg/mL of the extract. Compared to our results, they only achieved better results with the ribwort plantain extract.

Table 3. Antimicrobial activity of plant extracts with microdilution broth method

Microorganisms tested	*S. nigra* L.		*C. officinalis* L.		*U. dioica* L.		*P. lanceolata* L.	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
P. aeruginosa	36.92	45.36	18.96	30.32	9.56	10.24	8.44	10.82
S. enterica subsp. enterica	10.76	15.99	26.56	45.23	7.42	8.82	7.42	8.82
Y. enterocolitica	13.98	15.54	3.36	3.56	7.42	8.82	3.36	3.56
E. faecalis	8.53	9.54	13.75	35.24	12.78	13.59	4.26	4.76
S. aureus subsp. aureus	17.06	19.04	3.36	3.56	7.42	8.82	1.81	2.83
S. pneumoniae	7.42	8.82	3.36	3.56	8.53	9.54	13.75	35.24
C. albicans	23.56	36.44	23.56	36.44	45.36	55.24	45.36	55.24
C. krusei	45.36	55.24	45.36	55.24	23.56	36.44	23.56	36.44
C. tropicalis	23.56	36.44	23.56	36.44	45.36	55.24	45.36	55.24

4. Conclusions

In our findings *Sambucus nigra* L. extract showed the highest antioxidant activity. All our samples achieved an antioxidant activity higher than 60% and therefore all of them belong to plants with strong antioxidant activity. With disc diffusion method the best results were determined in the *Sambucus nigra* L. and *Urtica dioica* L. extracts. However, the most effective was *Calendula officinalis* L. extract against *Yersinia enterocolitica*. Minimum Inhibition Concentration (MIC) method determined *Plantago lanceolata* L. as the most effective extract. The marigold (*Calendula officinalis* L.) extract has also shown strong antimicrobial effects.

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