Medicinal Plants as Antimicrobial Agents Against Pathogenic Bacteria

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Abstract
This study was focused on monitoring of antimicrobial activity of selected medicinal plants: *Artemisia vulgaris, Geum urbanum, Euphorbia peplus, Rumex hydrolapathum, Portulaca oleracea, Geranium pratense, Polygonum aviculare*. The selected medicinal plants were evaluated for antimicrobial activity with disc diffusion method. Ethanolic extracts against 3 Gram-positive (G^+) bacteria (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*), 3 Gram-negative (G^-) bacteria (*Salmonella enterica*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*) and 3 yeasts (*Candida tropicalis*, *Candida krusei*, *Candida glabrata*, *Candida albicans*) were used as model microorganisms to determine antimicrobial activity. The *Portulaca oleracea* extract showed the largest zone of inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. *Artemisia vulgaris* extract formed the largest zone of inhibition against *Bacillus subtilis, Enterococcus faecalis* and one yeast *Candida krusei*. The extract from *Geum urbanum* and *Euphorbia peplus* did not create the maximum inhibition zone against any of the microorganisms that were tested.

Keywords: antimicrobial activity, plant extract, $Gram^+$, $Gram^-$, yeasts

1. Introduction

Plants are prospective source of antimicrobial agents [1]. Traditionally, crude plant extracts are used as herbal human medicine [2].
Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids. In plants, the antimicrobial properties have been found *in vitro* [3].
It is expected that plant extracts will be active against drug-resistant microbial pathogens because of different action model [4].
Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. Antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania, and insecticidal activities were also studied [5].

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Artemisia genus of Asteraceae family vary in characteristics and type of climate necessary for their growth, but some species share traits in common. Several *Artemisia* plants have diverse anti-inflammatory, anticancer, antiviral, antifungal, and antibacterial properties [6].

The genus *Geum* contains of about 70 plant species distributed in regions with temperate climate. Many *Geum* species are rich in biologically active compounds and therefore could be a source of new plant products with pharmacological potential. The roots and rhizomes have been applied for treatment of diarrhoea, dysentery, dyspepsia, gastroenteritis, but the aerial parts infusion was used for leucorrhoea, haemorrhages and fever [7].

The genus *Euphorbia* is the largest genus in the family Euphorbiaceae and comprises a large group of plants with over 2000 species in the world. Euphorbiaceae family is rich in medicinal phytochemicals including tannins, alkaloids, glycosides, flavonoids, and other phenolic compounds. *Euphorbia peplus* of Euphorbiaceae family is indigenous to Europe, North Africa, and western Asia [8].

Preparations from *Rumex*, especially due to their anti-inflammatory and antimicrobial activities, are used in dermatology for skin infections, in case of inflammation of the gastrointestinal tract and digestive disorders, as laxative or antidiarrheal, in treatment for upper respiratory tract diseases [9].

Portulaca oleracea L. (Purslane) contains plenty of bioconstituents, including catecholamines, noradrenaline, and dopamine and is used in treatment of urinary, digestive problems, and cardiovascular diseases. *P. oleracea* has a variety of pharmacological activities, including analgesic, anti-inflammatory, antimicrobial, wound healing, and hypoglycemic effects [10].

Plants from genus *Geranium* are known to contain flavonoids, tannins, lignans, and essential oils. Recent papers reported that flavonoids and tannins isolated from this genus have different biological activities such as antileishmanial, anti-inflammatory, antiprotozoal, antiinfluenza, antioxidant and antiproliferative [11].

Polygonum aviculare L. (Polygonaceae family) is an annual prostrate herb with small elliptic-lanceolate leaves. Plants of this family are known to produce a large number of biologically important secondary metabolites, such as flavonoids, anthraquinones, alkaloids, and steroids [12].

The aim of this study was to evaluate antimicrobial activity of different plants.

2. Materials and methods

Plants

The plants were picked and dried in October 2019 in Slovakia. The well-dried material was ground up to a powder. Extraction was performed on Dionex® ASE® 350 Accelerated Solvent Extractor (ThermoFisher Scientific). For preparation of extracts, 0.25 g of each plant sample was extracted with ethanol. The plant material was extracted at 40°C for 5 min and the cycle was repeated 2 times.

Microorganisms

In the experimental part of work were used three Gram-positive bacteria: *Bacillus subtilis* subsp. *Spizizenii* (CCM 1999), *Enterococcus faecalis* (CCM 4224), *Staphylococcus aureus* subsp. *aureus* (CCM 2461), three Gram-negative bacteria, *Yersinia enterocolitica* (CCM 5671), *Pseudomonas aeruginosa* (CCM 1959), *Salmonella enterica* subsp. *enterica* (CCM 3807) and four yeasts *Candida tropicalis* (CCM 8223), *Candida krusei* (CCM 8271), *Candida glabrata* (CCM 8270), and *Candida albicans* (CCM 8186). Before testing the antimicrobial activity of plant extracts, bacteria and yeasts were cultured in the Muller Hinton and Sabouraud broth for 24 h. The concentrations of microorganisms were measured with densilameter. Each culture was adjusted to a concentration of 0.5 McFarland. After adjusting the concentration, the bacteria were inoculated on Mueller Hinton agar and the yeast on Sabouraud agar and spread with an L-shaped cell spreader.

The antimicrobial activity of plant extracts against selected microorganisms was measured by the disk diffusion method. As the positive control, standard drugs cefepime (FEP 30µg/disc) for Gram-negative bacteria, tetracycline (TET 30µg/disc) for Gram-positive bacteria and fluconazole (FCA, 25mg/disc) for yeasts were used. Tests were performed in three separate experiments, and the means were calculated.

Disc diffusion method

Agar was inoculated with standard inoculum of the tested microorganisms. The disks, which were made of filter paper, with a diameter of 6 mm, were placed on the surface of the agar. A volume of 10 µl of extract was pipetted out on the disks. Inoculated agars were cultivated under suitable conditions.

Data statistical analysis

The inhibition zones were recorded, the mean and standard deviation were calculated. The Tukey's test was used for calculation of significantly different values. Statistically significant differences in the Tukey's test were observed at the 95% significance level.

3. Results and discussion

Table 1 shows the results of antimicrobial activity of plant extracts on Gram-positive bacteria. *Artemisia vulgaris* extract was the most effective extract against Gram-positive bacteria. It formed the largest inhibition zone against two bacteria (*B. subtilis*, *E. faecalis*) and was the second most effective extract against *S. aureus*. Comparison of the efficacy of the extracts on *Staphylococcus aureus* showed statistically significant differences. Statistically significant differences in the effectiveness of the extracts were also observed against *Enterococcus faecalis.* No statistically significant differences were recorded for *Bacillus subtilis*, so the detectability for all combinations of plant extracts was always P≥0.05.

Zoreky-Al [13] investigated natural inhibitors of pathogenic microorganisms and their antimicrobial activity - 80% methanol extract of pomegranate peels against *Listeria monocytogenes, S. aureus, Escherichia coli, Yersinia enterocolitica, Salmonella enterica, L. monocytogenes, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida utilis* and *Aspergillus niger*. The antimicrobial activity was measured using the disk diffusion method. The inhibition zone ranged from 0-20 mm and the largest inhibition zone of 20 mm was identified for *L. monocytogenes*. A 13 mm inhibition zone after application of extract (water-methanol) from the pomegranate peel of was formed against *Staphylococcus aureus*.

Portulaca oleracea showed 1.95-fold less inhibition than the above extract of Zoreky-Al study [13]. The 17 mm inhibition zone was measured for *Bacillus subtilis*, which is 4.63 times stronger antimicrobial activity of pomegranate extract than *Artemisia vulgaris* extract in our study.

Table 1. Antimicrobial activity against Gram-positive bacteria

Microorganisms	Plant extract	Inhibition zone (mm)	SD
Staphylococcus aureus	Control	0.00	0.00
	Artemisia vulgaris	4.00	0.82
	Geum urbanum	0.33	0.47
	Euphorbia peplus	1.33	0.47
	Rumex hydrolapathum	1.33	0.47
	Portulaca oleracea	6.67	2.05
	Geranium pratense	0.67	0.47
	Polygonum aviculare	2.33	0.47
Enterococcus faecalis	Control	0.70	0.00
	Artemisia vulgaris	5.33	0.47
	Geum urbanum	2.33	0.47
	Euphorbia peplus	0.67	0.47
	Rumex hydrolapathum	1.00	0.00
	Portulaca oleracea	2.67	0.47
	Geranium pratense	1.33	0.47
	Polygonum aviculare	1.00	0.00
Bacillus subtilis	Control	1.50	0.00
	Artemisia vulgaris	3.67	0.47
	Geum urbanum	2.67	1.70
	Euphorbia peplus	2.33	0.94
	Rumex hydrolapathum	3.00	0.00
	Portulaca oleracea	1.67	0.94
	Geranium pratense	1.33	0.47
	Polygonum aviculare	1.33	0.47

Table 2. Antimicrobial activity of Gram-negative bacteria

Microorganisms	Plant extract	Inhibition zone (mm)	SD
Salmonella enterica	Control	3.50	0.00
	Artemisia vulgaris	2.67	0.47
	Geum urbanum	1.33	0.47
	Euphorbia peplus	1.00	0.82
	Rumex hydrolapathum	1.67	0.94
	Portulaca oleracea	2.00	0.00
	Geranium pratense	2.00	0.82
	Polygonum aviculare	3.00	1.41
Pseudomonas aeruginosa	Control	2.70	0.00
	Artemisia vulgaris	2.00	0.00
	Geum urbanum	1.67	0.47
	Euphorbia peplus	1.67	0.94
	Rumex hydrolapathum	1.33	0.47
	Portulaca oleracea	3.00	0.82
	Geranium pratense	1.67	0.47
	Polygonum aviculare	1.33	0.47
Yersinia enterocolitica	Control	3.50	0.00
	Artemisia vulgaris	2.33	0.47
	Geum urbanum	1.33	0.47
	Euphorbia peplus	2.33	0.47
	Rumex hydrolapathum	1.67	0.94
	Portulaca oleracea	3.33	0.47
	Geranium pratense	1.00	0.00
	Polygonum aviculare	2.00	0.00

Antimicrobial activity by the disk diffusion method has also been investigated for aqueous extract of *Mentha* against various bacteria [14]. The plant extract did not show any inhibitory activity against *Staphylococcus aureus* in this study, but was effective against all other bacteria tested.

The antimicrobial efficacy of various parts of *Geum urbanum* was studied by Al-Snafi [15]. The methanol extract from the leaves showed the greatest antimicrobial activity against *Pseudomonas aeruginosa, Pseudomonas viridiflava, Bacillus subtilis, Rathayibacter toxicus*, *Xanthomonas campestris, Acidovorax avenae*, *Staphylococcus aureus*, *Pseudomonas syringae. syringae, Erwinia amylovora,* and *Escherichia coli*.

Table 2 shows the results of antimicrobial activity tested with disk diffusion method on Gram-negative bacteria. For *Pseudomonas aeruginosa, Yersinia enterocolitica*, an extract from *Portulaca oleracea* plant was the most active with 3.00 and 3.33 mm of inhibition zone, respectively. The antibiotic cefepime, was used as a positive control. In Gram-negative *Salmonella enterica* the antimicrobial activity of selected extracts showed statistically significant differences ($P \leq 0.05$) with Tukey’s test and 95% confidence interval. Statistically significant differences were also observed in *Pseudomonas aeruginosa*.

Tukey's test allowed to identify only one combination of extracts against *Yersinia enterocolitica*, which are characterized by a statistically significant differences ($P \leq 0.05$).

According to the study by Zoreky-Al [13], which compared the antimicrobial activity of pomegranate peel extract to different microorganisms, 18 mm the inhibition zone was observed against *Pseudomonas aeruginosa*. This was 5.3 times effective than the most active extract studied in the present study. Pomegranate extract formed an inhibition zone against *Yersinia enterocolitica* of 19 mm, but in the present work, the most effective extract was from *Portulaca oleracea* with inhibition zone of 3.33 mm against *Yersinia enterocolitica*.

Andogan et al. [16] used the disk diffusion method to determine the antimicrobial activity of essential oils. *Echinophora tenuifolia* essential oil was not active against *E. coli*, *S. aureus* and *P. aeruginosa*. *Origanum onites* revealed the strongest antimicrobial activity with inhibition zone of 35 mm. *Lavendula hybrida* exhibited the weakest antimicrobial activity with size of inhibition zones of 8 mm.

The antimicrobial activity of the essential oil of *Artemisia vulgaris* against various bacteria and yeasts was also tested in work Singh et al. [17]. There was not any effect *Salmonella enterica* ssp. *Indica*.

The methanol extract from the root part of *Geum urbanum* showed antimicrobial activity against *Pseudomonas aeruginosa,* but also *Escherichia coli, Pseudomonas viridiflava, Rathayibacter toxicus, Pseudomonas syringae. syringae, Bacillus subtilis, Acidovorax avenae, Xanthomonas campestris* [15].

Table 3. Antimicrobial activity of medicinal plants to yeasts

Microorganisms	Plant extract	Inhibition zone (mm)	SD
	Control	0.00	0.00
	Artemisia vulgaris	2.00	0.00
	Geum urbanum	1.33	0.47
Candida tropicalis	*Euphorbia peplus*	2.00	0.00
	Rumex hydrolapathum	2.00	0.00
	Portulaca oleracea	1.33	0.47
	Geranium pratense	2.00	0.82
	Polygonum aviculare	2.67	1.25
	Control	0.00	0.00
	Artemisia vulgaris	5.00	0.82
	Geum urbanum	2.33	0.47
Candida krusei	*Euphorbia peplus*	3.33	1.70
	Rumex hydrolapathum	4.00	0.82
	Portulaca oleracea	2.33	0.47
	Geranium pratense	1.67	0.47
	Polygonum aviculare	1.33	0,47
	Control	0.00	0.00
	Artemisia vulgaris	0.33	0.47
	Geum urbanum	1.67	0.94
Candida glabrata	*Euphorbia peplus*	2.00	0.82
	Rumex hydrolapathum	4.00	0.82
	Portulaca oleracea	2.00	0.00
	Geranium pratense	2.00	0.82
	Polygonum aviculare	1.00	0.00
	Control	1.70	0.00
	Artemisia vulgaris	1.67	0.47
	Geum urbanum	1.00	0.00
Candida albicans	*Euphorbia peplus*	2.33	0.94
	Rumex hydrolapathum	3.00	0.00
	Portulaca oleracea	2.33	0.47
	Geranium pratense	4.00	0.00
	Polygonum aviculare	3.00	0.00

In this work, the antimicrobial activity of plant extracts and their effectiveness on 4 yeasts was studied. The extracts showed different activity for each yeast, so it is not possible to generalize which extract is the most effective and which is the least effective against yeasts. The results of the antimicrobial activity of the extracts against yeast are shown in Table 3.

Using Tukey's test, statistically significant differences in the efficacy of the extracts against yeast were observed at a significance level of 95%. The efficacy of plant extracts was compared against *Candida tropicalis* and statistically significant differences ($P \geq 0.05$) have been shown.

The antimicrobial activity of the extracts against *Candida krusei*, *Candida glabrata*, *Candida albicans* shows statistically significant differences in the activity of the extracts.

Obistioiu et al. [18] on characterized the volatile fractions prepared from *Artemisia dracunculus, Artemisia abrotanum*, *Artemisia absinthium*, and *Artemisia vulgaris* and monitored their activity against *Candida albicans*. The results of the work stated that *Artemisia* oils were promising candidates for further research into new anti-*Candida* drugs. The antimicrobial activity of extracts from different parts of the *Calendula officinalis* by Mathur and Goyal [19] was with significant inhibitory activity against *Candida albicans* and *Candida parapsilosis*. However, the strongest inhibitory activity was recorded from the stem of *Calendula officinalis* against *E. coli*.

Andogan et al. [16] studied the antimicrobial activity of *Polygonum aviculare* in 13 species of bacteria and yeast. The aqueous, acetone extract of *Polygonum aviculare* did not form any inhibition zones against *Candida albicans*.

4. Conclusions

The antimicrobial activity studies of selected plant extracts showed that *Artemisia vulgaris* extract was the most effective against *Enterococcus faecalis*, *Bacillus subtilis* and *Candida krusei*. *Rumex hydrolapathum* had the highest inhibitory activity against *Candida glabrata*, but *Portulaca oleracea* had the strongest activity against 3 bacteria - *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The extract from the *Geranium pratense* revealed the highest antimicrobial activity against *Candida albicans*. *Polygonum aviculare* extract showed the best results against *Salmonella enterica* and *Candida glabrata*.

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