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Investigating the Antibacterial Effects of Plant Extracts on *Pseudomonas aeruginosa* and *Escherichia coli*

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Abstract

Background: Scientists are seeking an appropriate alternative method for curing infections caused by resistant bacteria, since drug resistance is continually increasing.
Objectives: This research aims to discover the function of some medicine plants on pestiferous *Pseudomonas aeruginosa* and *Escherichia coli* in humans.
Materials and Methods: Bacterial strains were obtained from a standard laboratory. The strains of *Pseudomonas aeruginosa* ATCC27853 and *E.coli* ATCC25922 bacteria were used for antimicrobial testing of the extractions.
Results: Our results showed that *Teucrium polium* extracts have the minimum density of inhibitory for *Escherichia coli*, 25 ppm, whereas the maximum of this is for *Peganum harmala* and *Prangos ferulaceae* with 100 ppm. The lowest minimum concentration inhibitory value of extracts *P. harmala*, *T. polium*, *T. pratensis* and *Rumex* was found in 25 ppm against *P.aeruginosa*.
Conclusions: The results of our study showed that plant extracts have good antibacterial properties against *Pseudomonas aeruginosa* and *Escherichia coli*.

Keywords: Antibacterial Activity, Extract Plant, *Pseudomonas aeruginosa*, *Escherichia coli*

1. Background

Recently, there has been a great deal of attention paid in medical treatments to plant extracts and compounds with biological features, because of the resistance and side effects that the micro-organisms of pathogens have shown in the face of antibiotics. The anti-bacterial compounds of plants are a significant medical resource. As a result of the spread of infectious diseases, exploration of more of these compounds will be useful. Anti-bacterial compounds with herbal sources have a wide range of therapeutic use. These compounds are not only efficient for the treatment of infectious diseases, but also concurrently diminish existing side effects via their anti-bacterial compounds (1). *Escherichia coli* is a pathogenic bacteria transmitted by infected food. Worldwide epidemics of this disease have been reported (2). The signs and symptoms of *E. coli* exposure include diarrhea, nausea, ulcerative colitis, abdominal pain and, in some cases, kidney disorders or death, especially among children (3). The third hospital-infection factor after *Escherichia coli* and *Staphylococcus aureus* is *Pseudomonas aeruginosa* (4). *Pseudomonas aeruginosa* is a Gram-negative bacteria that has shown an innate resistance to many antibiotics. However, it has shown sensitivity to various antibiotics, such as piperacillin, ciprofloxacin, tobramycin and imipenem (5). *Pseudomonas aeruginosa* is a factor for urinary tract infections and lung diseases, including cystic fibrosis.

Rumex alveollatus L [sorrel] is an herb in the polygonaceae family. Its leaves and flowers have already been used for lividness and biting treatments, and as an efficient anti-venom (6). Its impact on the treatment of peptic ulcers in animals has also been demonstrated (7). The herb *Teucrium polium* is used as anti-inflammatory and as an anti-diarrhea treatment (8, 9). In traditional medicine, it is used as an anti-bacterial. *Tragopogon graminifolius* is an herb from the asteraceae family that grows at elevations above 1400 m in the area of the Zagros mountains. In Bakhtiari and Lorestan, it is used as an anti-inflammatory for injuries in sheep and goats (10). The *prangos feralaceae* herb is used as a carminative, laxative, for soothing neuralgia, as an antiphlogistic, an antiviral, anti-parasite, an-

tifungal and antibacterial treatment (11). Leaves from this herb are useful for the treatment of stomach illnesses (12). For sexual desire disorders, its root has been proven useful (13). *Peganumharmala* L. (Syrian rue) is a wild-growing, flowering plant belonging to the zygophylaceae family. It is considered an important medicinal plant. The seeds are known to possess hypothermic and hallucinogenic properties (14, 15). *P. haramala* has been shown to possess anti-helmitic, lactogogue, antispasmodic, antipyretic, abortifient, emetic and emmenagogue properties (16).

2. Objectives

The aim of this study is to investigate the antibacterial effects of some plant extracts on pathogenic *Escherichia coli* and *Pseudomonas aeruginosa* bacteria.

3. Materials and Methods

3.1. Bacterial Strains and Culture Conditions

Bacterial strains were obtained from a standard laboratory. Evaluating the antibacterial activity of the plant extracts was conducted using strains of *Pseudomonas aeruginosa* ATCC27853 and *E. coli* ATCC25922 bacteria. The typed cultures of the bacteria were sub-cultured on nutrient agar and stored at 4°C until required for study.

3.2. Plant Materials

The plants were collected in the Zagros region of Iran. The plant materials were dried at 25°C. Samples were powdered and transferred into glass containers and preserved until the next experiment.

3.3. Preparation of Extracts

For each test, ten grams of samples were soaked for 48 hours in 96% ethanol and stored. The extracts were obtained using smooth filter paper and condensed using a rotary. The weight of a test tube was determined, and 1 mL of extract was transferred into it. The tube containing the extract was allowed to dry at room temperature. The weight difference of tube was equivalent to 1 mL of the extract. This was done three times, and the average of the three weights was calculated as the weight of the dried extract. Then, it was dissolved in the solvent DMSO and maintained at 4°C until use.

3.4. Antimicrobial Testing of Extracts

The susceptibility of bacterial isolates with multiple resistances to the plant extracts was investigated using a dilution plate. To seven micro-titter plates were given an amount of 100 mL nutrient broth, Mueller Hinton broth (MHB). The first well was given 100 mL of the diluted solution of the extract, after mixing; 100 mL of the first plate was removed and added to the second plate. The work was done in this way until the last plate. The end plate was removed and 100 mL of culture medium containing 10^7 units per mL, 100 mL of bacterial suspension equivalent to 0.5 McFarland was added to all wells and incubated at 37°C for 24 hours. The first well that prevented the growth of bacteria was considered as MIC and to ensure 10 mL of the clear plate was transferred to the Mueller Hinton agar medium. After 24 hours, the first dilution that could kill 99.9% of bacteria was deemed to have shown a minimum lethal concentration. The antibacterial activities of the plants' crude extracts were tested using the agar well diffusion method. The test inoculums (0.5 McFarland turbidity) were spread into Muller-Hinton agar using a sterile cotton swab. The wells were made by sterile well puncture, and 20 ìL of the extracts were added to each well and incubated at 37°C for 24 hours. The presence of an inhibition zone was regarded as the presence of antimicrobial action. The average diameter of the inhibition zone was measured in millimeters.

4. Results

In this study, *T. polium* extract had a minimum inhibitory concentration of *E. coli*, at 25 ppm, whereas the maximum of this is for *P. harmala* and *P. ferulaceae* with 100 ppm and the lowest minimum inhibitory concentration value of extracts *P.harmala*, *T.polium*, *T.pratensis* and *Rumex* were found to be 25 ppm against *P.aeruginosa*.

The highest MBC value of extract *H. feralaceae* was found to be 200, 100 ppm against *E.coli* and *P.aeruginosa*, and the lowest MBC values were found for extract *T. polium* against *E.coli* and *T. pratensis* against *P.aeruginosa* (Table 1).

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plant Extracts in Front of Pathogen

Extract Plant	*E. coli*		*P. aeruginosa*	
	MIC	MBC	MIC	MBC
Peganum harmala	100	100	25	50
Teucrium polium	25	50	25	50
Prangos feralaceae	100	200	50	100
Tragopogon pratensis	50	100	25	25
Rumex alveollatus	50	100	25	50

5. Discussion

This study showed that *T. polium* extract has a minimum inhibitory concentration of *E. coli*, at 25 ppm, whereas the maximum of this is for *P. harmala* and *P. ferulaceae* with 100 ppm. The lowest minimum inhibitory concentration value of extracts *P. harmala*, *T. polium*, *T. pratensis* and *Rumex* were found at 25 ppm against *P. aeruginosa*.

In this study, the extract of *T. polium*'s maximum inhibitory concentration was against *E.coli*, while that of the extracts of *P. harmala*, *T. polium*, *T. pratensis* and *Rumex* was against *P. aeruginosa*. The investigation of the antimicro-

bial activities of plant extracts showed that the extremity of side effects on chemical drugs leads to much effort in finding alternatives. Therefore, scientists have recently tried to find new herbal drugs without any side effects.

In one study, the antibacterial effects of four types of *Prangos ferulaceae* extracts (ethanol, methanol, aureus and n-hexane) before some Gram-positive bacteria like *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*. *Staphylococcus aureus* and Gram-negative ones, like *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Salmonella enteridis* were studied. The results showed that ethanol and methanol extracts have the most antibacterial features, and each of them has antibacterial features in a meaningful way (17). The results of another study on the antibacterial effects of the extract of *Prangos ferulaceae* on Gram-negative and Gram-positive bacteria, such as *Staphylococcus aureus*, *E.coli*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa* and *Bacillus cereus* was confirmed. In such studies, the existence of compounds like Limonene, α-pinene and α-humulene are defined as a reason for the affectivity of this herb in the emergence of antibacterial features (18, 19). Darabpour et al. studied the antibacterial effect of alcoholic extracts from the aerial parts of *Teucrium polium* native to Iran on some pathogenic bacteria. The ethanolic extracts' results showed that *Bacillus anthracis* was the most sensitive species, while *Escherichia coli* and *Proteus mirabilis* were more resistant than others. The minimal inhibitory concentration of *Staphylococcus aurous* and *Salmonella typhi* was 40 mg/mL, and that of *Bacillus anthracis* was 10 mg/mL. The minimal bactericidal concentration against *Bacillus anthracis* was 10 mg/mL, while its values against other species were not found (> 200 mg/mL) (20). The results obtained by Shahba showed that *Teucriumpolium* extracts were effective in *Enterococcus and Pseudomonas* bacteria. In general, the MIC rate of aqueous extract in *Enterococcus* was 1.25 - 5 mg/mL. The MIC rate of ethanolic extract for *Enterococcus* was calculated as 10 mg/mL. The MIC of aqueous and ethyl acetate extracts for *Pseudomonas* bacteria were achieved at 5 and 20 mg/mL, respectively. The MBC contents of aqueous and ethyl acetate extracts of *Teucrium* for *Pseudomonas* bacteria was 10 mg/mL in aqueous and 20 mg/mL in ethyl acetate extracts. The MBC content of extracts for *Enterococcus* bacteria were 10 mg/mL in aqueous extracts and 20 mg/mL in ethanolic extracts (21). A study on antioxidant features showed that ethanolic extracts have a stronger effect in comparison with aqueous extracts. These results were also true for phenol Tom. In an ascertainment of the nature of herbal compounds' existence of flavonoids, alkaloid, anthraquinone, glycosidic, tannin and reducing sugar is conformed. Darabpour considered the antibacterial effects of the methanolic extract of different parts of *P. harmala*, including its root, stem, leaves, flowers and seeds against some important human pathogenic bacteria, and tested against Gram negative bacteria. The obtained results were inconsistent. MIC and MBC (minimal bactericidal concentration) values for both extracts against MRSA (methicillin resistant *Staphylococcus aureus*) and for seed extract against *E. coli* and *S. typhi* were equal (0.625 mg/mL) (22).

In the study by Saeidi et al., *P. harmala* was proven effective against the selected isolates of ESBL-producing *E. coli*. The most frequent ESBL rate producing *E. coli* isolates (32 out of 50) had an MIC of 2.5 mg/mL in an ethanol extract of *P. harmala* (23).

In Bokaeian et al.'s study, the height of the MIC value of *Cuminum cyminum* essential oil at 250 ppm was observed (24).

The study of Sharifi Mood et al., antibacterial activities of Ajowan essential oil (AEO) have been evaluated against two gram negative bacteria; *Klebsiella* and *E. coli* and one gram positive bacteria; *Staphylococcus aureus* (*S. aureus*). Minimum inhibitory concentration value was determined against all mentioned bacteria. The essential oil was effective for *S. aureus* with MIC of 1.25 mg/mL, followed by, *E. coli* with MIC of 2.5 mg/mL and *Klebsiella* with MIC of 5 mg/mL (25).

5.1. Conclusions

The results of this study have represented the fine antibacterial activities of plant extracts that could be used as appropriate treatments for infections caused by *Escherichia coli* and *Pseudomonas aeruginosa*.

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