

Antibacterial Effect of *Plantago Ovata* and *Lallemantia Iberica* Seed Extracts against Some Bacteria

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Abstract

Background: Researchers are seeking new plant compounds as an alternative to chemical drugs and antibiotics due to the increasing resistance of pathogenic bacteria to antibiotics. This study investigated the antibacterial effect of *Plantago ovata* and *Lallemantia iberica* L. seed extracts on some foodborne human pathogenic bacteria.

Materials and Methods: Disk-diffusion antibiotic sensitivity testing, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were used to evaluate the antibacterial activity of plant extracts in comparison to the tetracycline, as a control antibiotic.

Results: The results of this experiment showed that the *L. iberica* seed extract had the greatest effect on *Bacillus subtilis*, *Bacillus sphaericus* and *Pseudomonas aeruginosa* and did not have inhibitory effect or moderate inhibitory effect against other bacteria. Also, *P. ovata* extract had a high and moderate effect against *Bacillus sphaericus* and *Pseudomonas aeruginosa*, respectively. This extract had no inhibitory effect on the other bacteria. Tetracycline also had a significant inhibitory effect on all tested bacteria.

Conclusion: According to the results of this study, it can be concluded that extracts of some Iranian native plants can be a suitable alternative to the existing antibiotics.

Keywords: Medicinal plants; Disk diffusion; *Plantago ovate*; *Lallemantia iberica* L

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Introduction

Bacteria resistant to antibiotics have been reported worldwide (1). These bacteria influence human digestive system by infected meats (2). *Bacillus* species are gram-positive, rod-shaped, aerobic or anaerobic bacteria which are found abundantly in water and soil. These bacteria also cause food poisoning and secondary infections. *Bacillus cereus* more frequently contaminates milk and meat and in this way enters the human body (3, 4). *Pseudomonas* is a gram-negative and aerobic bacterium and has the ability to grow in most environments (5). The bacteria from this group not only cause disease in animals, but also cause secondary infections in humans (6). The bacterial resistance to antibiotics has become a major clinical and public health problem in recent years (10). Given the ever growing human population, the increasing demand or a greater

diversity of healthy food is becoming increasingly a growing issue. On the other hand, the use of unauthorized chemicals and all kinds of antibiotics in various animal derived protein products such as milk, meat, eggs and other types of proteins has increased dramatically over the last few years (8). These foods cause health problems, mental illnesses and various types of diseases such as allergy, cancer, digestive problems and different types of genetic disorders in human societies (8). The excessive use of antibiotics can cause gastrointestinal resistant to these compounds and may lead to antibiotic-associated diarrhea (9). As the use of antibiotics in animals is the main reason of antimicrobial resistance promotion in humans, these trends in the antibiotic resistance may be reversed by switching to the production of organic food (10). The use of antibiotic

has been banned in the European Union since January 2006. So, scientists have become interested in evaluating other alternatives to control specific microbial populations to modulate rumen fermentation (8). Nowadays, herbal plants are used on a large-scale in medicine against drug-resistant bacteria, which are considered as one of the most important reasons for the lack of success in the treatment of infectious diseases. Herbal plants are the major sources of new medicines and may be considered as an alternative to the usual drugs (11).

Plantago ovata and *Lallemantia iberica* L. contain a lot of mucilage and oils. The *P. ovata* leaves are glabrous and elliptical; its seeds are transparent, pinkish-gray to brown, or white to pink (12). *P. ovata* is a member of Plantaginaceae family and is native to Iran and India (13). The seeds of this plant contain 20-30% mucilage, which produces D-xylosan, arabinose, D-galactose, and D-galacturonic acid by hydrolysis process (14). *P. ovata* is one of the most important medicinal plants that have been used since ancient times for various reasons in Iran and have high antibacterial properties. Its effect on various bacteria, including *Staphylococcus aureus*, *S. pyogenes* and *Bordetella bronchiseptica* has been determined (15). *P. ovata* seeds have been added to breakfast cereal in various countries such as United States, Canada and Australia, to increase the dietary fiber content and to decrease blood cholesterol. It should be noted that it sometimes causes allergies and anaphylaxis (13).

L. iberica contains compounds such as β -Cubebene, Linalool and Spathulenol. It is notable that this medicinal herb has a strong antioxidant effect (16).

In the present study, we have evaluated the antibacterial effects of *L. iberica* and *P. ovata* seed extracts on some human pathogenic bacteria capable of causing human illness and food spoilage.

Materials and methods

The seeds of plants were collected from their natural habitat in Bushehr province, located in the south of Iran, and then dried in a dry and dark environment. These dried seeds were also pulverized to give seed powder (15).

Extraction

Hydro-alcoholic extract (ethanol 70%) was prepared by seed soaking for 48 hours at room temperature and then filtered with filter paper (15). The following oils were used: *Plantago ovata* and *Lallemantia iberica* L. Species and genus of plants were diagnosed by expert Botanist.

Antimicrobial assay

Agar gel disk diffusion test, minimum inhibitory

concentration (MIC) and minimum bactericidal concentration (MBC) were used in this study.

Disk diffusion antibiotic sensitivity testing

The standard bacterial strains including *Pseudomonas aeruginosa*, *P. fluorescense*, *Bacillus subtilis*, *B. antheracoid*, *B. coagulans*, *B. cereus*, *B. sphericus*, *Escherichia coli* O157, *Salmonella liatica* and *S. typhymorium* ATCC3598 were obtained from the Department of Microbiology at the School of Veterinary Science of Shiraz University in order to investigate the antibacterial properties of the plant extracts. A bacterial suspension with a concentration of 1.5×10^6 CFU. ml⁻¹ was prepared for this test. Also, disk diffusion method was used in order to study the antibacterial activity of the plants. The bacterial suspension was grown on an agar medium, and then the prepared discs were placed on a bacterial culture medium. The tetracycline disk was used as a control. The treated Petri dishes were incubated at 37 °C for 24 h. The diameter of inhibition zone was measured and reported in millimeters (three replications each extract) (17).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

To determine the MIC and MBC, a set of 9 sterile test tubes was used for each extract. The stock solutions (500 mg/ml) were further diluted in a 2-fold serial dilution to obtain the following concentrations: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.91, 1.95 and 0.98 mg/ml. One test tube as a negative control and tetracycline as a positive control were used. An aliquot of 1 ml of the bacterial suspension was inoculated into each tube. The negative control tubes were inoculated with the same quantity of extracts. All tubes were incubated at 37 °C for 24 h. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration (MIC). The contents of all tubes that showed no visible growth were cultured on Muller Hinton agar and incubated at 37 °C for 24 h. The MIC was considered as the lowest concentration that could not produce a single bacterial colony and the MBC was defined as the lowest concentration of the extract at which 99.9% of the inoculated microorganisms were killed (18).

Statistical analysis

In order to determine whether there is a statistically significant difference among the obtained results from zone inhibition assays, MIC and MBC average analyses were carried out using the SPSS V16.0. The differences between any means were tested by the Duncan test and the results were considered significant when $P < 0.05$ (19).

Results

According to the previous studies, the zone of inhibition ranging ≥ 15 mm, 10-15mm and < 10 mm diameter are considered as high, moderate and low antibacterial effect of the essential oils or extracts, respectively [20]. Our results in this experiment showed that the seed extract of *L. iberica* had a high antibacterial effect against *P. aeruginosa*, *B. subtilis* and *B. sphaericus*. This extract also had a moderate

effect against *S. typhimorium* ATCC3598 and *B. cereus*. It is notable that other bacteria were not affected by *L. iberica* seed extract. Also, *P. ovata* seed extract had high and moderate antibacterial effect against *B. sphaericus* and *P. aeruginosa*, respectively (Table 1). Tetracycline, as a control group, had remarkable effects against all tested bacteria (Table 1).

Table 1. The inhibition zone (mm) of selected herbal extract against bacteria.

Bacteria	<i>L. iberica</i> L	<i>P. ovata</i>	Tetracycline
<i>B. coagulans</i>	0 ^a	0 ^a	24.6 \pm 5 ^d
<i>B. anthracoid</i>	0 ^a	0 ^a	28 \pm 0 ^e
<i>B. cereus</i>	11.3 \pm 2.5 ^b	0 ^a	26 \pm 6 ^{de}
<i>B. sphaericus</i>	15.3 \pm 3 ^c	0 \pm 16 ^c	41.3 \pm 1.1 ^f
<i>B. subtilis</i>	16.6 \pm 4.1 ^c	14.6 \pm 1.4 ^c	15.3 \pm 1.1 ^c
<i>E. coli</i> O157	9.6 \pm 1.5 ^b	0 ^a	34.3 \pm 3 ^{fg}
<i>S. typhimorium</i> ATCC3598	14.6 \pm 2.3 ^c	0 ^a	31.3 \pm 3 ^e
<i>S. liatica</i>	0 ^a	0 ^a	32.6 \pm 6.4 ^{ef}
<i>Ps. Fluorescence</i>	0 ^a	0 ^a	33.3 \pm 4.1 ^{efg}
<i>Ps. Aeruginosa</i>	18.3 \pm 6.5 ^d	10 \pm 2 ^b	35 \pm 3 ^g

Different letters indicate significant difference (P-value \leq 0.05)

The results of the MIC and MBC methods are reported in Table 2. These results indicate that *L. iberica* extract had the highest MIC and MBC effects against *P. aeruginosa*. The *P. ovata* extract had the

highest MIC effect against *B. sphaericus* and *B. subtilis*. It is notable that *B. sphaericus* was affected most by *P. ovata* extract according to the MBC test.

Table 2. MIC and MBC (mg.ml⁻¹) results of seed extracts.

Bacteria	MIC			MBC		
	<i>P. ovata</i>	<i>L. iberica</i> L.	Tetracycline	<i>P. ovate</i>	<i>L. iberica</i> L.	Tetracycline
<i>B. coagulans</i>	No	No	0.39	No	No	3.125
<i>B. anthracoid</i>	No	25	0.39	No	No	1.562
<i>B. cereus</i>	No	12.5	0.39	No	No	1.562
<i>B. sphaericus</i>	6.25	6.25	0.195	12.5	25	0.39
<i>B. subtilis</i>	6.25	6.25	1.562	25	50	3.125
<i>E. coli</i> O157	No	25	0.195	No	No	0.78
<i>S. typhimorium</i> ATCC3598	No	6.25	0.39	No	25	1.562
<i>S. liatica</i>	No	No	0.39	No	No	1.562
<i>Ps. Fluorescence</i>	No	No	0.39	No	No	0.78
<i>Ps. Aeruginosa</i>	25	3.125	0.195	No	12.5	0.78

No: no effect

Discussion

Infectious diseases are one of the most common diseases in the world which impose huge financial burdens on human societies. Synthetic antibiotics have played an important role in the treatment of infectious diseases in recent decades. However, excessive use of these kinds of antibiotics and the problems associated with their antimicrobial resistance has led to an increase in the use of more herbal medicines as antibiotic alternatives (21, 22). The antimicrobial activity of plants is generally related to the presence of phenolic compounds, saponins, tannins and flavonoids in their structures. The antibacterial properties of these compounds are related to their effects on the cellular membrane or inhibition of structural enzymes (23).

According to the results of this study, the highest growth inhibition zone in the presence of *L. iberica* extract was related to *P. aeruginosa* with diameter of 18.3 mm. It is notable that the inhibition zone diameter in the presence of *P. ovata* extract against *B. subtilis* was 14.1 mm (the difference was not statistically significant). The plant seed extracts did not have a significant effect on the growth inhibition zone of other bacteria.

The results of MIC and MBC tests showed that *L. iberica* extract had the highest effect on *P. aeruginosa*. In a similar study, the antibacterial effect of various extracts of herbs such as nettle (*Urtica Dioicas*) on *B. subtilis*, *P. aeruginosa*, *E. coli* and *S. aureus* was confirmed (24). *L. iberica* contains compounds such as β -cubebene, Linalool and Spathulenol (16). The medicinal plants and their metabolites such as linalool and thymol exterminate the microorganisms' membrane and cause liposaccharides leakage and increase the cytoplasmic membrane permeability to ATP. Eventually, the exit of ATP leads to the waste of cellular energy storage; and consequently causes cell death (25).

According to the results of this study, *B. sphericus* with the inhibition zone diameter of 16 mm was affected more than the other bacteria by the alcoholic extract of *P. ovata*. The inhibition zone diameter for *B. Subtilis* and *P. aeruginosa* were 14.6 and 10 mm, respectively. Other bacteria were not affected by *P. ovata* extract.

The mechanism of plant compounds action on the prevention of bacterial growth involves the destructive effect on the cell wall, which results in the separation of the cell wall components and the exposure of the cell contents and ultimately the cell death. The antibiotic effect of *P. ovata* extract may be related to their secondary metabolites (26). The *P. ovata* extract had the highest MIC effect against *B. sphericus* and *B. subtilis* and the highest MBC effect against *B. sphericus*. The antibacterial effects of *P.*

ovata extract have been previously studied against various bacteria such as *S. aureus*, *S. pyogenes* and *B. bronchiseptica* (15). Sharif et al. (2010) reported that the MIC of Plantago Psyllium against Staphylococcus aureus and Staphylococcus epidermidis were 20 and 18 mm, respectively (27).

Conclusion

According to the results of this study, it can be concluded that the medicinal plant extracts of *P. ovata* and *L. iberica* are suitable for use in poultry feeds and human foods as herbal antibiotics. However, further studies are needed for the definitive administration of these extracts as the source of antibiotic plants.

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Author Contributions

The present study was funded by HH and NGH. HH and NGH were also involved in the collection of data, statistical analysis and drafting of the manuscript. LK read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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