

Antibacterial Effect of Hydroalcoholic Extracts of Herbal Plants Against Some Hospital-acquired Infections



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ABSTRACT

Background: Due to increased bacterial resistance to common antibiotics, the tendency towards using herbal extracts is increasing. *Plantago major* L, *Plantago ovata*, *Lallemantia iberica* L, *Sinapis arvensis* L, and *Ficus carica* are widely used as herbal plants in traditional medicine. They were known to have a variety of therapeutic effects. The current study aimed to evaluate the antibacterial activity of hydroalcoholic extract of these herbs against some hospital-acquired infections.

Materials and Methods: Disk-diffusion antibiotic sensitivity testing, minimum inhibitory concentration, and minimum bactericidal concentration of hydroalcoholic extracts were applied to assess the antibacterial activity compared with tetracycline, as a control antibiotic.

Results: The results of this experiment showed that the *L. iberica* and *S. arvensis* extract had the greatest effect on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus vulgaris*. All the tested medicinal plants had a high antibacterial effect on *P. vulgaris*, except *P. ovata*.

Conclusion: The results of this study show that the replacement of chemical drugs with herbal extract could be effective in the elimination of bacterial growth.

Introduction

Wound infection is a common and serious complication of surgical operations. It is the second most frequent nosocomial infection in hospitals. Although eradication of wound infections in surgeries seems impossible, decreasing the

number of infection victims will result in significant benefits such as higher patient satisfaction and lower medical costs [1]. The bacterial agents, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* cause several human infections [2].

Staphylococcus aureus is one of the main causes of surgical wound infections in hospitalized patients and

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medical device-related contaminations. *S. aureus* is predominantly colonized on the surface of the skin and mucosa, and can also survive in all tissues of the body [3, 4]. *Pseudomonas aeruginosa* is a multidrug-resistant pathogen, recognized early for its ubiquity, and its mechanism of advanced inherently antibiotic resistance [5]. *Proteus vulgaris* is one of the normal flora of the human digestive system that can cause urinary tract infection, especially in patients with a weak immune system [6].

The antibiotics are among the most common methods used to eliminate pathogenic bacteria and of great benefit in maintaining human health. However, the resistance of many pathogenic bacteria to these antibiotics is one of the biggest challenges facing humans let alone their adverse effects in humans' bodies [7, 8]. During the last several decades, natural products with antimicrobial effects have been investigated to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects on human health [9]. Aromatic plants are known for a very long time and they are used in phototherapy and food preservation [10]. Plant extracts are the potentially useful sources of antimicrobial compounds. Many studies have been conducted on the antimicrobial activities of plant compounds against many different types of microorganisms, including hospital-acquired infections [11].

Plantago ovata Forssk (Plantaginaceae family) is a winter annual plant [12]. The seeds of this plant contain 20%-30% mucilage, which produces D-xyloosan, arabinose, D-galactose, and D-galacturonic acid by hydrolysis process [13]. *Plantago ovata* can reduce the level of cholesterol in the blood, anti-diarrheal effect that can set blood sugar [14]. Its effect has been determined on various bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bordetella bronchiseptica* [12].

Plantago major is a perennial medicinal herb. It has been reported to cure numerous diseases from common cold to hepatitis, skin diseases, infectious diseases, fever, problems concerning the digestive organs, respiratory organs, reproduction, the circulation [15].

Lallemantia iberica L contains compounds such as β -cubebene, linalool, and spathulenol. Notably, this medicinal herb has a strong antioxidant effect [16].

The *Ficus carica* Linn leaves contain several active compounds such as flavonoids, tannins, sesquiterpenes, alkaloids, and saponins [17], which have antioxidant, anticancer, anti-inflammation, antiviral, and antibacterial activities [18]. *Sinapis arvensis* L, a genus of Brassicaceae, has a

long history of use as condiments and as herbal medicines. In many developing countries, *Sinapis* species are used as food, fodder to livestock, and herb in folklore medicine [19]. The objective of the current study was to evaluate the antibacterial activity of hydroalcoholic extract of *Plantago major* L, *Plantago ovata*, *Lallemantia iberica* L, *Sinapis arvensis* L, and *Ficus carica* against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus vulgaris*.

Materials and Methods

Preparation of herbal samples

The aerial parts of plants were collected from their natural habitat in Bushehr Province, Iran. The samples were dried out in a dry and dark environment (far from sunlight). Samples were also pulverized to obtain a powder. Noticed in the voucher that a specimen from each variety was deposited in the Herbarium of Agriculture and Natural Resources Faculty of Persian Gulf University (Bushehr, Iran).

Extraction

Hydroalcoholic extract (ethanol 70%) was prepared by seed soaking for 48 hours at room temperature and then filtered with filter paper. The following oils were used: *Plantago major* L (*P. major*), *Plantago ovata* (*P. ovata*), *Lallemantia iberica* L (*L. iberica*), *Sinapis arvensis* L (*S. arvensis*) and *Ficus carica* (*F. carica*) [16]. Species and genus of plants were diagnosed by an expert in botany.

Microorganisms

Pseudomonas aeruginosa, *Staphylococcus aureus*, and *Proteus vulgaris* were used to study the antibacterial properties of plants. By morphocultural and biochemical characteristics, the isolated bacterial strains were identified. Gram staining and biochemical tests were done as per the guidelines of the Clinical and Laboratory Standards Institute [20]. A pure culture of the strains was maintained at -20°C in brain heart infusion broth (Merck) containing 10%-15% glycerol, which was thawed before use. To prepare bacterial inocula, 0.1 mL of the thawed cultures were transferred to 10 mL of sterile Tryptic soy broth (TSB, Merck) and was incubated overnight at 37°C [12].

Antimicrobial assay

Agar gel disk diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used in this study.

Disk diffusion susceptibility

Antibacterial susceptibility assay of Mueller-Hinton Broth (MHB, Merck) medium was used to grow the test isolates at 37°C for 22 h. Final bacterial numbers were standardized to 1×10^6 CFU/mL. A total of 0.1 mL of bacterial suspension was poured into each plate containing Mueller-Hinton Agar. The surface culture was prepared by sterile L shape pipet Pasteur and allowed to remain in contact for 1 min. Thereafter, a 5% concentration of each plant essential oils was prepared. The sterile filter paper disks (6-mm diameter) were placed on the cultures and 24 h after incubation at 37°C, the zone diameter of inhibition (ZDI) was measured in mm. To determine the sensitivity of each bacterial species tested, tetracycline was used as a positive control standard (three replications for each extract) [21].

MIC and MBC

For each extract and essential oil, a set of 9 sterile test tubes were used. The stock solutions (500 mg/mL) were further diluted in a 2-fold serial dilution to obtain the following concentrations of 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 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. Then the tubes were incubated for 24 h at 37°C. Based on the MIC definition, the last tube with no turbidity was considered as MIC. To determine the MBC, the solution of the transparent tubes of the previous step was cultured in the TSA medium. A plate with no bacterial growth was considered as MBC (three replications for each extract) [21]. Pseudomo-

nas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 were used as the control strain in this test.

Statistical analysis

To determine the significant difference among the obtained results from zone inhibition assays, MIC and MBC average analyses were carried out in SPSS v. 16.0. The differences between any means were tested by the Duncan test and the results were considered significant when $P < 0.05$.

Results

Antimicrobial activity of the tested extracts

Results indicated that all five plants had high antibacterial effects on *S. aureus*. Also, *P. aeruginosa* was highly affected by *L. iberica* and *S. arvensis*. All the tested medicinal plants had a high antibacterial effect on *P. vulgaris*, except *P. ovata* (Table 1).

MIC and MBC

The MIC values of the hydroalcoholic extract of medicinal plants, at different concentrations ranging from 0.98 mg/mL to 250 mg/mL, in comparison with the activity of tetracycline has been shown in Tables 2 and 3.

Discussion

According to the previous studies, the ZDI values of >15 mm, 10-15 mm, and <10 mm diameter are considered as high, medium, and low antibacterial effects of the essential oils or extracts, respectively [22].

Table 1. Inhibition zone (Mean \pm SD, mm) results of hydroalcoholic extracts against some hospital-acquired infections

Treatment	Mean \pm SD		
	<i>Pseudomonas Aeruginosa</i>	<i>Staphylococcus Aureus</i>	<i>Proteus Vulgaris</i>
<i>Plantago major</i>	0.00 \pm 0.00 ^c	20.33 \pm 1.52 ^c	21.66 \pm 1.52 ^c
<i>Plantago ovata</i>	0.00 \pm 0.00 ^c	13.33 \pm 0.57 ^e	0.00 \pm 0.00 ^d
<i>Lallemantia iberica</i>	16.33 \pm 1.15 ^b	30.00 \pm 1.00 ^b	32.00 \pm 0.00 ^b
<i>Sinapis arvensis</i>	16.00 \pm 1.00 ^b	30.33 \pm 0.57 ^b	35.00 \pm 1.00 ^a
<i>Ficus carica</i>	0.00 \pm 0.00 ^c	15.16 \pm 0.28 ^d	20.33 \pm 1.52 ^c
Tetracycline	36.00 \pm 0.00 ^a	39.33 \pm 1.15 ^a	35.16 \pm 0.28 ^a

Means within same column with different superscripts differ ($P < 0.05$). Non-similar alphabets in each column indicate a significant difference between treatments

Table 2. MIC (mg/mL) results of hydroalcoholic extracts against some hospital-acquired infection-causing bacteria

Treatment	<i>Pseudomonas Aeruginosa</i>	<i>Staphylococcus Aureus</i>	<i>Proteus Vulgaris</i>
<i>Plantago major</i>	No	31.22	62.5
<i>Plantago ovata</i>	No	62.5	No
<i>Lallemantia iberica</i>	62.5	15.62	31.22
<i>Sinapis arvensis</i>	31.22	7.81	3.91
<i>Ficus carica</i>	No	125	125
Tetracycline	3.91	7.81	7.81

NO: No effect; P≤0.05

**Table 3.** MBC (mg/mL) results of hydroalcoholic extracts against some hospital-acquired infection-causing bacteria

Treatment	<i>Pseudomonas Aeruginosa</i>	<i>Staphylococcus Aureus</i>	<i>Proteus Vulgaris</i>
<i>Plantago major</i>	No	125	250
<i>Plantago ovata</i>	No	62.5	No
<i>Lallemantia iberica</i>	125	31.22	62.5
<i>Sinapis arvensis</i>	62.5	7.81	15.62
<i>Ficus carica</i>	No	250	125
Tetracycline	15.62	7.81	15.62

NO: No effect; P≤0.05



Results of this experiment showed that the seed extract of *L. iberica* and *S. arvensis* have a high antibacterial effect against *P. aeruginosa*, *S. aureus*, and *P. vulgaris*. In the present study, *S. arvensis* hydroalcoholic extract had the strongest antibacterial effect against *P. vulgaris* (35 mm inhibition zone). Oil seeds have been used for making soap, cooking, and lubricating, and the whole plant used as green fodder [23]. The antibacterial effect of *L. iberica* seed extracts has studied against *Pseudomonas aeruginosa* (18.3±6.5 mm inhibition zone), *Bacillus subtilis* (16.6±4.1 mm inhibition zone), *Bacillus cereus* (11.3±2.5 mm inhibition zone), *Bacillus sphaericus* (15.3±3 mm inhibition zone), *Escherichia coli* O157 (9.6±1.5 mm inhibition zone), *Salmonella liatica*, and *Salmonella typhimurium* ATCC3598 (14.6±2.3 mm inhibition zone) and it had a significant effect on all the mentioned bacteria [16]. *L. iberica* produced many secondary metabolites such as phenolic acids, flavonoids, tannins, triterpene, mucilage, and oil [24]. The secondary metabolites such as linalool and thymol exterminate the bacterial membrane, cause liposaccharides leakage, and increase the cytoplasmic membrane permeability to ATP. Eventually, the exit of ATP leads to the loss of cellular energy storage and cell death [16, 25].

Ficus carica exhibited high inhibition zones of 15.16 and 20.33 mm on *S. aureus* and *P. vulgaris*. The MIC value for the *F. carica* was 125 mg/mL against *S. aureus* and *P. vulgaris*. The MBC values for the *F. carica* were 250 mg/mL against *S. aureus* and 125 mg/mL against *P. vulgaris*. The major components of *F. carica* were arabinose, β-amyrins, β-carotenes, glycosides, β-setosterols, and xanthotoxo [26]. The obtained results indicate the therapeutic virtue of *F. carica* leaves as an antimicrobial agent against some microbial infection, such infection by methicillin-resistant *S. aureus* which recognized as a global nosocomial problem [27]. Jung reported that the methanol extract from the leaves of *F. carica* exhibited a strong effect against *Escherichia coli* but weak effect against *S. aureus* [28].

P. major contains biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, alkaloids, and terpenoids [29]. This plant is an important medicinal plant with different pharmacological properties, including anti-inflammation, analgesic, wound healing, antipyretic, antitussive, anti-infective, anti-hemorrhagic, laxative, astringent, hemostatic activity and diuretic [30] to manage a wide

range of diseases including constipation, coughs and wounds. The aim of this study is to review the traditional application, botanical characterization, pharmacological activities, phytochemistry effects and toxicity of *Plantago major*. In this review study, medicinal properties of *Plantago major* are collected from credible pharmacopoeias, textbooks of traditional Persian medicine (TPM). In this experiment, *P. major* had a significant effect on *S. aureus*. Also, *P. vulgaris* showed a significant difference from the control antibiotic. Mohammadsadeghi et al. reported that different extracts (hydroalcoholic extracts) of *P. major* leaves exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* [20].

Investigations showed that *P. ovata* can inhibit *B. sphaericus* (16 mm inhibition zone) and *B. subtilis* (14.6 mm inhibition zone) [16]. *P. ovata* showed less activity than *P. major*, *L. iberica* L, *S. arvensis*, and *F. carica* against all tested pathogenic bacteria. Studies showed that the extracts made from *P. ovata* had antibacterial activity against many bacteria such as *S. aureus*, *Strep. pyogenes*, and *B. bronchiseptica* [12]. Sharif et al. reported that the MIC of *Plantago Psyllium* against *Staphylococcus aureus* and *Staphylococcus epidermidis* were 20 mm and 18 mm, respectively [31].

Conclusion

In conclusion, the seed extract of *L. iberica* and *S. arvensis* have a high antibacterial effect against *P. aeruginosa*, *S. aureus*, and *P. vulgaris*. Our results support the use of these plants in traditional medicine and suggest that some of the plant essential oils possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Authors' contributions

Financial support: Hassan Habibi, Noorsaadat Saajedi and Najmeh Ghahtan; Collection of data, statistical anal-

ysis and drafting of the manuscript: Hassan Habibi, Najmeh Ghahtan; Writing – original draft: Hassan Habibi, Saajedah Habibi

Conflict of interest

The authors declared no competing interests.

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