

Antimicrobial Activity of *Peganum harmala* and *Heracleum persicum* Against *Acinetobacter baumannii*

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Abstract

Background: The extracts of many plants have been used for their applications in the prevention of bacterial growth; however, these applications need more investigations.

Objectives: The major aim of the current study was to investigate the antimicrobial activity of an extract of the *Peganum harmala* flower and *Heracleum persicum* against *Acinetobacter baumannii*.

Materials and Methods: The minimum inhibitory (MIC) concentrations Minimum Bactericidal Concentration of extract and essential oil were investigated by microdilution method and antibiotic resistance was evaluated using the disk diffusion test.

Results: In this study, the levels of MIC extract and essential oil of *Peganum harmala* were observed in ranges from 6.25 ppm to 12.5 ppm and 3.1 ppm to 25 ppm, respectively. The highest MIC value was observed as 12.5 ppm in *A. baumannii*. The levels of MIC extract and essential oil of *Heracleum persicum* were observed in ranges from 5 ppm to 20 ppm and 12.5 ppm to 10 ppm, respectively. The highest level of MIC extract and the highest essential oil value of *Heracleum persicum* were observed as 20 ppm and 10 ppm, respectively, in *A. baumannii*.

Conclusions: Results of this study suggest that the extract alone of *Peganum harmala* and *Heracleum persicum* may be useful to treat bacterial infections.

Keywords: Minimum Bactericidal Concentration, *Peganum harmala*, *Heracleum persicum*, *Acinetobacter baumannii*

1. Background

Medicinal plants are used in treating diseases because they are low-risk, readily available, and inexpensive natural materials and have a higher consumption by people compared to synthetic drugs (1, 2).

Herbal medicine represents one of the most important fields of traditional medicine all over the world. Medicinal plants are traditionally used for the treatment of pain. The formation of free radicals may play an important role in the origin of life and biological evolution, implying both their beneficial effects on the aging of organisms, as well as cancer promotion. Espand (scientific: Zygophyllaceae), also called Harmal and Suryin, is a perennial, bushy, wild-growing flowering plant with short creeping roots that may grow to 30 - 100 cm high (3-5). Espand contains alkaloids such as harmine, Harmalyn, harmol, and harmalol (6). The seed extract has antispasmodic, antihistaminic (7), and vasorelaxant effects (8).

Acinetobacter is a gram-negative coccobacillus separated from many human and environmental resources (9). This bacterium is known as a tropical and humid pathogen, and the prevalence of infection in the summer is higher than in other seasons (10, 11). Over the past decade,

the incidence of nosocomial infections caused by these bacteria has been on the rise. Although this bacterium usually has low virulence, infected catheters cause a variety of infections and are often related to this respiratory system infection (9). *Acinetobacter baumannii* causes different hospital-acquired infections, such as bacteremia, urinary tract infections, and secondary meningitis, but its prominent role is in hospital pneumonia, especially pneumonia of the upper respiratory tract of patients in intensive care units.

Heracleum persicum flowering plants are plants of the Apiaceae family. This plant is native to moist mountainous regions of Iran and its margins grow. *Heracleum persicum* seeds are very thin and have a spicy taste.

Angelica has a copper color and its most important pharmaceutical active ingredients are essential oils and resin. Angelica, due to the antiseptic and germicidal compounds, such as Anatole strong, can have an antimicrobial effect (12).

2. Objectives

The purpose of this study was to examine the evolution

of the antimicrobial activity of extracts of *Peganum harmala* and *Heracleum persicum* against *Acinetobacter baumannii*.

3. Materials and Methods

The different environmental samples were processed for the isolation of bacteria by methods described elsewhere. Morphologically distinct colonies obtained from different plates were streaked on Nutrient agar (NA), MacConkey agar (MCA) and blood agar to purify. The bacterial isolates were identified on the basis of standard cultural, morphological, and biochemical characteristics. Antibiotic susceptibility testing was performed using the Kirby-Bauer test on Mueller-Hinton agar, according to CLSI protocols. The tested drugs (μg) and their potencies are as follows: nalidixic acid (30 μg), penicillin (10 μg), amikacin (10 μg), and tetracycline (30 μg) (13).

3.1. Plant Materials

The seed of *Peganum harmala* and the *Heracleum persicum* leaf were collected in Iran and dried at room temperature. The specimens were ground and stored in a glass container and preserved until used.

3.2. Preparation of Extracts

Samples were justly dried and ground into fine particles and into a crude powder. In the first step, 10 g of each sample was drenched in 60 ml of 95% ethanol for one day (agitation sporadically with a shaker). Then supplies were strained (Whatman No. 1 filter paper). In the next step, filtrates were condensed with a rotary evaporator. Finally 0.97 g of prepared extracts were acquired and were stored at 4°C in an airtight screw-cap tube.

3.3. Distillation of Essential Oil

The seed of *Peganum harmala* and the *Heracleum persicum* leaf were ground prior to the distillation operation, and then 300 g of ground *Peganum harmala* and *Heracleum persicum* were submitted to water distillation for four hours using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered, and stored at 4°C.

3.3.1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Extract and Essential Oil

For calculated minimum inhibitory concentration (MIC) and MBC, broth microdilutions were utilized. All examinations were carried out in a Mueller-Hinton broth complement with Tween 80 at a definitive concentration of 0.5% (v/v). In short, a two-fold serial dilution of the extract was prepared in a microtiter 96 ranged from 6.25 ppm to 100 ppm. In short, serial doubling dilutions of the extract were provided in a 96-well microtiter plate. After

preparation of an Indices solution, 10 μL , including a 10 mg extract in 2 mL DMSO, was added to the wells. Next, 10 μL of Mueller-Hinton broth was transferred into the wells. Finally, bacterial suspensions (106 CFU/mL) were mixed with them to provide a concentration of 104 CFU/mL. With cling film, plates were complicated freely to ensure that the bacteria did not get dehydrated. Three replications were made for each treatment and were put in an incubator at 37°C for 18 - 24 hours. The color changes were examined. The minimum concentrations at which the changing of colors had taken place was used as a MIC value. Then average of the three replicates was calculated as the MIC for the samples. The point that growth of bacteria was inhibited by the lowest concentrations of extract was used for the MIC value. Microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

4. Results

In this study, the minimum inhibitory concentration (MIC) was evaluated. The levels of MIC extract and essential oil of *Peganum harmala* were observed in ranges from 6.25 ppm to 12.5 ppm and 3.1 ppm to 25 ppm, respectively. The highest MIC value was observed at 12.5 ppm against *A. baumannii* (Table 1), and the levels of MIC extract and essential oil of *Heracleum persicum* were observed in ranges from 5 ppm to 20 ppm and 12.5 ppm to 10 ppm, respectively. The highest MIC for extract and essential oil values of *Heracleum persicum* were observed at 20 ppm and 10 ppm, respectively, against *A. baumannii* (Table 2).

Table 1. Minimum Inhibitory Concentration of *Peganum harmala* Extract and Essential Oil Against *A. baumannii* (PPM)

Bacterial Code	MIC Extract/ Essential Oil	Bacterial Code	MIC EXTRACT/ Essential Oil
1	6.25/3.1	7	12.5/12.5
2	12.5/12.5	8	6.25/25
3	6.25/12.5	9	6.25/12.5
4	6.25/3.1	10	6.25/6.25
5	6.25/3.1	11	6.25/6.25
6	12.5/6.25	12	6.25/12.5

Table 2. Minimum Inhibitory Concentration of *Heracleum persicum* Extract and Essential Oil Against *A. baumannii* (PPM)

Bacterial Code	MIC Extract/ Essential Oil	Bacterial Code	MIC Extract/ Essential Oil
1	10/5	7	20/5
2	10/5	8	10/2.5
3	5/5	9	20/10
4	10/2.5	10	20/5
5	5/1.25	11	10/2.5
6	10/10	12	20/10

The levels of MBC extract and essential oil of *Heracleum persicum* were in observed ranges from 10 ppm to 20 ppm and 2.5 ppm to 20 ppm (Table 3), respectively. The levels of MBC extract and essential oil of *P. harmala* were observed in ranges from 12.5 ppm to 25 ppm and 6.25 ppm to 50 ppm, respectively. The highest MBC values for extract and essential oil of *P. harmala* were observed at 25 ppm and 50 ppm (Table 3), respectively.

An antibiotic susceptibility test of *Acinetobacter baumannii* was evaluated for four antimicrobial agents. However, overall *Acinetobacter baumannii* were resistant to all four of the antimicrobial agents, including nalidixic acid (100%), penicillin (100%), amikacin (83.3%), and tetracycline (58.3%).

Table 3. Minimum Bactericidal Concentration of *P. harmala* L and *H. persicum* Extract and Essential Oil Against *A. baumannii* (PPM)

Bacterial Code	MBC <i>P. harmala</i> Extract/Essential Oil	MBC <i>H. persicum</i> Extract/Essential Oil
1	12.5/6.25	20/10
2	25/25	20/10
3	12.5/25	10/5
4	12.5/6.25	20/5
5	12.5/6.25	10/2.5
6	25/12.5	20/10
7	25/25	20/10
8	12.5/50	20/5
9	12.5/25	20/20
10	12.5/6.25	20/10
11	12.5/12.5	10/5
12	12.5/25	20/20

5. Discussion

In this study, *Acinetobacter baumannii* were resistant to four agents including nalidixic acid (100%), penicillin (100%), amikacin (83.3%), and tetracycline (58.3%). Shah cheraghi et al. observed that *Acinetobacter baumannii* was the most and least resistant to the antibiotics cefixime (95 isolates, 100%) and colistin (4 isolates 2/4%), MICs to ceftazidime in 79 (83%) isolates was 64 micrograms per ml and 18 samples (18.9%) were ESBL-producing isolates (14).

The results of a study by Vafee et al. showed that 100 isolates of *Acinetobacter baumannii* were resistant to imipenem (100%), ceftriaxone (95%), amikacin (95%), imipenem (76%), piperacillin-tazobactam (70%), meropenem (69%), gentamicin (63%), tobramycin (56%), and tetracycline (51%) (15).

Results of a study by Sadeh et al. showed that *Acinetobacter baumannii* isolated from surfaces of medical equipment in Tehran were resistant to imipenem (100%), meropenem (100%), ceftazidime (99%), ciprofloxacin (98%), gentamicin (97/85%), tetracycline (2/70%), ampicillin (2/70%), and cefotaxime (4/69%) (16).

Herbal medicine represents one of the most important fields of traditional medicine all over the world. Medicinal plants are traditionally used for the treatment of pain. The formation of free radicals may play an important role in the origin of life and biological evolution, implying both their beneficial effects on aging of organisms and in cancer promotion (17).

In a study by Edziri, ethyl acetate, chloroform, butanol, and methanol extracts from the aerial section of *Peganum harmala* were tested for antibacterial, antioxidant, and antiviral activities. Results showed that a chloroform extract had the best antibacterial activity against gram-positive and gram-negative bacteria. The methanol extract showed significant antiviral activity against the CoxB-3 virus. The chloroform extract may be a significant fount of antibacterial compounds against gram-positive bacteria (18).

In a study by Darabpour, the amounts of MIC and MBC for *Peganum harmala* on MRSA (methicillin-resistant *Staphylococcus aureus*) and for seed extract on *E. coli* and Salmonella was similar (0.625 mg/mL) (19).

In the Hayat study, the chloroformic, ethyl acetate, butanolic, and methanolic extracts of *P. harmala* leaves all showed acceptable antifungal activity, with a MIC of 2.5 mg/mL. Chloroformic and methanolic extracts represented significant antibacterial activity on gram-positive bacteria rather than gram-negative bacteria, with MIC values ranging between 0.251 mg/mL and 2.5 mg/mL (20).

Peganum harmala seed extracts are reported to contain alkaloids, flavonoids, and anthraquinones (21, 22). The alkaloids in the seed extract have been utilized to control hemosporeidial infection in naturally and experimentally infected cattle (23, 24). An ethanolic *P. harmala* extract has been represented to have high an antibacterial role on MRSA (methicillin-resistant *Staphylococcus aureus*) (25) and CRSA (cefixime-resistant *S. aureus*) (22).

Findings of Hassan Ali showed that *Peganum harmala* was effective on *Staphylococcus aureus*, *Acinetobacter calcoaceticus*, and *Candida albicans*. Ampicillin, velosef, sulfamethoxazole, tetracycline and ceftazidime, cefotaxime, and cefixime, which were applied as controls, had MIC \geq 50 and 1.5 μ g/mL, respectively, for organisms sensitive to extracts (26).

the result show that the ethanol extract of *P. harmala* has exhibited antibacterial activity on MRSA (28) and CRSA (29). A study by Nazemi showed a minimum inhibitory concentration (MIC) of *H. Persicum* against *Bacillus polymixa*, *Baxillus subtilis*, *Enterococcus faecalis*, *Nocardia*, and *Staphylococcus aureus* of 50, 100, 500, 200 and 500 mg/mL, respectively (27).

In another study, phytochemical analysis showed that the main components of the essential oil of Angelica included ethylhexyl Bvtanvat (98/25%), octyl 2-methyl butyrate (37/14%), Penylt cyclopropane (77/12%) and a minimum inhibitory concentration for *Escherichia coli* and *Listeria monocytogen* as 5 and 5.2 mg/mL, respectively (28).

A study by Pirbaloutl showed that fruit oil *H. persicum* snow against anti-bacterial effect against *Campylobacter*

coli and *jejuni* (29), and a study by Dadfar showed the antibacterial properties of the essential oil of *Angelica* extract have a low effect on *Pseudomonas aeruginosa* (30).

In a study by Dehghan Noudeh, the antibacterial activity of *H. persicum* was exhibited against *B. subtilis* (MIC = 6.25 mg/mL). The other fractions were inactive against tested strains and showed no significant difference ($P > 0.05$) (31).

The study of Scheffer, the essential oil from the fruits of *H. persicum* was investigated and result show that oil contains about 95% aliphatic esters, 4% aliphatic alcohols, and 1% monoterpenes. In addition, 37 esters and 17 monoterpenes were identified (32).

Pimpinellin, isopimpinellin, bergapten, isobergapten, and sphondin are furanocoumarins that are found in the roots of *H. persicum*. Hexyl butyrate (56.5%), octyl acetate (16.5%), Hexyl 2-methyl butanoate (5.2%), and hexyl isobutyrate (3.4%) were identified as the major constituents of the *Heracleum persicum* essential oil (33).

Finally, reports suggest that *H. persicum* and *P. harmala* have potential for newer therapeutic applications in the future.

Footnotes

Authors' Contribution: Freshteh Javadian, Saeide Saeidi and Somayeh Jahani had equal roles in designing the study, gathering data, statistical analysis and manuscript writing.

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