



Antibacterial Activity of Ethanolic Extract of *Matricaria chamomilla*, *Malva sylvestris*, and *Capsella bursa-pastoris* against Multidrug-Resistant *Pseudomonas aeruginosa* Strains

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Abstract

Background: This study aimed to determine antibacterial activity of ethanolic extract of *Matricaria chamomilla* (chamomile), *Malva sylvestris*, and *Capsella bursa-pastoris* against multidrug-resistant (MDR) clinical isolates of *Pseudomonas aeruginosa*.

Methods: The plants were collected from Ziarat village of Gorgan, Iran in April 2019. The required parts of the plants were separated and completely dried in the shade. After grinding, extraction was performed by maceration method. The extract was dried at 37°C for 24 hours. To obtain a concentration of 50 mg/mL of each extract, 500 mg of the dried plant extract was dissolved in 10 mL 5% dimethyl sulfoxide and sterilized by filtration through a 0.45 µm membrane filter. For the antibacterial assay, agar well diffusion and broth microdilution methods were used.

Results: Based on the results, ethanolic extracts of *M. sylvestris* and *Capsella bursa-pastoris* did not show any antibacterial activity against MDR *P. aeruginosa* isolates in both antibacterial assays. No inhibitory effect was observed for ethanolic extract of chamomile against *P. aeruginosa* isolates in agar well diffusion method as well. In broth microdilution method, the extract of chamomile leaves showed inhibitory effect and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as 12.5 and 25 mg/mL, respectively.

Conclusions: In this study, the extract of ethanolic chamomile leaves showed antibacterial activity against the MDR *P. aeruginosa* isolates. Thus, it can be used in the production of antibacterial agents, and it is a good option for protection against pathogenic microorganisms, as well as *P. aeruginosa*.

Keywords: Antibacterial activity, *Matricaria chamomilla*, *Malva sylvestris*, *Capsella bursa-pastoris*, *Pseudomonas aeruginosa*

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Background

Pseudomonas aeruginosa, as an opportunistic gram-negative pathogen, is one of the most important pathogens responsible for 9%-10% of all nosocomial infections (1-3). It is the major cause of hospital-acquired infections all over the world, particularly in intensive care units (4). Immune-deficient patients, such as cancer patients, HIV-infected patients, individuals with cystic fibrosis, and severe burns patients are susceptible to infections caused by this bacterium and the mortality rate approaches 50% in these patients (5). *P. aeruginosa* is intrinsically resistant to many antibiotics with the capacity to obtain more resistance mechanisms to various classes of antibiotics (6). Overuse and misuse of antibiotics have led to the rapid emergence of multidrug-resistant (MDR) bacteria including *P. aeruginosa*. Today, development of resistance to multiple antibiotics by this organism and declining discovery of new antibiotics has created a global health crisis due to the limited treatment options. Thus, there is an urgent need to find novel and safe antibacterial substances as alternatives for antibiotics (7,8).

In recent years, the use of medicinal plants for the prevention and treatment of bacterial infections, as an inexpensive natural source with lower side effects compared to synthetic antibiotics, has received attention of many researchers (9,10). The antibacterial effect of many medicinal plants has been investigated by a number of researchers worldwide. There are various medicinal plants in Iran, as well as in Golestan province. One of these plants is *Matricaria chamomilla* (German chamomile), a well-known medicinal flowering plant belonging to the Asteraceae family that grows in temperate regions of Europe, Asia, America, and Africa; it has an extensive range of effects including antioxidant and antimicrobial activities (11). *Capsella bursa-pastoris*, a common weed belonging to the Brassicaceae, is indigenous to Europe, West Africa, and Asia; it has been reported to have several useful medicinal properties such as wound-healing, antioxidant agent, and antibacterial effects (12). *Malva sylvestris* is also a native medicinal plant of Europe, North Africa, and Asia and its leaves are reported to possess anti-inflammatory and antioxidant activities (13). This

study aimed to evaluate antibacterial activity of ethanolic extract of *M. chamomilla* (chamomile), *M. sylvestris*, and *C. bursa-pastoris* collected from Ziarat village of Gorgan, Iran against drug-resistant clinical isolates of *P. aeruginosa*.

Materials and Methods

The plants were collected from Ziarat village of Gorgan in May 2019 and approved in the herbarium of Islamic Azad University of Gorgan. The characteristics of plant species used in this study are shown in Table 1. The required parts of the plants were separated and completely dried in the shade. After grinding, extraction was performed by the maceration method. Then, 10 g of the plant powders was soaked in 200 mL of pure ethanol and left in the dark for 3 days. Next, the resulting solution was filtered through filter paper. The extract was concentrated using a rotary apparatus at 45°C and dried at 37°C for 24 hours. To obtain a concentration of 50 mg/mL of each extract, 500 mg of the dried plant extract was dissolved in 10 mL 5% dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.45 µm membrane filter. Different concentrations of the extract (25, 12.5, 6.25, 3.12 mg/mL) were prepared by serial dilution method (14).

The antibacterial effect of different concentrations of each extract against MDR *P. aeruginosa* isolates from our previous study (15) was determined by agar well diffusion and broth microdilution methods. In both methods, *P. aeruginosa* ATCC 27853 strain was used as a control.

Agar Well Diffusion Method

In this study, 30 µL of dilutions of 50, 25, 12.5, 6.25, 3.12 mg/mL of the prepared extract was poured in each 6-mm-deep wells punched into the Müller-Hinton agar plates previously seeded with 10⁶ CFU/mL of the test bacteria pre-cultured in nutrient broth. After 24 hours of incubation at 37°C, the diameter of the clear inhibitory zone formed around each well was measured in millimeters. Amikacin (30 µg) and DMSO were used as a positive (with inhibitory zone) and negative control (without inhibitory zone), respectively. This test was done in triplicate and the mean values was recorded (14).

Broth Microdilution Method

In this method, a microtiter plate was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). First, 100 µL of Müller-Hinton broth was poured into sterile

round bottom 96 microplates No. 1 to 9. Then, 100 µL of different dilutions of each extract were added from the highest concentration in 1 to 9 microplate wells, respectively. Thereafter, 1/100 dilutions of the pre-cultured test bacteria in nutrient broth with 10⁶ CFU/mL were prepared and added to all wells. The well series 10 containing culture medium and bacterial suspension (positive control), well series 11 containing sterile Müller-Hinton broth culture medium (no growth), and well series 12 containing culture medium and extract (no growth) were considered as negative control. After incubation for 24 hours at 37°C, growth of bacteria in 630 nm was determined using ELISA microplate reader. The MIC was considered as the minimum concentration of the extract in which no growth or a decrease in optical density (OD) was observed. To determine the MBC, the contents of the wells in which no growth was observed were cultured on Müller-Hinton agar and placed in the incubator for 24 hours at 37°C. The lowest concentration of the extract at which no bacterial growth was observed was considered as MBC (14).

Data Analysis

Data were analyzed by SPSS 16 using the Kruskal-Wallis nonparametric test. A *P* value of less than 0.05 was considered statistically significant.

Results

Based on the results of agar well diffusion method, none of the extracts had inhibitory effect against the MDR *P. aeruginosa* isolates (Table 2). As shown in Table 3, chamomile leaves extract demonstrated a MIC of 12.5 mg/mL against all the MDR *P. aeruginosa* isolates (*P*=0.38). This extract had a bactericidal effect on the isolates at a concentration of 25 mg/mL, which was considered as MBC.

Discussion

In recent years, an increase in antibiotic resistance and emergence of MDR bacteria necessitates efforts to find new antimicrobial agents. Recent studies have focused on herbs as a source of natural antimicrobial agents and mostly have reported their effectiveness against various pathogenic bacteria which cause different infectious diseases. In the present study, we investigated antibacterial activity of ethanolic extract of *M. chamomile*, *M. sylvestris*, and *C. bursa-pastoris* collected from Ziarat village of

Table 1. The Characteristics of Plant Species Used in the Study

Plant Part(s) Used	Natural Habitat in Iran	Plant Family	Botanical Name
Leaves and flowers	Different parts of Iran	Asteraceae	<i>Matricaria chamomilla</i>
Leaves	Alborz areas, around Tehran, northern Iran, Azerbaijan, Astara, Isfahan	Malvaceae	<i>Malva sylvestris</i>
Whole plant	Different parts of Iran	Brassicaceae	<i>Capsella bursa-pastoris</i>

Table 2. Results of Different Concentrations of the Plant Extracts in Agar Well Diffusion Method against *Pseudomonas aeruginosa* Isolates

Plants	Concentration of the Plant Extracts (mg/mL)				
	3.12	6.25	12.5	25	50
Chamomile (leaves)	-	-	-	-	-
Chamomile (flower)	-	-	-	-	-
<i>Malva sylvestris</i>	-	-	-	-	-
<i>Capsella bursa-pastoris</i>	-	-	-	-	-

Table 3. Minimum Inhibitory Concentrations of Test Extracts by Broth Microdilution Method on the MDR *Pseudomonas aeruginosa* Isolates

Plants	Concentration of the Plant Extracts (mg/mL)				
	3.12	6.25	12.5	25	50
Chamomile (leaves)	+	+	-	-	-
Chamomile (flower)	+	+	+	+	+
<i>Malva sylvestris</i>	+	+	+	+	+
<i>Capsella bursa-pastoris</i>	+	+	+	+	+

Gorgan against drug-resistant *P. aeruginosa* strains isolated from clinical specimens.

Based on the results, ethanolic extracts of *M. sylvestris* and *C. bursa-pastoris* were not effective against *P. aeruginosa* isolates in both methods. In similar studies conducted by Aminnezhad et al and Hassanpour et al, the mean diameter of inhibitory zone of ethanolic extract of *M. sylvestris* at a concentration of 50 mg/mL against *P. aeruginosa* ATCC 27853 was 6 mm. The MIC and MBC of the extract reported by Aminnezhad et al were both 62.5 mg/mL; meanwhile, Hassanpour et al reported these values as 25 and 50 mg/mL, respectively (5,16). In another study, the mean diameter of inhibitory zone of this extract was 8.6 mm at a concentration of 52.2 mg/mL and the MIC and MBC were 13 and 26.1 mg/mL, respectively (17). A study from Turkey showed antibacterial effect of ethanolic extract of *M. sylvestris* against *P. aeruginosa* ATCC 27853 in disk diffusion method at a concentration of 10 mg/mL (18). In Pakistan, Walter et al studied the antibacterial activity of the methanolic extract plant against some pathogens, including *P. aeruginosa* and reported the maximal diameters of inhibition zone 1.6 mm at a concentration of 15 mg/mL for this organism (19).

About antibacterial activity of *Capsella bursa-pastoris*, Birinci Yildirim et al used water, ethanol, and methanol as extraction solvents; but only a concentration of 100 mg/mL of the ethanolic extract was effective against *P. aeruginosa* ATCC 27853 (7 mm) and others did not show any inhibitory effect. Consistent with the findings of our study, Bazzaz and Haririzadeh reported no inhibitory effect for this extract on this organism (21). In a study from Baghdad, water and ethanol were used as extraction solvents and both extracts showed antibacterial activity against *P. aeruginosa* at a concentration of 3000 µg/mL,

but aqueous extract (19 mm) was more effective than ethanolic extract (13 mm) (22).

In the current study, in agreement with a study conducted by Saderi et al, no inhibitory effect was observed for ethanolic extract of chamomile against *P. aeruginosa* isolates in agar well diffusion method (23). In our study, the MIC and MBC were determined 12.5 and 25 mg/mL, respectively, but a study from Iraq reported different values (32 and 64 mg/mL) (24).

We found that the antibacterial effect of the extracts is significantly influenced by some factors, including plant habitat, plant part(s) used, solvent extraction methods, extract concentration, test organism, and antibacterial assay procedure. Therefore, further study is recommended to investigate the antibacterial activity of these extracts. Future studies may use higher concentrations and different solvents extraction methods.

Conclusions

In this study, the ethanolic extract of chamomile showed antibacterial activity against the *P. aeruginosa* isolates. Thus, it can be used in the production of antibacterial agents and it is a good option for protection against pathogenic microorganisms, as well as *P. aeruginosa*.

Conflict of Interests

No competing interest was declared by any of the authors.

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Ethical Approval

Not applicable.

Authors' Contribution

AAA; Data curation and formal analysis: AAA, LF; Investigation: FP; Methodology and project administration: AAA; Supervision: AAA; Validation: AAA; Writing of original draft: AAA; Writing, reviewing, and editing: AAA, LF.

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