ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2021;7(1):29-35 10.29252/iem.7.1.29

Antibacterial Effect of *Matricaria chamomilla* Alcoholic Extract against Drug-Resistant Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ARTICLEINFO

Article Type
Original Research

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How to cite this article
Ahani Azari A., Danesh A. Antibacterial Effect of *Matricaria chamomilla* Alcoholic Extract against Drug-Resistant Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Infection Epidemiology and Microbiology. 2021;7(1): 29-35

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Article History
Received: October 25 ,2020
Accepted: December 15 ,2020
Published: January 23 ,2021

ABSTRACT

Background: This study aimed to determine antibacterial activity of ethanolic extract of *Matricaria chamomilla* (chamomile) against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains isolated from clinical specimens.

Materials & Methods: The plant samples were collected, and the flowers and leaves were separated and dried completely in the shade. After grinding, extraction was performed using the maceration method. The extracts of both flowers and leaves were dried at 37°C for 24 hrs. About 500 mg of the dried plant extract was dissolved in 10 mL of 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.

Findings: No inhibitory effect was observed for both extracts against MDR *P. aeruginosa* isolates in agar well diffusion method. In broth microdilution method, the leaves extract showed inhibitory effect, and its MIC and MBC were determined at 12.5 and 25 mg/mL concentrations, respectively. The flowers extract showed antibacterial activity against most MRSA isolates. The extract of leaves demonstrated inhibitory effect on 7 MRSA isolates. The MIC and MBC of flowers extract were determined at concentrations of 6.25 and 12.5 mg/mL for most MRSA isolates, while MIC and MBC of leaves extract were 12.5 and 25 mg/mL for a few MRSA isolates, respectively.

Conclusion: In this study, the ethanolic extract of chamomile leaves showed antibacterial activity against MDR *P. aeruginosa* isolates; meanwhile, the flowers extract showed better activity against MRSA isolates.

Keywords: Antibacterial effect, Drug-resistance, Matricaria chamomilla, *Pseudomonas aeruginosa, Staphylococcus aureus*.

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Introduction

The emergence of drug resistant bacteria and the decline in the discovery of new antibiotics has created a global health crisis due to limited treatment options. Thus, there is a crucial need to find new and effective antibacterial agents as alternatives to antibiotics [1-2]. Herbs and plant-derived products have a long history of safe use as natural products in the treatment of various diseases [3]. The use of medicinal plants lessens antibiotic usage and thus inhibits rapid emergence and spread of resistant bacteria [4].

One such plant is *Matricaria chamomilla* (German chamomile), a well-known medicinal flowering plant belonging to the Asteraceae family, which grows in temperate regions of Europe, Asia, America, and Africa [5-6]. This plant has a branched, straight, and smooth stem, growing to a height of 15–60 cm, with long and narrow bipinnate or tripinnate leaves and flowers borne in paniculate flower heads [6]. Chamomile has an extensive range of effects, including antioxidant, antimicrobial, antitumor, anti-inflammatory, and antiviral activities [7]. The chamomile flowers contain many chemical constituents including volatile terpenoids (e.g., α-bisabolol, bisabolol oxide A and B, β-trans-farnesene and chamazulene), sesquiterpene lactones such as matricin, and phenolic compounds (flavonoids, coumarins and phenolic acids) [8]. There is a large number of published data on the antibacterial effects of this herb and its chemical constituents [6]; however, there are differences in the extent of these effects because antimicrobial activity is attributed to its chemical composition that largely depends on the type of plant variety, growth stage or collection time of plant, and many ecological factors such as plant habitat [9]. Therefore, due to the wide geographical distribution of chamomile in Iran, the study of antimicrobial properties of this plant in different regions is of great importance.

Objectives: The aim of this study was to evaluate antibacterial activity of ethanolic extract of German chamomile collected from Ziarat village, situated in southern heights of Gorgan, Golestan province, against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains isolated from clinical specimens.

Materials and Methods

Plant materials and preparation of extracts: The chamomile leaves and flowers were collected from Ziarat village located 17 km south of the city of Gorgan, Golestan province in May 2019 and approved in the herbarium of Islamic Azad University of Gorgan. Then the required parts of the plants were seperated and placed in the shade to dry. After grinding, extraction was performed using the maceration method. To do so, 10 g of plant powder was soaked in 200 mL of pure ethanol and left in the dark for 72 hrs. After that, the resulting solution was filtered through a filter paper. The extract was concentrated using a rotary evaporator at 45 °C and dried at 37 °C for 24 hrs. To obtain a concentration of 50 mg/mL of the extract, 500 mg of the dried plant extract was dissolved in 10 mL of 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 [10].

Microorganisms: The antibacterial effect of each extract at different concentrations was assessed against MRSA (n=24) and MDR *P. aeruginosa* (n=16) isolates from our previous study [11-12]. In previous study, methicillin resistance was evaluated using 30 µg cefoxitin disk (≤21 mm indicated MRSA) and 1 µg oxacillin disk (≤10 mm indicated

[DOR: 20.1001.1.25884107.2021.7.1.7.2]
[DOI: 10.52547/iem.7.1.29]

MRSA) [13]. In addition, *P. aeruginosa* isolates that were resistant to one or more antimicrobial agents in three or more antimicrobial categories were considered as MDR [14]. To determine the antibacterial effect of extracts, agar well diffusion and broth microdilution methods was used. In both methods, *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 strains were used as control.

Agar well diffusion method. About 30 µL of the prepared extract at different dilutions (50, 25, 12.5, 6.25, 3.12 mg/mL) was poured in each of the 6-mm-deep wells punched into the Müeller-Hinton agar plates previously seeded with 10^6 CFU/mL of the test bacteria pre-cultured in nutrient broth. After 24 hrs of incubation at 37°C, the diameter of the clear inhibition zone formed around each well was measured in millimeters. In this study, vancomycin antibiotic (30 µg) as well as amikacin (30 µg) as a positive control (with inhibition zone) and DMSO as a negative control (without inhibition zone) were used. This test was done in triplicate, and the mean values were recorded [10].

Broth microdilution method. In this method, a microtiter plate was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In this test, 100 µL of Müeller-Hinton broth was poured into 1 to 9 wells of a sterile round-bottom 96-well microplate. Afterwards, 100 µL of different dilutions of each extract were added from the highest to the lowest concentration into 1 to 9 microplate wells, respectively. Thereafter, 1/100 dilutions of the pre-cultured test bacteria with 10^6 CFU/mL in nutrient broth were prepared and added to 1 to 9 wells. In each series, the well No. 10 containing culture medium and bacterial suspension was considered as positive control, and the well No. 11 containing sterile Müeller-Hinton broth culture medium (no growth) and the well No. 12 containing culture medium and extract (no growth) were considered as negative control. After incubation at 37 °C for 24 hrs, bacterial growth rate at 630 nm was determined using ELISA microplate reader. The lowest extract concentration at which no growth or a decrease in OD was observed, was considered as MIC. To determine the MBC, the contents of the wells in which no growth was observed were cultured on Müeller-Hinton agar and placed in an incubator at 37 °C for 24 hrs. The lowest extract concentration at which no bacterial growth was observed, was considered as MBC [10].

Findings

The chamomile flowers extract showed antibacterial activity against 20 MRSA isolates (Table 1). The chamomile leaves extract demonstrated an inhibitory effect on 7 MRSA isolates. The MIC and MBC of chamomile flowers extract were determined at 6.25 and 12.5 mg/mL concentrations for 14 MRSA isolates, while these values were 12.5 and 25 mg/mL for 6 MRSA isolates, respectively. The MIC and MBC of chamomile leaves extract were 12.5 and 25 mg/L for 7 MRSA isolates, respectively (Table 2).

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

As a result, the chamomile leaves extract showed antibacterial activity against MDR *P. aeruginosa* isolates; meanwhile, the flowers extract showed better activity against MRSA isolates.

[DOR: 20.1001.1.25884107.2021.7.1.7.2]
[DOI: 10.52547/iem.7.1.29]

Table 1) Mean diameter of inhibitory zone (mm) in different concentrations of the test extracts in agar well diffusion method against the MRSA isolates

Microorganism	Plant part used	Concentration of the chamomile flower and leaves extracts (mg/ml)					Negative control: DMSO	Positive control: Vancomycin
		3.12	6.25	12.5	25	50		
MRSA (n=6)	Flower	-	-	-	-	12.3	-	13.3
MRSA (n=14)	Flower	-	-	-	10.3	12.7	-	13.1
MRSA (n=4)	Flower	-	-	-	-	-	-	13
MRSA (n=7)	Leaves	-	-	-	-	10.1	-	13
MRSA (n=17)	Leaves	-	-	-	-	-	-	13.3
S. aureus ATCC 29213	Flower	-	-	-	-	12.1	-	15
S. aureus ATCC 29213	Leaves	-	-	-	-	9.8	-	15

Table 2) Minimum inhibitory concentrations (mg/ml) of the test extracts on the MRSA isolates by using the microdilution method

Microorganism	Plant part used	Concentration of the flower and leaves extracts (mg/ml)				
		3.12	6.25	12.5	25	50
MRSA (n=6)	Flower	+	+	-	-	-
MRSA (n=14)	Flower	+	-	-	-	-
MRSA (n=4)	Flower	+	+	+	+	+
MRSA (n=7)	Leaves	+	+	-	-	-
MRSA (n=17)	Leaves	+	+	+	+	+
S. aureus ATCC 29213	Flower	+	+	-	-	-
S. aureus ATCC 29213	Leaves	+	+	+	+	+

[DOR: 20.1001.1.25884107.2021.7.1.7.2]
[DOI: 10.52547/iem.7.1.29]

Table 3) Minimum inhibitory concentrations (mg/ml) of the test extracts on the MDR *P. areuginosa* isolates by using microdilution method

Microorganism	Plant part used	Concentration of the plant extracts (mg/ml)				
		3.12	6.25	12.5	25	50
MDR *P. areuginosa*	Leaves	+	+	-	-	-
MDR *P. areuginosa*	Flower	+	+	+	+	+
P. aeruginosa ATCC 27853	Leaves	+	+	-	-	-
P. aeruginosa ATCC 27853	Flower	+	+	+	+	+

Discussion

In recent years, an increase in antibiotic resistance and the emergence of drug-resistant bacteria have necessitated efforts to find novel antimicrobial agents. Recent studies have focused on herbs and plant-derived products as a source of natural antimicrobial substances and mostly reported their effectiveness against various pathogenic bacteria causing different infectious diseases. In this study, antibacterial activity of the chamomile flowers and leaves ethanolic extracts was tested against MRSA and MDR *P. areuginosa* strains isolated from clinical specimens.

According to the results, the chamomile flowers extract showed antibacterial activity against most MRSA isolates, and its MIC and MBC were 6.25 and 12.5 mg/mL, respectively; however, a study in Iraq reported MIC and MBC values of 15mg/mL for *S. aureus* NCIM 2243 [15]. In a study by Dadgar et al. (2007), the mean inhibition zone diameter of the chamomile flower ethanolic extract (4 mg/mL) against MRSA and methicillin-resistant *S. aureus* (MSSA) was reported as 10.6 and 8.8 mm, respectively [16]. Carvalho et al. (2014) reported that flower ethanolic extract of chamomile had no effect against *S. aureus* isolates [17].

In the current study in agreement with the studies conducted by Saderi et al. (2007) and Owlia et al. (2010), no inhibitory effect was observed for the chamomile ethanolic extracts against *P. aeruginosa* isolates in agar well diffusion method [18-19]. In contrast, a study in Brazil reported that the growth of *P. aeruginosa* was inhibited by crude flower ethanolic extract using broth dilution (1g/mL), and inhibition zone diameter was reported as 10 mm using agar diffusion [15]. Solidônio et al. (2015) in Brazil used disk diffusion method to evaluate antibacterial activity of ethanolic extract of chamomile, but no inhibitory activity was observed against *P. aeruginosa* strains [20].

Consistent with the present study findings, in a study by Eslami et al. (2016), the MIC and MBC of chamomile leaves ethanolic extract were reported 12.5 and 25 mg/mL for all metallo-beta-lactamase-producing *P. aeruginosa* isolates, respectively; however, in another study in Iraq, different values were reported for MIC and MBC of chamomile (32 and 64 mg/mL) [21]. In Belgium, Oliveira Ribeiro et al. (2020) reported antibacterial activity of chamomile flower essential oil against *P. aeruginosa* and *S. aureus* isolates at a concentration of >1000 ug/mL [22]. In another study, methanolic extract of chamomile leaves presented a MIC of 78 µg/mL against *P. aeruginosa* isolates [23].

Considering the results of the aforementioned and the present studies, there are different

[DOR: 20.1001.1.25884107.2021.7.1.7.2]
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reports about antibacterial activity of the chamomile flowers and leaves ethanolic extracts, which could be attributed to the differences in some factors as follows: geographical location of plant collection, season of harvest, method of extraction, extract concentration, plant part (s) used, test organism, and antibacterial assay [6, 9]. Therefore, further studies are recommended to be performed in order to investigate the antibacterial activity of these herbal extracts. In future studies, the use of higher concentrations of extracts as well as different solvents and extraction methods is suggested.

Conclusion

In this study, the chamomile leaves extract showed antibacterial activity against MDR *P. aeruginosa* isolates; meanwhile, the flowers extract showed better activity against MRSA isolates.

Acknowledgements: The Department of Microbiology of the Islamic Azad University, Gorgan Branch is acknowledged for providing facilities to accomplish the present study.

Ethical Permissions: This study was approved by the Academic Committee of the Islamic Azad University, Gorgan Branch.

Conflicts of Interests: The authors declared no conflict of interests.

Authors' Contribution: Conceptualization: AAA; Data curation and formal analysis: AD; Investigation: AAA; Methodology and project administration: AAA; Supervision: AAA; Validation: AD; Writing of original draft: AAA; Writing, reviewing, and editing: AD.

Fundings: None declared by authors.

Consent to participate: A written informed consent was obtained from all patients.

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