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Evaluation of the Antibacterial Activity of Ethanolic Extract of *Matricaria chamomilla*, *Malva sylvestris*, and *Capsella bursa-pastoris* against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: The study aimed to determine the antibacterial activity of ethanolic extract of *Matricaria chamomilla* (chamomile), *Malva sylvestris*, and *Capsella bursa-pastoris* against Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens. **Methods:** The plants were collected from Ziarat Village, southern heights of Gorgan, and the required parts were separated and then thoroughly dried in the shade. After grinding, extraction was performed by the maceration method. The extract was dried at 37°C for 24 h. A concentration of 50 mg/ml of each extract was obtained in 10 ml 5% dimethyl sulfoxide and sterilized. For the antibacterial assay, agar well diffusion and broth microdilution methods were used. **Results:** Our results showed no inhibitory effect for the ethanolic extracts of *M. sylvestris* and *C. bursa-pastoris* against the MRSA isolates in both antibacterial assays. The chamomile flower extract showed antibacterial activity against the 20 MRSA isolates at 50 and 25 mg/ml concentrations. The extract from chamomile leaves demonstrated an inhibitory effect on the 7 MRSA isolates. The extracts from chamomile flowers demonstrated MIC and MBC at a concentration of 6.25 and 12.5 mg/ml for most MRSA isolates, while these values for the extracts from chamomile leaves were 12.5 and 25 mg/ml for a few MRSA isolates, respectively. **Conclusion:** In this study, the ethanolic flower extract of chamomile showed significant antibacterial activity against the MRSA isolates. Hence, this extract may be an alternative to antibiotic therapy and a good option to control infections caused by MRSA and pathogenic bacteria.

INTRODUCTION

Staphylococcus aureus is a major human pathogen that causes a broad spectrum of infections, including skin infections, food poisoning, and nosocomial infections [1, 2]. Its potential for rapid resistance to many antibiotics has led to the emergence of methicillin-resistant *S. aureus* (MRSA) [3]. In recent years, MRSA has become a public health challenge posing a significant morbidity and mortality burden; therefore, infections caused by this pathogen and related problems require particular attention [4, 5]. MRSA is a significant nosocomial pathogen contributing to 44% of all hospital-associated infections [6]. The hospitalization cost imposes a high economic burden on patients and the hospital [7].

Today, the emergence of MRSA and the declining discovery of new antibiotics have 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 [8, 9]. Recently, medicinal plants as an inexpensive natural source with lower side effects than antibiotics have received considerable attention to prevent and treat bacterial infections [10, 11]. This approach reduces antibiotic use and prevents the rapid emergence and spread of resistant bacteria and MRSA [12]. Many researchers have studied the antibacterial properties of many medicinal plants worldwide. There are numerous medicinal plants in different parts of Iran as well as in Golestan province.

One such plant 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 [13, 14]. Chamomile has an extensive range of effects, including

antioxidant, antimicrobial, antitumor, anti-inflammatory, and antiviral activity [15]. *Malva sylvestris* is also a native medicinal plant of Europe, North Africa, and Asia, which wildly grows in the Mediterranean region. Its Leaves are reported to possess anti-inflammatory, anticomplementary, antioxidant, anticancer, and potent gastric antiulcer activities [16]. *Capsella. bursa-pastoris* is a common weed belonging to the Brassicaceae and indigenous to Europe, West Africa, and Asia. It has been reported to have several useful medicinal properties such as anti-bleeding, anticancer, antithrombin, wound-healing, antioxidant, and antibacterial agents [17]. This study aimed to evaluate the antibacterial activity of ethanolic extract of *Matricaria chamomilla* (German chamomile), *Malva sylvestris,* and *Capsella bursa-pastoris* collected from Ziarat Village situated in southern heights of Gorgan City, Golestan Province, against MRSA strains isolated from clinical specimens.

MATERIALS AND METHODS

Plant materials and preparation of extracts: The plants were collected from Ziarat Village, located 17 km south of Gorgan, Golestan province, in May 2019 and were approved in the herbarium of Islamic Azad University, Gorgan. The characteristics of plant species and plant part(s) used in this study are shown in Table 1. The required parts of the plants were separated and allowed to dry completely in the shade. After grinding, extraction was performed by the maceration method. Ten grams of the plant powders were soaked in 200 ml of pure ethanol and left in the dark for 3 days. 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 h. To obtain a 50 mg/ml concentration 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 [18].

Microorganisms: The antibacterial effect of each extract in different concentrations against 24 MRSA isolates from our previous study [19] and *S. aureus* ATCC 29213 as a standard strain was determined by agar well diffusion and broth microdilution methods.

Agar well diffusion method. An amount of 30µL of dilutions 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 10^6 CFU/ml of the test bacteria pre-cultured in nutrient broth. After 24h of incubation at 37°C, the apparent inhibitory zone diameter formed around each well was measured in millimeters. Vancomycin (30µg) and DMSO were used as the positive (with an inhibitory zone) and negative controls (with no inhibitory zone), respectively. The tests were performed in triplicate, and the mean values were recorded [18].

Table 1. Characteristics of plant species used in this study

Plant part(s) used	Natural habitat in Iran	Plant family	Botanical name
Leaves and flower	Different parts of Iran	Astraceae	*Matricaria chammomila*
Leaves	Alborz areas, around Tehran, northern Iran, Azerbaijan, Astara, Isfahan	Malvaeceae	*Malva sylvestris*
Whole plant	Different parts of Iran	Brassicaceae	*Capsella bursa-pastoris*

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. After that, 1/100 dilutions of the pre-cultured test bacteria in nutrient broth with 10^6 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 the negative control. After incubation for 24 h at 37 °C, the growth of bacteria in 630nm was determined using an ELISA microplate reader (BioTek, USA). The minimum concentration of the extract with no growth and a decrease in OD was considered MIC. 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 h at 37 ° C. The lowest concentration of the extract with no bacterial growth was considered as MBC [18].

RESULTS

Based on the results, no inhibitory effect was observed for the ethanolic extracts of *M. sylvestris,* and *C. bursa-pastoris* against the MRSA isolates in both antibacterial assays. The chamomile flower extract showed antibacterial activity against the 20 MRSA isolates at concentrations of 50 and 25 mg/ml (Table 2). The extract from chamomile leaves demonstrated an inhibitory effect on the 7 MRSA isolates. The extracts from chamomile flowers demonstrated MIC and MBC at a concentration of 6.25 and 12.5 mg/ml for the 14 MRSA isolates, while these values were 12.5 and 25 mg/ml for the 6 MRSA isolates, respectively. The MIC and MBC for the extracts from chamomile leaves were 12.5, and 25 mg/ml for the 7 MRSA isolates, respectively (Table 3).

DISCUSSION

In recent years, an increase in antibiotic resistance and the emergence of MDR bacteria necessitate 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 that cause different infectious diseases.

In the present study, we investigated the antibacterial activity of ethanolic extract of *M. chamomile*, *M. sylvestris*, and *C. bursa-pastoris* against MRSA strains isolated from clinical specimens.

Based on the results, ethanolic extracts of *M. sylvestris* and *C. bursa-pastoris* were not effective against the 24 MRSA isolates in both methods. In agreement with our findings, no inhibitory effect was reported for these extracts in a similar study [20]. In similar studies, the mean diameter of the inhibitory zone of ethanolic extract of *M. sylvestris* (50 mg/ml) against *S. aureus* ATCC 25923 was 6.32 mm, and the MIC and MBC of the extract were 12.5 and 25 mg/ml, respectively [21]. In another study, Mohammad Eini et al. (2014) studied the antibacterial activity of hydro-alcoholic extracts of aerial parts of *M. sylvestris* against *S. aureus* PTCC 1112 and reported 24.75 mg/ml as a MIC value [22]. In Turkey, the antibacterial effect of ethanolic extract of *M. sylvestris* against *S. aureus* ATCC 6538p at a concentration of 10mg/ml exhibited a 12 mm inhibition zone [23]. In Pakistan, the methanolic extract plant against some pathogens, including *S. aureus*, showed a mean diameter of inhibition zone of 3.1 mm at a concentration of 15 mg/ml for this organism [24].

Table 2. Mean diameter of the inhibitory zone (mm) in different concentrations of chamomile extract in agar well diffusion method

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

Table 3. Minimum inhibitory concentrations (mg/ml) of the chamomile flower and leaves extracts by broth microdilution method on the MRSA isolates

Concentration of the flower and leaves extracts (mg/ml)						
3.12	6.25	12.5	25	50	**Plant part used**	**Organism**
+	+	-	-	-	Flower	MRSA (6)
+	-	-	-	-	Flower	MRSA (n=14)
+	+	+	+	+	Flower	MRSA (n=4)
+	+	-	-	-	Leaves	MRSA (7)
+	+	+	+	+	Leaves	MRSA (17)
+	+	-	-	-	Flower	Control
+	+	+	+	+	Leaves	Control

In a study, Birinci Yildirim et al. used water, ethanol, and methanol as extraction solvents to prepare extracts from C. *bursa-pastoris*, but none of the extracts showed an inhibitory effect against *S. aureus* ATCC 27853 [25]. In Iraq, water and ethanol were also used as extraction solvents, and both resulting extracts showed no antibacterial activity against S. *aureus* [26].

In a study by Dadgar et al., the mean diameter of the inhibitory zone of ethanolic extract of chamomile (4 mg/ml) against MRSA and Methicillin-resistant *S. aureus* (MSSA) was 10.6 and 8.8 mm, respectively [27]. In our study, the MIC and MBC were determined as 6.25 and 12.5 mg/ml for the most MRSA isolates, respectively, but a study from Iraq reported different values against *S. aureus* NCIM 2243 (both 15mg/ml) [28].

According to the findings of this study and other studies, there are different reports about the antibacterial activity of the plant extracts used in the present study. Some factors may be involved in these differences, such as the geographical location of plant collection sites, season variations, extraction methods, extract concentration, test organism, and antibacterial assay.

Therefore, further study is recommended to investigate the antibacterial activity of these herbal extracts. In future studies on higher concentrations of the extracts and different solvents and extraction methods are suggested.

In this study, the ethanolic flower extract of chamomile showed significant antibacterial activity against most MRSA isolates. It may be a possible alternative for

antibiotic therapy and a suitable option to control infections caused by MRSA and other pathogenic bacteria.

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CONFLICT OF INTEREST

None of the authors declared any competing interest associated with this article.

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