Antibacterial In-Vitro Activities of Selected Medicinal Plants against Methicillin Resistant Staphylococcus Aureus from Libyan Environment

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Abstract

Medicinal plants are valuable natural sources effective against various infectious agents. Extracts from Libyan traditional medicinal plants were investigated for antibacterial activity. In this study, the potential antibacterial activity of extracts from eight Libyan traditional medicinal plants against methicillin-resistant Staphylococcus aureus (MRSA) was investigated in-vitro. Susceptibility assays using disc diffusion and broth microdilution test for the determination of minimum inhibitory concentration (MIC) were used to assess the antibacterial activity of methanolic extracts from medicinal plants. Extracts from all eight plants showed anti-MRSA activity with MIC values ranged between 25-50 mg/ml. Cistus salvifolius, Salvia officinalis, Pistacia atantica, Arbutus pavarii, and Myrtus communis exhibited the most potent anti-MRSA activity, whereas extracts from Teucrium polium, Thymus capitellatus, and Euphorbia dendroides showed weak anti-MRSA activity. Medicinal plants may serve as useful bactericidal agents and warrant further investigation to better evaluate their particular therapeutic potentials and optimize their application.

Keywords: Medicinal plants; in-vitro antibacterial activity; Methicillin-resistant Staphylococcus aureus (MRSA); Libya

Introduction

Medicinal plants can be valuable therapeutic resources [1-3]. The treatment of infections with plant-derived compounds is an age-old practice that is employed throughout the world, especially in developing countries where traditional medicines are used to treat a variety of diseases [1]. Interest in plants with antimicrobial properties has been revived as a result of current resistance profiles associated with over- and inappropriate use of antibiotics [3,4].

Staphylococcus aureus is a gram-positive bacterium responsible for morbidity and mortality [5]. It is one of the leading causes of human infections of the skin, soft tissues, bones and joints, abscesses, and normal heart valves. Staphylococcus aureus flourishes in the hospital setting and is associated with bloodstream and surgical wound infections [6] and has become important nosocomial organism. Methicillin Resistant Staphylococcus aureus' (MRSA) first recognized in the 60s [7] is a multiresistant strain that has been documented worldwide showing risen resistance to different classes of antimicrobials [8].

Over the past decades, MRSA has spread throughout the world and has become highly endemic in many geographic areas [9]. MRSA infections are difficult to treat because of their resistance to many of the commonly used antibiotics (e.g., macrolides, tetracyclines, aminoglycosides). Some of these MRSA strains are resistant to even the most powerful antibiotics, including vancomycin [8,10]. The WHO has acknowledged the need to identify new antibiotics and/or new approaches to overcome the growing problems associated with such infectious agents.

In Libya, MRSA and other epidemic diseases have emerged as a serious emerging problem [11,12]. Limited studies in Libya have assessed the antibacterial activity of medicinal plants against different types of bacteria [13]. In the current study, only eight Libyan traditional medicinal plants commonly used for the treatment of several clinical conditions were tested for in-vitro antimicrobial activity against the pathogenic virulent bacteria methicillin resistant Staphylococcus aureus (MRSA).

Materials and Methods

Plants

Eight Libyan traditional medicinal plants were collected in the period of spring 2010 from north-east region (Al Jabal al Akhdar" Green Mountains") of Libya (Table 1). Fresh plants material (Table1) were collected and processed within hours after collection. The collected plants were identified and classified according to the herbarium at the biotechnology research centre Tripoli, Libya.

Preparation of extracts

Plants parts (Table1) were first cleaned, shade dried and grounded to a fine powder and extraction process was next carried out using a conventional solvent extraction procedure. The extraction method was adapted from Hassawi and Karma [14] and in accordance with Motamedi et al. [3] as following: 10grams of each plant materials were extracted with 100 ml of alcohol (i.e. methanol with concentration of alcohol:water = 8:2, v/v), filtered through Whatman No. 1 filter paper, and the residual material was re-extracted with methanol. The two resulting filtrates were combined and dried using a rotary evaporator.

6-Bacterial strain

The MRSA strain used in the current study was in-house confirmed clinical isolate from Libyan hospital environment provided by MO

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Ahmed from the department of Microbiology and parasitology, faculty of veterinary medicine at University of Tripoli, Libya.

Antimicrobial Susceptibility Assays

Disk diffusion assay

The anti-MRSA activity of the plant extracts was initially determined using the disc diffusion assay [15]. Each dried plant extract was dissolved and prepared as described by Gangoué-Piéboji [16]. MRSA were over night cultured (24 h) at 37°C on nutrient agar for the preparation of cell suspensions. MRSA cell suspensions adjusted to 0.5 McFarland standards (10^7 CFU/ml) were prepared and 0.1 ml of the suspension was poured on Muller Hinton agar (MHA) (Oxoid, UK) and dispersed using a cotton swab to ensure robust cell growth. Plates were then allowed to dry for 5 min prior incubation.

The sterile filter paper discs (Whatman No. 3, diameter=6 mm) were soaked in 30 μl of each plant extract for 30 min. The extract-soaked filter paper discs were then placed on the inoculated MHA plates, allowed prior incubation to stand for 30 min at room temperature to permit proper diffusion of the extract. All plates were incubated at 37°C for 24 h, and the resulting inhibition zones were measured in mm for each of the medicinal plant extracts. This experiment was done in triplicate and the antimicrobial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts. As positive controls, discs (oxoid) containing oxacillin 1 μg were used.

Minimum inhibitory concentration (MIC)

The determination of the lowest concentration of the extracts capable of inhibiting the growth of (MRSA) (i.e. minimum inhibitory concentration (MIC)) for each of the eight medicinal plant extracts was performed using broth microdilution test [17] as following: A stock solution of 200 mg/ml was prepared for each extract by dissolving 200 mg of each extract in 1 ml dimethyl sulfoxide solvent (DMSO: Water, 2:4 v/v). Two hundred microliters of each of the eight extract stock solutions were pipetted into the first row of a 96-well plate, and 100 μl of nutrient broth (Oxoid) were pipetted into each of the remaining wells. Serial dilutions of the stocks were performed such that each of the following wells contained 100 μl of the stock solution in serially descending concentrations (1:2) until a final concentration of 6.25 mg/ml was achieved. One hundred microliters of MRSA culture (10^7 cells/ml) were added to each well. All of the plates were incubated at 37°C for 24 h. The least concentration of the extract showing no visible growth was initially taken as the MIC. The MIC was established by determining the lowest extract concentration corresponding to a lack of turbidity in the wells, and further confirmed by culturing at 37°C for 24 h on nutrient agar. MIC values were evaluated based on positive interpretation for both no visible turbidity and negative culturing.

Results

The antimicrobial activities for each of the eight extracts according to a zone of inhibition ranged from 6-18 mm (Table 2). Only five out of the eight plants (Cistus salvifolius, Salvia officinalis, Arbutus pavarii, Pistacia atantica, and Myrtus communis) exhibited broad spectrum activity against MRSA with corresponding inhibition zones of 18 mm, 14 mm, 9 mm, 11 mm, and 9 mm respectively. The remaining crude methanolic extracts from Teucrium polium Thymus capitellatus, and Euphorbia dendroides showed selective activity (inhibition zone =6mm) against MRSA (Table 2).

The MIC values obtained using methanolic extracts derived from the eight plants exhibited anti-MRSA activity ranged between 25-50 mg/ml (Table 2). MIC values for the Cistus salvifolius extract was the most active against MRSA with an MIC value of 25 mg/ml. In contrast, Salvia officinalis, Arbutus pavarii, Pistacia atantica, and Myrtus communis exhibited an MIC value of 50 mg/ml. Teucrium polium Thymus capitellatus, and Euphorbia dendroides had no effect on MRSA.

Discussion

Medicinal plants are commonly available resources, have less if no side effects, economic and have antimicrobial properties [14]. The majority of these medicinal plants used in this study are applied in traditional medicine in many Libyan regions to cure different disorders. Medicinal plants are considered one of the most valuable resources for antibiotic development. Pathogenic strains of antibiotic-resistant bacteria have emerged due to the misuse of antibiotics. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics capable of treating such antibiotic-resistant bacterial strains. In recent years, publications from several countries have reported the use of active compounds extracted from medicinal plants, which may benefit antibiotic development [4,18]. Many studies have extracted active compounds from medicinal plants that exhibit a synergistic effect against some bacterial species [4,18].

Table 1: Plants names and plant parts used in the anti-MRSA activity assays.

<table>
<thead>
<tr>
<th>Plant Botanical Name</th>
<th>Family</th>
<th>Local name</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cistus salvifolius</td>
<td>Cistaceae</td>
<td>Parshib</td>
<td>Leaves</td>
</tr>
<tr>
<td>Salvia officinalis</td>
<td>Labiatae</td>
<td>Tea apple</td>
<td>Leaves</td>
</tr>
<tr>
<td>Arbutus pavarii</td>
<td>Ericaceae</td>
<td>Shmary</td>
<td>Leaves</td>
</tr>
<tr>
<td>Pistacia atantica</td>
<td>Anacardiaceae</td>
<td>Bathom</td>
<td>Leaves</td>
</tr>
<tr>
<td>Myrtus communis</td>
<td>Myrtaceae</td>
<td>Marseen</td>
<td>Leaves</td>
</tr>
<tr>
<td>Teucrium polium</td>
<td>Labiatae</td>
<td>Jeheda</td>
<td>Leaves and flowers</td>
</tr>
<tr>
<td>Thymus capitellatus</td>
<td>Labiatae</td>
<td>Zahter</td>
<td>Leaves and flowers</td>
</tr>
<tr>
<td>Euphorbia dendroides</td>
<td>Euphorbiacea</td>
<td>Halabilib</td>
<td>Leaves and flowers</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant</th>
<th>Disk diffusion(mm)</th>
<th>Minimum inhibitory concentration (MIC) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>37.5</td>
</tr>
<tr>
<td>Cistus salvifolius</td>
<td>18</td>
<td>No growth</td>
</tr>
<tr>
<td>Salvia officinalis</td>
<td>14</td>
<td>No growth</td>
</tr>
<tr>
<td>Arbutus pavarii</td>
<td>9</td>
<td>No growth</td>
</tr>
<tr>
<td>Pistacia atantica</td>
<td>11</td>
<td>No growth</td>
</tr>
<tr>
<td>Myrtus communis</td>
<td>9</td>
<td>No growth</td>
</tr>
<tr>
<td>Teucrium polium</td>
<td>6</td>
<td>Growth</td>
</tr>
<tr>
<td>Thymus capitellatus</td>
<td>6</td>
<td>Growth</td>
</tr>
<tr>
<td>Euphorbia dendroides</td>
<td>6</td>
<td>Growth</td>
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Table 2: Results of disk diffusion and minimum inhibitory concentration (MIC) assays to determine the anti-MRSA activity of methanolic extracts from eight Libyan traditional medicine plants.
MRSA is one of the leading causes of skin, soft tissue, bone, joint, abscess, and normal heart valve infections. Herein, we examined the anti-MRSA activity of extracts from eight different Libyan traditional medicinal plants. Taken together, the results of disk diffusion and MIC assays show that five out of the eight plant extracts (i.e., Cistus salvifolius, Salvia officinalis, Arbutus pavarri, Pistacia atlantica, and Myrtus communis) exhibited bactericidal activity against MRSA. Previously observation indicated that the relationship between inhibition zone diameters and the MIC values was not correlated [18] however this was not the case in our study. It is generally held that for evaluating bactericidal activity of medicinal plants, both MIC and MBC values are often near or equivalent [19]. Previously it has been acknowledged that in the determination of the antibacterial activity of plant extracts, methanolic extract had a better efficacy than its ethanol extract [3].

The results obtained from this study provide evidence that methanolic extracts of the Libyan traditional medicinal plants Cistus salvifolius, Salvia officinalis, Arbutus pavarri, Pistacia atlantica, and Myrtus communis exhibit useful bactericidal activities against locally isolated MRSA strain, suggesting that they may be clinically useful. Further search in respect of these findings are needed and promising.

However, while in vitro results are encouraging and merit further study, the in-vivo efficacy remains to be confirmed. In general, the results revealed significant antibacterial activity of the studied medicinal plants, which could be a potential source of new antibacterial agents. To our knowledge, there was no previous report on the antimicrobial activities on selected resistant bacteria in Libya.

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References