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Original Article

Antimicrobial Impact of Hibiscus Sabdariffal Extract Against Acinetobacterbaumannii

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1.INTRODUCTION

Herbal medicines are the major remedy in traditional medical systems from thousands of years and made a great contribution in maintaining human health and in preventing many infectious diseases(1). The recent reports of the medical community has confirmed that infection with Gram-negative bacteria is increasing because of the resistance of bacteria to antibiotics (2-5). Hibiscus sabdariffa L. (family Malvaceae) is an annual, tropical or subtropical shrub that is native to an area from Malaysia to India. Hibiscus is grown in many countries including Sudan, Mexico, India, and Thailand. While many species of Hibiscus are used as ornamentals, the red calyces of H.sabdariffa are used in the preparation of a flavorful and tartcold or hot beverage(6, 7). H. sabdariffa is a common herbal drink consumed both hot and cold by people around the world and used in traditional medicine in the treatment of hypertension. The infusion is made from the calyces of the H. sabdariffa (8). Relatively few studies have been carried out to evaluate the antimicrobial activities of this plant against common human pathogens (9, 10). Extracts of the plant significantly enhanced the inhibitory effects of antibacterial agents against different bacterial species (11). The infection caused by Acinetobacteris

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difficult to control due to multidrug resistance, which limits therapeutic options in critically ill and debilitated patients, specifically those in intensive care units(12). Hospital outbreaks have been described from various geographic areas and this organism has become endemic in some of them. The role of the environmental contamination in the transmission of nosocomial infections in general and in *A. baumannii* infections in particular is well recognized (13, 14, 15).

### 1.1. Objective

According to the medicinal properties of Hibiscus sabdariffa, there was no comprehensive study in Southeastern Iran; thus, we aimed to study the antibacterial effect of this plant.

### 2. MATERIAL AND METHODS

The different environmental samples were processed for the isolation of bacteria by methods described elsewhere (16). 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 (17).

#### 2.1. Agar Disk Diffusion Assay

Resistance to tetracycline was tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocols. Antibiotic disks were obtained from the Patan ted-Iran. *Acinetobacter baumannii* isolates were evaluated based on the size of the zones of inhibition and classified as susceptible (S), intermediate resistant (I) or resistant (R) according to the CLSI criteria.

#### 2.2. Plant materials

The flowers of *Hibiscus sabdariffa* were collected in the region of Iran. The plant materials were dring at 25 °C. Samples were powdered and transferred into glass containers and preserved until the next experiment.

#### 2.3. Preparation of extracts:

For each test, 50 grams of samples soaked for 48 hours in 96% ethanol and stored. The extract obtained with smooth filter paper was condensed using rotary. The weight of a test tube determined and one ml of extract was transferred into it. Tube containing extract was dried at room temperature. The weight difference tube was equivalent one ml of the extract. Average three times, was calculated as weight dried extract. Then dissolved in the solvent DMSO and was maintained at 4 °C until used.

#### 2.4. Antimicrobial testing of extraction:

Susceptibility of bacterial isolates with multiple resistance to the plant extract of Achillea, green tea and Ajowan was investigated using the dilution plate. To seven micro-titter plates was added amount of the 100 ml nutrient broth Mueller Hinton Broth (MHB). The first well was added 100 ml of the diluted solution of the extract, after mixing, 100 ml of the first plate is removed and added to the second plate, this work was done in this way until the last plate. Remove the end plate 100 ml of culture medium containing 10^7 units per ml, 100 ml of bacterial suspension equivalent to 0. 5 McFarland added to all wells and incubated at 37 °C for 24 h. The first well that prevent the growth of bacteria is considered as MIC and to ensure 10 ml of the clear plate was transferred to the Mueller Hinton agar medium. After 24 hours the first dilution that could kill 99.9% of bacteria as shown Minimum lethal concentration.

Agar Well Diffusion Assay for Extracts. Antibacterial activity of the plant crude extracts was tested using agar well diffusion method. The test inclusions (0.5 McFarland turbidity) were spread into Muller-Hinton agar using a sterile cotton swab. The wells were made by sterile well puncture and 20 μL of the extracts were added to each well and incubated at 37°C for 24 hours. The presence of inhibition zone was regarded as the presence of antimicrobial action. The average diameter of the inhibition zone was measured in millimeter.

#### 2.4. Statistical Analysis

For all samples, experiments have been repeated three times. Data were imported in Excel then analyses using SPSS. All data were studied with mean representational for bacterial strains and completely random design statistics for fungi samples. A value of *P*<0.05 was regarded as statistically significant.

### 3. RESULT

Antibiotic susceptibility *Acinetobacter baumannii* was evaluated for 4 antimicrobial. However, overall *Acinetobacter baumannii* were resistance to 4 of the agent including nalidixic acid(100%), penicillin(100%), tetracyclin(58.3%), amikacin(83.3%). As was shown in table 1, the most frequent resistance was observed for nalidixic acid and penicillin.

<table>
<thead>
<tr>
<th>NA</th>
<th>P</th>
<th>AN</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
</tr>
<tr>
<td>R</td>
<td>100</td>
<td>100</td>
<td>83.3</td>
</tr>
</tbody>
</table>

NA=nalidixic acid, P=penicillin, TE=tetracyclin, AN=amikacin

In this study, the minimum inhibitory concentration (MIC) was used. The levels of MIC were in ranges from 6.25 to 25 ppm. The highest MIC value was observedat
6.25 ppm against one. *A. baumannii*(Table 2). The highest MBC for *Hibiscus sabdariffa* leave was 50 ppm, while the lowest MBC was 12.5 ppm. The maximum diameter of inhibition zone (mm 14) for *Hibiscus sabdariffa* leaves was observed against *A. Baumannii*.

**Table 2:** Minimum inhibitory concentration of *Hibiscus sabdariffa* extract against *A. Baumannii*

<table>
<thead>
<tr>
<th>Bacterial cod</th>
<th>MIC and MBC(PPM)</th>
<th>Bacterial cod</th>
<th>MIC and MBC(PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.5/25</td>
<td>7</td>
<td>6.25 / 12.5</td>
</tr>
<tr>
<td>2</td>
<td>25/50</td>
<td>8</td>
<td>12.5/25</td>
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<tr>
<td>3</td>
<td>25/50</td>
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<tr>
<td>5</td>
<td>12.5/12.5</td>
<td>11</td>
<td>12.5/25</td>
</tr>
<tr>
<td>6</td>
<td>12.5/12.5</td>
<td>12</td>
<td>12.5/12.5</td>
</tr>
</tbody>
</table>

**Table 3:** Antimicrobial Activity of the Plant Crude Extracts as. Mean of Inhibition Diameter Zone Against *A. Baumannii*

<table>
<thead>
<tr>
<th>Bacterial cod</th>
<th>Diameter Zone for Leaf Extract, mm</th>
<th>Bacterial cod</th>
<th>Diameter Zone for Leaf Extract, mm</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>7</td>
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</tr>
<tr>
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<td>5</td>
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</table>

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**Authors’ Contributions**

All the authors had equal roles in design and writing of the manuscript.

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