Inhibitory Action of Aqueous Extract of Anethum graveolens on Staphylococcus aureus

Karzan M. Khalid

Abstract—Ten isolates of bacteria were collected from Rezgari hospital in Erbil city (KRI). The identity of isolates were emphasized as they demonstrated culturally, microscopically and some biochemical properties. Among these isolates, only two isolates (S3 and S7) characterized as Staphylococcus aureus. The susceptibility of S3 and S7 isolates against five different antibiotics (Amp, Amc, Amx, Cip, Tet) was screened, and isolate (S3) was resistant to (Amp and Amc), while isolate (S7) was resistant to (Amc). Furthermore, the antibacterial effect of aqueous extract of Anethum graveolens on (S3) was screened, the minimum and maximum inhibition zone at 9000µg/100µg and 24000µg/100µg was 24mm and 32mm in diameter, respectively.


Keywords: Antibacterial activity, Antibiotic resistant, Anethum graveolens, Staphylococcus aureus.

1. INTRODUCTION

In the last few decades, many bacterial species have developed resistance to antimicrobial agents that have been commonly used to treat them (1). Staphylococcus aureus is one of the pathogens known to rapidly develop resistance to antimicrobial agents as new antibiotics are introduced (2). S. aureus is a gram-positive, spherical bacterium approximately 1µm in diameter. Its cells form grape-like clusters, since cell division takes place in more than one plane (3). S. aureus colonized mainly the nasal passage, skin, oral cavity and gastrointestinal tract. It is often appears golden yellow on media and hemolytic on blood agar, facultative anaerobic, catalase positive and oxidase negative. It also can grow at 15-45°C and NaCl concentration as high as 15 percent (4). S. aureus known as clumping factor, which reacts with fibrinogen to cause organisms to aggregate, and extracellular staphylococcus reacts with prothrombin to form staphylotrombin (5).

Control of antibiotic resistant bacteria and the treatment of infections caused by them is a major problem worldwide (6 and 7). Elderly, medicinal plants have been used as treatment of various human ailments. A revolution came in the medicinal world with the discovery of antibiotics for treatment of bacterial infections. Various plants were documented as enrolled in development of novel drugs by monitoring and evaluating their antimicrobial activity. It is believed that, crude extract from plants have higher efficiency than isolated components due to synergistic effect (8).

Dill (Anethum graveolens) also known as shapt or dill-weed and Sowa, the name came from Greek word aneeson or aneeton which means strong smelling. It is an annual herb with the height about 1.5 meter (9 and 10). It is indigenous to southern Europe. It is an annual herb growing in the Mediterranean region, central and southern Asia. Now dill is cultivated around the world (11,12 and 13). Traditionally, dill used as an aromatic herb and spice in many countries such as India. Moreover, it is used as an anti-convulsion, anti-emetic, anti-cramp especially in children, as a wound healer and to increase the appetite and strengthen the stomach (14 and 15). Biological activity of A. graveolens oil displays an important role in anti-microbe, anti-bacteria and anti-fungi (16 and 17).
2. MATERIAL AND METHODS

2.1 Plant extraction

2.1.1 Bacteria under study
Ten isolates were collected from Rezgari Hospital in Erbil city. The isolates were microscopically, morphologically demonstrated, and they were characterized by performing some biochemical tests.

2.1.2 Collection of plant
*Anethum graveolens* was obtained from Market, and the leaves were washed with tap water. Furthermore, it was washed with distilled water and left for air drying until completely dried. Then, the plant was converted to powder form and kept in polyethylene sacks in refrigerator at 4°C for further use.

2.1.3 Preparation of aqueous extract
The aqueous extract of *A. graveolens* was prepared according to (18), with slight modification. Sixty gram of ground dried plant was added to 600ml of sterilized distilled water, and heated until boiling point with stirring for three hours. Then, the extract was filtered by both muslin cloth and filter paper (Whatman NO.1), the product evaporated to dryness and stored in refrigerator at 4°C.

2.2 Preparation of inoculum
Two to three colonies from pure culture of isolated bacteria were transferred into test tube which contain 5ml nutrient broth, and incubated overnight at 37°C.

2.3 Stock solution preparation of plant extract
2.4gm of aqueous extract of *A. graveolens* was dissolved in 10ml sterilized distilled water.

2.4 Susceptibility to antimicrobial agents
The two isolates (S3 and S7) was subjected to antibiotic susceptibility test, using cup plate agar diffusion, consisted of nutrient agar enriched with Amp, Amc, Amx, Cip and Tet. Then, they were poured to plate and solidified. After that, S3 and S7 were inoculated and streaked on prepared medium(19).

2.5 Antibacterial activity test of plant extract
Susceptibility of identified *S. aureus* (S3) to aqueous extract of *A. graveolens* was measured by the cup plate agar diffusion method on sterilized nutrient agar. After solidification, 0.1ml of bacterial suspension was inoculated and left for about 30 minutes. Cups of 8mm diameter were made in the medium using sterile cork borer. Nine different concentrations of aqueous extract were added into cups, after 24hours incubation at 37°C, the diameter per inhibition zone were measured (20).

3. RESULTS AND DISCUSSION
Ten isolates were collected from Rezgari hospital in Erbil city. Morphological and microscopical characteristics of isolates were demonstrated. Morphologically, among ten collected isolates only two isolates (S3 and S7) were gave features of *S. aureus* as colonies appeared circular, large, golden yellow in color on manitol salt agar, while they had β-hemolytic property on blood agar (21). The two isolates were appeared purple and cocci shape under microscope.

Fig. 1. *S. aureus*, cluster in arrangement, purple color and cocci shape.

Furthermore, several biochemical tests were performed and isolate S3 and S7 were identified as *S. aureus*, due to catalase positive, oxidase positive, gelatinase negative and non-motile (22) as shown in the following table.
TABLE 1: RESULTS OF SOME BIOCHEMICAL, MORPHOLOGICAL AND CULTURAL INDICATORS.

<table>
<thead>
<tr>
<th>S. aureus isolates</th>
<th>Manitol Salt Agar</th>
<th>Blood agar</th>
<th>Gelatinase</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Motility</th>
<th>Gram Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No change</td>
<td>α-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No change</td>
<td>β-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>S aureus</td>
<td>β-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Purple, 2002</td>
</tr>
<tr>
<td>4</td>
<td>No change</td>
<td>Y-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>No change</td>
<td>Y-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>No change</td>
<td>Y-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S aureus</td>
<td>β-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Purple, cocci</td>
</tr>
<tr>
<td>8</td>
<td>No change</td>
<td>β-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>No change</td>
<td>Y-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>No change</td>
<td>Y-hemolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

The table shows that only isolate S3 and S7 changed color of MSA medium from pink to golden yellow as indicator to differentiate S. aureus from other species. They were also β-hemolysis, (gelatinase and oxidase) negative and catalase positive. They were non-motile and purple cocci shape microscopically.

The susceptibility of isolates (S3 and S7) to five different common antibiotics was tested, it showed that S3 was resistant to (Amp and Amc) and sensitive to (Amx, Cip and Tet). While S7 was resistant to Amc and sensitive to remained antibiotics (table 2).

TABLE 2: RESISTANCY OF S. AUREUS (ISOLATE S3 AND S7).

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration µg/ml</th>
<th>S. aureus isolate (S3)</th>
<th>S. aureus isolate (S7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp</td>
<td>30</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Amc</td>
<td>30</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Amx</td>
<td>30</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cip</td>
<td>30</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tet</td>
<td>15</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

R: Resistant  S: Sensitive

According to the previous studies, the main reason of S. aureus resistance to antibiotics is the presence of β-lactamase enzyme (23), which fights antibiotics by deactivating β-lactam binding proteins (24).

Antibacterial activity of aqueous extract of Anethum graveolens against (S3) of S. aureus was performed as shown in (table 3), and it was demonstrated that plant extract had ability to inhibit growth of this bacterium at different concentrations. In first five concentrations (375µg/100µl, 750µg/100µl, 1500µg/100µl, 3000µg/100µl and 6000µg/100µl), no effect was appeared. Moreover, with the use of higher concentrations, they were suppressed growth of S3. The resistancy of S. aureus (S3) was decreased by providing higher concentration of aqueous extract of A. graveolens.

TABLE 3: EFFECT OF AQUEOUS EXTRACT OF A. GRAVEOLENS ON S. AUREUS.

<table>
<thead>
<tr>
<th>Concentration of A. graveolens extract µg/100µl</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>375</td>
<td>-</td>
</tr>
<tr>
<td>750</td>
<td>-</td>
</tr>
<tr>
<td>1500</td>
<td>-</td>
</tr>
<tr>
<td>3000</td>
<td>-</td>
</tr>
<tr>
<td>6000</td>
<td>-</td>
</tr>
<tr>
<td>9000</td>
<td>24</td>
</tr>
<tr>
<td>12000</td>
<td>27</td>
</tr>
<tr>
<td>18000</td>
<td>30</td>
</tr>
<tr>
<td>24000</td>
<td>32</td>
</tr>
</tbody>
</table>

-= no effect

Furthermore, the minimum inhibitory zone was 24mm in diameter at 9000µg/100µl, while the highest effect was recorded at 24000µg/100µl and produced inhibition zone was 32mm.

Fig. 2 Antibacterial activity of A. graveolens extract on S. aureus (S3) at different concentrations.
Activity of Anethum aqueous extract on S. aureus may returns to the presence of chemical compositions such as essential oil, terpenoid, oxypencedanin, and falcarindiol (25,26 and 27). In this study, it was concluded that Anethum graveolens can be used as an antibacterial agent against S. aureus, due to possess of antimicrobial property in it is structure.

4. Conclusion
Isolate S3 and S7 were identified as S. aureus and antibiotic susceptibility test was performed. As a result, S3 was resist to (Amp and Amc) and sensitive to (Amx, Cip and Tet). While isolate 7 was resist to Amc and sensitive to other antibiotics. Furthermore, the effect of aqueous extract of A. graveolens S. aureus (S3) was appeared at 9000µg/100µl, 12000µg/100µl, 18000µg/100µl and 24000µg/100µl, which was 24mm, 27mm, 30mm and 32mm in diameter, respectively. In conclusion, despite of using dill (A. graveolens) as a tasty diet, it is beneficial to inhibit infectious microorganisms such as S. aureus.

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References


