Phytochemical Screening and Antibacterial Activity of Methanol Extracts of *Suaeda aegyptiaca* Leaf on *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*

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**Abstract**

The screening of plant extracts for natural products and antimicrobial activity has revealed the potential of higher plants as a source of new anti-infective agents. In the current study, the antibacterial activity of methanol extract of *Suaeda aegyptiaca* leaf was tested against four bacteria species: two Gram-positive bacteria (*Staphylococcus aureus* and *S. epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The Well diffusion method was used to assess the antibacterial activity of the plant extract. The results showed that the methanolic extract of *Suaeda aegyptiaca* leaves was more effective against Gram-positive bacteria, with an inhibition rate of 18.28 mm, as measured by the spectrum of antimicrobial activity against *S. epidermidis* bacteria, where the zone of inhibition ranged between (24.7 - 12.7) mm and (20 - 13.3) mm against *S. aureus*, when compared to Gram-negative bacteria, which had a mean of inhibition rate of 14.28 mm, which were indicated by zones of inhibition ranging between (18.7 and 11.3 mm) against *E. coli* and (16.3-9.3 mm) against *P. aeruginosa* at concentrations Extract(10- 2.5 % ) as the mean of inhibition increases with increasing concentration of the extract. This suggests that this plant extract could be used to treat a variety of diseases caused by these pathogens. The presence of alkaloids, flavonoids, saponins, sterols/terpenes, and tannins in the leaf was validated by phytochemical screening, confirming the potential good source of antibacterial agents with the highest sensitivity observed. This indicates that this plant extract could be used to treat a variety of diseases.

**Keywords:** Antibacterial activity, *Suaeda aegyptiaca*, Medicinal plants, Antibiotics, Plant extract
1. Introduction

Medicinal plants are thought to be a rich source of medicinal compounds that have been shown to treat infectious diseases. Traditional remedies are well documented and widely used in developing nations, where medicinal plants have been used as a replacement for antibiotics, which have side effects as well as the emergence of resistance to numerous therapies (1-5). Because most plants include various active chemicals such as flavonoids, tannins, and alkaloids, they provide a plentiful supply of antimicrobial agents against infectious diseases such as bacteria, fungi, viruses, insects, anti-inflammatory activity and immunomodulatory effect (6-12). *Suaeda aegyptiaca*, a member of the Chenopodiaceae family, is found throughout Europe, the Canary Islands, and the Mediterranean region, Australia, Asia, Argentina and North America. It’s found throughout Arabia in saline settings, particularly along the beaches, and grows in a variety of plant groups and fields (13). Because the plant appears deeper or blackish when dried, Suaeda takes its name from the Arabic word suwaid (14). The herb *S. aegyptiaca* has been used in folk medicine to treat stomach ache, wounds, and skin infections (15). Considering the significance of therapeutic herbs, the goal of the study was to investigate the antibacterial activity of a methanol extract of *Suaeda aegyptiaca* leaf in vitro by evaluating its effects on bacterial biological activity inhibition as well as screening phytochemical ingredients in it with the goal of finding new compounds as well as alternate treatments for diseases caused by harmful microorganisms.

2. Materials and Methods

*Suaeda aegyptiaca* leaves were collected in Thi-Qar from a variety of sources. The plants were powdered and stored in a sterile container until they were needed.

2.1. Preparation of crude extracts

A powdered sample of 20 g was extracted in a Soxhlet device with 200 mL of methanol at room temperature. Until the usage, the samples were maintained at 40°C. A 20 mg/mL stock solution was made. One day ahead of time, stock solutions were prepared. For initial testing and retests,
several aliquots of each sample were stored (16, 17). Stock solutions were sterilized after being filtered. On the day of the assay, thaw an aliquot of frozen stock solution in the refrigerator. On the day of the assay, thaw an aliquot of frozen stock solution in the refrigerator. By serial dilution, a concentration of 100 g/ml of extract was obtained. The crude extracts were then diluted to reach concentrations of (2.5, 5, and 10%).

2.2. Screening of phytochemical constituents

*Suaeda aegyptiaca* phytochemical components were evaluated qualitatively utilizing various standardized test methodologies (18, 19) as shown in table 1.

Table 1. Phytochemical screening methods

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Methodology used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>A few drops of dilute HCL and 0.5 mL Wagner's reagent have been added. A brown flocculent precipitate indicates the presence of alkaloid (18).</td>
</tr>
<tr>
<td>Anthraquinons</td>
<td>The plant extracts were given a few drops of 2% HCl. The generation of red precipitate revealed the presence of anthraquinones in the samples (19), while another test procedure (20) using a 10% ammonia solution revealed the presence of anthraquinones in the samples. The presence of anthraquinones was shown by the production of a red tint.</td>
</tr>
<tr>
<td>Coumarins</td>
<td>To one ml of plant material, one ml of a 10% NAOH solution was added. The presence of coumarins in the examined samples was confirmed by the production of a yellow color (19).</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1 g powder + 10 ml ethyl acetate, heated for 5 minutes over a steam bath (40–50°C), filtrate + 1 ml dilute ammonia A positive test was indicated by yellow coloration (20).</td>
</tr>
<tr>
<td>Saponins</td>
<td>The extract was briskly agitated after boiling 1 g powdered in 10 ml distilled water for 15 minutes to record froth formation (19).</td>
</tr>
<tr>
<td>Sterols/ Terpenes</td>
<td>The test of Liebermann-Büchard: An aliquot of extract was diluted in 1 ml of acetic anhydride in a test tube. Then 0.5 ml of concentrated H2SO4 was slowly poured on the tube's walls. A positive reaction is indicated by the emergence of a purple tint changing blue to green (18).</td>
</tr>
</tbody>
</table>
Tannins FeCl3 test: 0.5 g powdered was heated in 20 ml distilled water with three drops of five percent FeCl3 for a few minutes. A favorable sign was the emergence of a blue-black or brownish green color (20).

2.3. Test microorganisms and their sources

*S. aureus, S. epidermides, E. coli* and *P. aeruginosa* isolates were obtained from the Department of Biology at Thi-Qar University College of Science. API Staph. and API Enterobacteracea (21) were used to confirm the bacterial species. The bacteria were obtained from clinical specimens. Nutrient agar slants were used to subculture the pure cultures. They were kept at 40°C until they were needed for the investigation.

2.4. Antibacterial Assay

The bauer, Kirby well diffusion method of (22) was used to screen methanolic extracts of *Suaeda aegyptiaca* leaves for antibacterial activity in vitro. The sterile petri-plates were filled with sterilized nutritional agar medium (20 ml) and allowed to harden. The bacterial broth cultures were swabbed individually on an agar plates using a sterile bud. Wells (5 mm in diameter) were drilled into the agar with a sterile borer. Plant organic extracts (30 l) were placed into each well aseptically and incubated for 24 hours at 37°C. A measurement of the inhibitory zone was made. Antibacterial activity was measured using the diameter of the inhibition zone (DIZ) of the examined bacterium. The DIZ was measured in millimeters. All experiments were carried out in triplicate using the methods described in (23).

2.5. Statistical Analysis

Each experiment was carried out triplicates, with the means from absolute data being reported. According to Box, Hunter (24), statistical analysis of the acquired data was performed using the (ANOVA) test and the (SPSS) program.

3. Results and Discussion

The phytochemical screening of *Suaeda aegyptiaca* revealed the presence of alkaloids, tannins, sterols and/or terepenes, flavonoids, and saponins, but no anthraquinones or coumarins, as shown in table 2.

### Table 2. Phytochemical constituents of Suaeda aegyptiaca

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td></td>
</tr>
</tbody>
</table>
+ presence of phytochemicals; - absence of phytochemicals

According to the results of an antimicrobial assay using the agar diffusion method (wells of medium agar are filled with sample extracts), methanol extracts of the *S. aegyptiaca* plant show potent antibacterial inhibitory zones against *E. coli, P. aeruginosa, S. aureus*, and *S. epidermidis*. *S. aegyptiaca*’s crude methanolic extract has a significant inhibitory effect on *S. epidermidis*. Figure 1 showed that the inhibitory zones had diameters of (24.7, 21 and 12.7%) mm at concentrations of 10%, 5% and 2.5%, respectively. Figure 2 showed that the sizes of the inhibition zones against *S. aureus* ranged from 20 to 13.3 mm at concentrations of 10%, 5% and 2.5%, respectively. The extract’s inhibitory impact was stronger as the concentration of the extract was raised.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Presence</th>
</tr>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinons</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Sterols/Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
</tbody>
</table>

![Figure 1](image1.png)  
**Figure 1.** The effect of crude methanol extract of *S. aegyptiaca* against *S. epidermidis*.  

![Figure 2](image2.png)  
**Figure 2.** The effect of crude methanol extract of *S. aegyptiaca* against *S. aureus*.  

<table>
<thead>
<tr>
<th>Concentration of extract</th>
<th><em>S. epidermidis</em></th>
<th>Sig. = 0.002</th>
<th>df = 2</th>
<th>Mean ± Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>24.7 ± 2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>21 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5%</td>
<td>12.7 ± 1.2</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of extract</th>
<th><em>S. aureus</em></th>
<th>Sig. = 0.007</th>
<th>df = 2</th>
<th>Mean ± Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>20 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>18 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5%</td>
<td>13.3 ± 0.6</td>
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</table>
Figure 3 shows the effect of *S. aegyptiaca* extracts on *Escherichia coli*. The higher mean zone of inhibition was determined to be 18.7 mm at 10% con. At 5% and 2.5%, the additional zones of inhibition are 16.7 and 11.3 mm, respectively. As shown in figure 4, the mean inhibition zone against *P. aeruginosa* was (16.3, 13.3, and 9.3) mm at 10%, 5%, and 2.5% con, respectively.

The extract under investigation was examined for inhibitory effect against tested microorganisms that cause human diseases, as the extract's efficiency varied depending on the type of bacteria. As shown in figure 5, *Staph. epidermidis* had the highest inhibition zone of 19.4 mm when compared to the other bacteria, which had inhibition areas of 17.1, 15.6, and 13, respectively, for *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*.

Figure 6 shows that ethanolic extracts of *S. aegyptiaca* have antibacterial efficacy against both Gram-positive and Gram-negative human pathogenic bacteria. The antibacterial efficiency of ethanolic extracts of *Suaeda aegyptiaca* against both Gram-positive and Gram-negative human pathogenic bacteria is shown in figure 6. Positive bacteria were more effective, with a mean inhibition of 18.28 mm compared to negative bacteria, which inhibited by 14.28 mm.
The results reveal that the current extracts were more efficient against Gram positive bacteria, which is consistent with earlier publications (25-27) that the presence of outer membrane lipopolysaccharides is responsible for Gram-negative bacteria's higher resistance to plant extracts. Many investigations have found that herbal antimicrobial compounds are unable to stop the growth of Gram-negative bacteria due to the presence of a sophisticated cell wall construction that prevents herbal extracts from penetrating bacterial cells. However, extracts inhibited the growth of numerous bacteria in the current investigation, demonstrating their potential to penetrate bacterial cells (28, 29). Bacteria that are Gram-negative *P. aeruginosa* has a high level of natural resistance to nearly all known antimicrobials and antibiotics, and is even resistant to manufactured medicines, thanks to its outer membrane barrier.

In the current results, however, extracts prevented the growth of this bacteria, revealing its' ability to permeate bacterial cells (30). Plant-derived flavonoids, terpenoids, and steroids have sparked a lot of attention in recent years due to their wide range of pharmacological effects, such as antibacterial, antioxidant, and anticancer activities.

This extract's effectiveness is due to its active chemical compounds, which are consistent with previous studies showing the presence of alkaloids, stimulants, coumarins, catechins, tannins, phenols, flavonoids, saponins, glycosides, and the bioactive xanthoprotein of the leaves of
**Suaeda monoica**, which were used as a treatment for hepatitis and as an ointment for wounds and antiviral (31, 32).

The current extract inhibited both Gram positive and negative bacteria, indicating that they have a broad spectrum inhibitory effect, according to the findings of this investigation. Extracts rendered Gram positive bacteria more susceptible to extract than Gram negative bacteria, indicating that it could be utilized to treat infections caused by resistant germs.

**References**