Phytochemistry and Antibacterial Activity of *Prosopis juliflora* (SW.) DC

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**Introduction**

Traditional medicine is widely practiced in Egypt, where has been documented by ethnobotanical surveys. The main objective of the current study is to evaluate phytochemistry and antibacterial potential of ethanolic and aqueous extracts of *P. juliflora* cultivated in Matruh region with a view to considering their contribution to human health. Qualitative and quantitative phytochemical screening of pods and leaves was carried out. Five pathogenic bacteria were selected as recipients including four Gram-negative and one Gram-positive bacteria. Chocolate and blood agar solid media were applied for agar diffusion method. Extracellular proteins before and after treatment were measured and total protein assay was performed. Cytological effect of the ethanolic extracts was investigated via TEM. Phytochemical screening provides the occurrence of alkaloids, cardiac glycosides, coumarins, flavonoids, phenolics, steroids, tannins and terpenes in pods and leaves ethanolic and aqueous extract. In agar diffusion experiment, data showed that pod ethanolic extract (5%) was more active than the other three types of extracts. The extract elicited a decrease in the growth of *Staphylococcus aureus* in culture medium in comparison to the control. Both pods and leaves ethanolic extracts showed a decrease in the extracellular protein content of the tested *S. aureus* compared with control. TEM showed that pod ethanolic extract affected the growth of *S. aureus* cells. It caused severe damage in the cytoplasmic membrane and also the cell wall. The present study concluded that the pods and leaves extracts of *P. juliflora* possesses antibacterial potential against target bacterial type cultures.

**Keywords:** Prospis juliflora, Pathogenic bacteria, Phytochemistry, Growth, extracellular proteins, Cytogenic effect.
Australia and elsewhere (CABI, 2017). This herb is well-known in the folk medicine because of its ethnobotanical importance (Hebbar et al., 2004). Badri et al., (2017) reported that, the leaf extracts of *P. juliflora* showed the various degree of inhibitory activity against different bacterial species. The present study is a trial to explore phytochemistry and antibacterial potentiality of ethanolic and aqueous extracts of *P. juliflora* cultivated in Matriugh region with a view to assessing their contribution to human health.

**METHODS**

**Phytochemical screening**

**Preparation of plant extracts**

Pods and leaves of *P. juliflora* were collected from El-Kasr region, Matriugh governate. Phytochemical screening was performed using standard phytochemical procedures. Thirty-gram dry powder was taken and ethanol or water was added so that the plant material got totally immersed in the solvent (Khan et al., 2010). The extraction period was prolonged for 72 hours. At the completion of the extraction, the extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated. Later, each of the test samples was processed further to use to evaluate the presence of saponins, tannins, flavonoids, alkaloids, terpenes and sterols. Before doing so, each test sample was reconstituted in the respective solvents and divided into aliquots to perform the qualitative tests (Deepa et al., 2013).

**Qualitative and quantitative phytochemicals screening of pods and leaves**

Qualitative test for saponins, tannins and terpenes were performed according to Odhiambo et al., (2014) while for steroids, alkaloids and flavonoids were according to Kavishankar et al., (2011). Furthermore, coumarins and phenol were identified according to Harborne, (1998). On the other hand, quantitative evaluation of flavonoids, alkaloids, saponins, phenol and tannins were according to Okwu and Ukanwa (2007), Poornima and Ravishankar (2009), Aliyu et al., (2008), Hussain et al., (2011) and Price and Butler (1977) respectively.

**Bacterial species**

Five pathogenic bacterial species (*Acinetobacter baumannii* complex, *Klebsiella pneumonia SSP pneumonia, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) were selected as recipients to carry out the present study including only *Staphylococcus aureus* as Gram-positive bacteria. All of these bacterial species were obtained from Microbiology lab, High Institute of Public Health, Alexandria University and were maintained at 4°C on both chocolate agar slants and blood agar slants as well as Luria-Bertani (LB) slants.

**Culture media**

Chocolate agar and blood agar solid media were applied for agar diffusion method while Luria-Bertani (LB) broth medium was used for growth measurement of bacteria, extracellular protein and total protein assay.

**Agar well-diffusion method**

The agar diffusion method was used to test the antagonistic activity of the plant extracts against the five recipient pathogenic bacteria. The resulting inhibition zones were measured in centimeters (Bauer et al., 1966 and Balouiri et al., 2016).

**Measurement of extracellular protein**

Extracellular proteins of the five different pathogenic bacteria before and after treatment were measured according to (Lowry et al., 1951).

**Total protein assay**

*Preparation of cell lysate*

The method assay was conducted according to the method described by Bradford (1976).

**Transmission Electron Microscopy (TEM)**

The freshly samples of cells not exposed to the plant extract (control) and others subjected to ethanolic and aqueous extracts were fixed using a universal electron microscope fixative. Series of dehydration steps were followed using ethyl alcohol and propylene oxide. The samples were then embedded in labeled beam capsules and polymerized. This section of cells was cut using LKB 2209-180 ultra-microtome and stained with a saturated solution of uranyl acetate for 30 min and lead acetate for 2 min (Mc Dowell and Trump, 1976). Electron micrographs were taken using a transmission electron microscope (JEM-100 CX JOel), at the Electron Microscope Unit, Faculty of Science, Alexandria University.

**Statistical Analysis**

Data collected on different parameters were analyzed statistically by using the COSTAT 2.00 statistical analysis software manufactured by Co-Hort Software Company. For analysis of variance and means data were separated using Fisher's protected least significant difference (LSD) test at 5% probability level (Steel et al., 1997).

**RESULTS**

**Phytochemical Screening**

Data in Table 1 provides the occurrence of some phytochemicals (alkaloids, cardiac glycosides, coumarins, flavonoids, phenolics, steroids, tannins and terpenes) in pods and leaves ethanolic and aqueous extract of *P. juliflora*. It was recorded that alkaloids attained moderate concentration in both pods and leaves of the study species either in ethanolic or aqueous extract. Cardiac glycosides were absent in leaves but...
reached low concentration in pods with both extracts. Coumarins attained low concentration in all cases except in pods with aqueous extract, it attained low moderate concentration. In the same context, flavonoids achieved low concentration in all cases except in pods with ethanolic extract it attained moderate concentration. It was clear that phenolics attained high and moderate concentration in pods and leaves, respectively in both extracts. Proteins attained moderate and low concentration in pods and leaves, respectively in the two extracts. Steroids were detected in leaves only either in ethanolic or aqueous extracts with high concentration. Clearly, tannins and terpenes attained low concentration in both pods and leaves with the two types of extracts.

Table 1: Qualitative phytochemical screening of pods and leaves of *Prosopis juliflora* in ethanolic and aqueous extracts

<table>
<thead>
<tr>
<th>No.</th>
<th>Constituent</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pods</td>
<td>Leaves</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenolics</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+; low concentration, ++; moderate concentration, +++; high concentration, -; Absent

Data in Table 2 provides the concentration (%) of some secondary metabolites such as alkaloids, flavonoids, phenols, saponins and tannins in pods and leaves of *P. juliflora*. It was recorded that flavonoids attained the highest concentration values in pods (22%) and leaves (16 %). In contrast, tannins achieved the lowest values (0.66 and 0.33 %) in pods and leaves, respectively.

Table 2: Quantitative phytochemical screening of leaves and pods of *Prosopis juliflora*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pods</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>5.2 ± 0.09</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>22 ± 0.58</td>
</tr>
<tr>
<td>Phenols</td>
<td>0.87 ± 0.21</td>
</tr>
<tr>
<td>Saponins</td>
<td>3.92 ± 0.33</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.66 ± 0.08</td>
</tr>
</tbody>
</table>

3.1. Antagonistic activity of plant extracts

The concentration of the different plant extracts applied in the present study was 5%. In the agar diffusion experiment, data showed that pod ethanolic extract (5%) was more active than the other three types of extracts. The maximum effect of both ethanolic and aqueous extracts was demonstrated against *Staphylococcus aureus* (Figure 1) while the minimum was established on *Acinetobacter baumannii*, *Klebsiella pneumonia*, *E. coli*, and *Pseudomonas aeruginosa* (Figure 2).

Figure 1: Inhibition zone for the effect of pod ethanolic extract against *Staphylococcus aureus* in chocolate (A) and blood (B) agar media
Figure 2: Inhibition zone for the effect of leaf ethanolic extract against *Acinetobacter baumannii* complex, *Klebsiella pneumonia* SSP pneumonia, *Escherichia coli*, and *Pseudomonas aeruginosa* in chocolate (A) and blood (B) agar media

Effect of plant extracts on bacterial growth in culture media

Pod ethanolic extract elicited a decrease in the growth of *Staphylococcus aureus* in culture medium in comparison to the control (Figure 3).

![Graph showing growth comparison](image)

Figure 3: Effect of pod ethanolic extract on the growth of *Staphylococcus aureus*

Effect of ethanolic plant extract on *Staphylococcus aureus* extracellular protein

Both pods and leaves ethanolic extracts showed a decrease in the extracellular protein content of the tested *S. aureus* compared with control (Figure 4).

![Graph showing extracellular protein content](image)

Figure 4: Effect of pod ethanolic extract on extracellular protein content of *Staphylococcus aureus*
Data showed that total protein decreased by about 82% compared to control in case of pod ethanolic extract and approximately 50% in case of leaf ethanolic extract. With respect to leaf aqueous extract, total proteins were reduced by about 27% compared to control (Table 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance (1)</th>
<th>Absorbance (2)</th>
<th>Absorbance (3)</th>
<th>Average</th>
<th>Cells weight (1ml)</th>
<th>Protein concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.531</td>
<td>0.534</td>
<td>0.536</td>
<td>0.533</td>
<td>20.1</td>
<td>1.021</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pods</td>
<td>0.118</td>
<td>0.116</td>
<td>0.118</td>
<td>0.117</td>
<td>0.521</td>
<td>0.218</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.254</td>
<td>0.251</td>
<td>0.254</td>
<td>0.253</td>
<td>0.813</td>
<td>0.480</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pods</td>
<td>0.641</td>
<td>0.642</td>
<td>0.643</td>
<td>0.642</td>
<td>2.14</td>
<td>1.022</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.388</td>
<td>0.387</td>
<td>0.389</td>
<td>0.388</td>
<td>1.18</td>
<td>0.741</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Prosopis juliflora yields several compounds including alkaloids, tannins, phenolics, steroids, terpenes, flavonoid, proteins, sugars, and fatty acids (Singh, 2012). Some of these compounds may exhibit therapeutic activities such as antibacterial activity. For example, juliprosinene and juliflorinine isolated from *P. juliflora* exhibit antibacterial effect on bacteria such as *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Shigella sonnei* (Prabha et al., 2014). Tajbakhsh (2015) stated that the extract of *P. juliflora* seed pods was effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, and *Pseudomonas aeruginosa* but the Minimum Inhibitory Concentration (MIC) for gram-positive species was lower than that of for gram-negative species. In present study, five different pathogenic bacterial species including Gram-negative and Gram-positive bacteria were used. The effect of the four plant extracts (pods and leaves; aqueous and ethanolic) were tested by the agar diffusion method. Ethanolic extract was more active than aqueous one. The maximum effect of the ethanolic extracts was shown against *S. aureus* but has a minimum effect on the other types of bacteria. The aqueous extract had no effect on all types of the recipient bacteria. Contrariwise, Thakur et al (2014) reported that cold and hot extracts of *P. juliflora* leaves at 100 mg/ml concentration significantly inhibited the growth of all test bacteria viz., *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Salmonella typhi* and *Salmonella typhimurium*.

Both ethanolic extracts (pods and leaves) showed a decrease in the extracellular protein content of the tested *S. aureus* compared with control and the decrease was prominent in case of pod ethanolic > leaf ethanolic > leaf aqueous. It produced severe damage in the cytoplasmic membrane and cell wall and elicited abnormalities in morphology and cell volume. The leaf methanolic extract of *P. juliflora* were screened for...
antibacterial activity against seven Gram negative bacteria (Escherichia coli, Escherichia coli ESBL, Shigella flexneri, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis), and three Gram positive bacteria (Enterococcus faecalis, Listeria monocytogenes and Bacillus cereus) using the cup-plate agar diffusion method. The extract employed pronounced activity against the tested bacteria as indicated by diameter of growth inhibition zones that varied from 12 to 41 mm except S. typhi has no inhibition zones (Badri et al., 2017). P. juliflora pod methanolic crude extract tested on two tested Gram- negative bacteria (E. coli and Klebsiella spp.) and three Gram-positive bacteria (Staphylococcus aureus, Bacillus spp. and Streptococcus spp.) indicated an inhibition of all tested bacteria except Klebsiella spp. The tests on Gram-positive bacteria (S. aureus, Streptococcus spp. and Bacillus spp.) showed higher sensitivity than for Gram-negative bacteria (E. coli and Klebsiella spp.). The findings generally indicated that Gram-positive organisms were more susceptible to the extract of P. juliflora pods than Gram-negative organisms (Tajbakhshe et al., 2008, and 2011). The less susceptibility of Gram-negative bacteria to antibacterial substances in such studies may be associated with their outer membrane and lipopolysaccharide molecules which provide the barrier against penetration of antimicrobial molecules. Gram-positive bacteria do not have this type of outer membrane and cell wall construction (Willey et al., 2008). Moreover, Singh and Verma, (2011) investigated the antibacterial effect of alkaloid rich fractions of P. juliflora taken from different parts of plant including leaf, pods and flower. The leaf extract showed the highest antibacterial properties compared to the other organs. Thakur et al. (2014) stated that cold and hot extracts at 100 mg/ml concentration significantly inhibited the growth of all test bacteria viz., Bacillus subtilis, E. coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi and Salmonella typhimurium. Overall, 100% bacteria produced the zone of inhibition during the screening process showing appreciable inhibitory effect.

The present study concluded that the pods and leaves extracts of P. juliflora possesses antibacterial potential against target bacterial type cultures. Therefore, further investigations regarding the mode of action and other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended. Isolation and characterization of the bioactive components can be further done by systematic screening of the most active solvent fraction which could lead to the possible source of new antibacterial agents.

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