Antimicrobial effect of Lagenaria siceraria (Mol.) Standley, against certain bacteria and fungal strains

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Abstract: This study was performed to evaluate the antibacterial and antifungal potency of Lagenaria siceraria (Mol.) Standley, commonly known as bottle gourd belonging to the family Cucurbitaceae. Petroleum Ether, Chloroform, Methanol, Absolute alcohol and Water showed a majority of the compound including Steroids, Alkaloids, Tannins, Flavonoids, Lectones and Carbohydrates. All the five extracts were prepared by using soxhlet apparatus and the extracts showed moderate to potent antimicrobial activity against the bacterial strains: Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus and antifungal strains: such as Aspergillus flavus, Aspergillus oryzae and Trichoderma harzianum.

Keywords: Lagenaria siceraria, Antibacterial, Antifungal, Phytochemicals, Leaf extracts

INTRODUCTION

The use of plant extracts and phytochemicals both with known antimicrobial properties can be of great significance in therapeutic treatments (Shapoval et al., 1994). Many plants are used as medicines because of their antimicrobial nature, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances (Geethalahakshmi et al., 2010). The demand on plant based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, easily biodegradable producing minimum environmental hazards, no side effects and easily available at affordable price. India is the largest producer of medicinal herbs and traditional practitioners of this country use more than 6000 medicinal plants in primary health care (Shaniff et al., 2008). Lagenaria siceraria (Mohl.) Standley fruit (Syn. L. vulgaris Ser., Cucurbita legenaria, Linn., L. leucantha Rusbey, belongs to the family Cucurbitaceae), commonly known as Bottle gourd is distributed in Africa, Asia and America. The first and foremost important use of bottle gourd was as a water carrier. Medicinally it is used as a purgative, an antidote for certain poisons and a cure for coughs (Uphof, 1959). An infusion of the seeds has been used to cure chills and for headaches, and juice from the leaves was taken against jaundice and to cure baldness (Moerman, 1998 and Deshpande et al., 2008). The present study was undertaken to evaluate the potentiality of aqueous and organic solvent extracts of L. siceraria against some medically important bacterial and fungal strains.

MATERIALS AND METHODS

Test plant material: The fresh plant material of the L. siceraria was collected from Hoskote forest ranges near Bangalore in Karnataka, India during February 2009.

Test organisms: To find out the antimicrobial activity of various extracts of L. siceraria, the different strains of pathogenic bacteria used are Escherichia coli ATCC25922, Enterococcus faecalis ATCC29212, Klebsiella pneumonia ATCC31488, Salmonella typhi ATCC6539, Staphylococcus aureus ATCC2923 and antifungal strains includes Aspergillus flavus ATCC32611, Aspergillus oryzae ATCC76080, Trichoderma harzianum ATCC20476.

Extraction and phytochemical analysis: The surface of the leaves were washed in distilled water to remove the surface microflora and are then shade dried, pulverized by a mechanical grinder and passed through a 40 µm mesh sieve to get the fine powder and stored in an airtight container. The dried powder (50g) was extracted by soxhlet extraction method using different solvents namely, Petroleum Ether, Chloroform, Methanol, Absolute alcohol and Water sequentially. The solvents from various extracts were then concentrated in a rotavapour at reduced pressure below 40°C and the extract thus obtained was stored in airtight bottles at 4°C for further experiments.

Qualitative tests for phytochemical analysis: The phytochemicals in the plants were investigated by performing standard qualitative tests for the presence of...
The values are mean ± S. D. of zone of inhibition including standard was taken in discs. The discs were Muller Hinton agar medium (Hi-media). All the test extracts for Clinical Laboratory Standards (NCCLS, 1993) using by dilution method as described by National Committee Minimal Inhibitory Concentration assay was performed.

**Minimal inhibitory concentration (MIC) assay:**

\[
\text{Radial growth of control (mm)} \times 100
\]

\[
\text{Radial growth of treatment (mm)}
\]

\[
\text{Inhibition} = \frac{\text{Radial growth of control (mm)}}{\text{Radial growth of treatment (mm)}} \times 100
\]

The relative growth inhibition of treatment compared to control was calculated by percentage, using the following formula:

In the case of antifungal activity, the growth of inhibition was less.

The results for tannins. The phytochemical analysis of water, methanol and ethanol extracts respectively, whereas in case of Klebsiella pneumonia 11.4±1.34, 04.6±6.39, 11.8±1.79, 7.0±2.24 and Salmonella typhi15.2±6.40, 04.8±6.72, 11.6±2.19, 5.6±3.21, the growth of inhibition was less. In the case of antifungal activity, the growth of inhibition was comparatively more with respect to Aspergillus flavus 13.2±2.28, 13.4±2.19, 10.8±1.10, 5.0±3.81, Aspergillus oryzae 12.8±3.35, 13.0±1.00, 10.4±0.89, 3.6±3.36, but the growth of inhibition was less in case of Trichoderma harzianum 00.0±0.00, 14.2±2.28, 11.2±1.79, 4.2±3.03. Thus the different solvent extracts have shown different results with different microorganisms. The petroleum ether extracts has showed MIC at 0.25 x 10^-3 mg/ml in bacterial strains whereas in fungi MIC was shown positive for A. oryzae. The chloroform extract in bacteria dipped in extracts having the concentrations of 0.0625, 0.125, 0.25 and 0.5mg/10^-3. The plates were incubated at 37°C for 24h. The assay was repeated twice and the antimicrobial potency of different microbial strains was determined by observing the inhibitory zone of microbial strains. The lowest concentration showing no inhibitory zone was considered as the MIC.

**RESULTS**

Extraction yield and phytochemical estimation: The results of phytochemical analysis of petroleum ether, chloroform, methanol, absolute alcohol and water extract has revealed the positive results for alkaloids, steroids, flavonoids, lactones and carbohydrates and negative results for tannins. The phytochemical analysis of water and ethanol extract has shown the presence of saponins and terpenoids respectively (Table 1).

The results of antibacterial activity of L. siceraria extract in vitro are given in Tables 2 and 3. It was observed that, the plant is possessing more activity against Escherichia coli 14.2±3.77, 14.4±3.13, 14.4±2.88, 9.8±1.48, Enterococcus faecalis 13.6±3.21, 12.4±2.88, 17.0±4.00, 8.0±1.41, and Staphylococcus aureus 14.6±2.61, 13.6±2.00, 13.8±3.03, 6.6±2.07 in petroleum ether, chloroform, methanol and water extracts respectively, whereas in case of Klebsiella pneumonia 11.4±1.34, 04.6±6.39, 11.8±1.79, 7.0±2.24 and Salmonella typhi15.2±6.40, 04.8±6.72, 11.6±2.19, 5.6±3.21, the growth of inhibition was less.

Table 1. Preliminary phytochemical screening of Lagenaria siceraria.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Tests</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Steroids +</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids +</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Tannins -</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Lactones +</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Carbohydrates +</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity of various extracts of L. siceraria on different microbes.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Methanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial Strains</strong></td>
<td>14.2±3.77</td>
<td>13.4±3.13</td>
<td>14.4±2.88</td>
<td>9.8±1.48</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13.6±3.21</td>
<td>12.4±2.88</td>
<td>17.0±4.00</td>
<td>8.0±1.41</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>11.4±3.34</td>
<td>04.6±6.39</td>
<td>11.8±1.79</td>
<td>7.0±2.24</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>15.2±6.40</td>
<td>04.8±6.72</td>
<td>11.6±2.19</td>
<td>5.6±3.21</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>14.6±2.61</td>
<td>13.0±2.00</td>
<td>13.8±3.03</td>
<td>6.6±2.07</td>
</tr>
<tr>
<td><strong>Fungal Strains</strong></td>
<td>13.2±2.28</td>
<td>13.4±2.19</td>
<td>10.8±1.10</td>
<td>5.0±3.81</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>12.8±3.35</td>
<td>13.0±1.00</td>
<td>10.4±0.89</td>
<td>3.6±3.36</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>00.0±0.00</td>
<td>14.2±2.28</td>
<td>11.2±1.79</td>
<td>4.2±3.03</td>
</tr>
</tbody>
</table>

The values are mean ± S. D. of zone of inhibition.

The values are mean ± S. D. of MIC.

Table 3. Antimicrobial activity of various extracts of L. siceraria on different microbes.

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Alcohol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.43</td>
<td>0.66</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>0.66</td>
<td>0.25</td>
<td>0.66</td>
<td>0.25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.25</td>
<td>0.8</td>
<td>0.43</td>
<td>0.25</td>
</tr>
<tr>
<td>S. typhi</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.25</td>
</tr>
<tr>
<td>Fungal Strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>0.43</td>
<td>0.66</td>
<td>0.11</td>
<td>0.8</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>0.11</td>
<td>0.11</td>
<td>0.66</td>
<td>0.8</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>0.8</td>
<td>0.11</td>
<td>0.11</td>
<td>0.8</td>
</tr>
</tbody>
</table>

showed MIC in E. coli, E. faecalis and S. aureus at 0.25 x 10^3 mg/ml and in case of fungi, the extract was able to show the MIC at 0.11 x 10^3 mg/ml and 0.25 x 10^3 mg/ml. The methanol extract has showed MIC in 0.25 x 10^3 mg/ml and 0.43 x 10^3 mg/ml in bacterial strains and 0.11 x 10^3 mg/ml and 0.66 x 10^3 mg/ml in the case of fungi (Table 3).

DISCUSSION

The results obtained in this study showed the presence of antimicrobial activity of L. siceraria, in the preliminary screening of antimicrobial activity using disc diffusion method. The antibacterial activity of L. siceraria water extract was lower than the organic solvent extracts however the results are concurrent with the earlier report done by Toholope (2007) in Hibiscus sabdariffa against S. aureus, E. coli and K. pneumoniae. Ghosh et al. (2008) have also used similar bacterial sources in their investigation. It includes, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi and Staphylococcus aureus and got more zone of inhibition in the methanol extract. However, in the present study also it is revealed that the extracts of petroluem ether, chloroform and methanol have showed more zone of inhibition than the water extract and it is also suggesting that, the inhibition is more in E. coli, E. faecalis and S. aureus but it was comparatively less in case of K. pneumonia and S. typhi.

The antifungal property of the plant species has been done by using three different strains amongst A. flavus which showed more zone of inhibition than the other two strains. This is due to the chemical property of the extract which inhibited the growth of the fungi. Similar types of results were also observed by Cichewicz and Thorpe (1996). Many plants have been used to screen the antimicrobial property because of their antimicrobial traits, which are due to chemical compounds synthesized in their secondary metabolism of the plant such as alkaloids (O. T. A. Plants, 1985 and Omulokoli et al., 1997); flavonoids (Bhatia et al., 1994). Incase of petroleum ether extract, the MIC was found to be more at 0.25 x 10^3 mg/ml. Even in case of fungi A. oryzae showed MIC at 0.11 x 10^3 mg/ml and A. flavus at 0.25 x 10^3 mg/ml. The results are similar to the report mentioned by the earlier workers like Geethalakshmi et al., (2010). It was concluded that, the most susceptible bacterial was E. faecalis while most resistant was S. typhi and in case of fungi the most susceptible fungal strain was A. flavus while most resistant was T. harzianum.

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REFERENCES


they can be obtained (mainly N. American nurseries but also research institutes and a lot of other nurseries from around the world.


