Phytochemical screening and antibacterial activity of Bombex ceiba

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Abstract

Bombex ceiba is a traditional medicinal plant used by tribal people in different diseases. The present study was carried out for providing the scientific evidence of its traditional medicinal usage on antibacterial capacity and presence of different phytochemical constituents on B. ceiba seeds. Qualitative phytochemical screening, quantitative estimation of total phenolics and alkaloids was carried out using different standard test procedures and antibacterial activity was tested using cup plate method and measured its capacity on zone of inhibition measurement. The all extracts of B. ceiba revealed the presence of phenols, alkaloids, carbohydrates, steroids, terpenoids and glycosides and gave negative result to saponins. The hydroalcoholic and ethyl acetate extracts revealed the presence of flavonoids and tannins but the hexane extract gave negative results. The Quantified phenolic contents of B. ceiba extracts were ranging from 13.85±1.22 to 34.10±2.62 (mg/gm). The quantitative alkaloid content was ranging from 16.24±2.38 to 31.86±1.88 (mg/gm). All the extracts (hexane, ethyl acetate and hydroalcoholic) of selected medicinal plants at different concentrations (50µg, 100µg, 150µg and 200µg/cup) exhibited antibacterial activity along with standard drug (Rifampicin) against tested bacterial strains. Ethyl acetate and hydroalcoholic extract showed equal antibacterial activity on all bacterial strains.

Keywords: Bombex ceiba, Seeds, Phenolics, Alkaloids and Antibacterial activity.

1. Introduction

Bombex ceiba is a traditional medicinal plant used by tribal people in different diseases. The present study was carried out for providing the scientific evidence of its traditional medicinal usage on antibacterial capacity and presence of different phytochemical constituents on B. ceiba seeds. Qualitative phytochemical screening, quantitative estimation of total phenolics and alkaloids was carried out using different standard test procedures and antibacterial activity was tested using cup plate method and measured its capacity on zone of inhibition measurement. The all extracts of B. ceiba revealed the presence of phenols, alkaloids, carbohydrates, steroids, terpenoids and glycosides and gave negative result to saponins. The hydroalcoholic and ethyl acetate extracts revealed the presence of flavonoids and tannins but the hexane extract gave negative results. The Quantified phenolic contents of B. ceiba extracts were ranging from 13.85±1.22 to 34.10±2.62 (mg/gm). The quantitative alkaloid content was ranging from 16.24±2.38 to 31.86±1.88 (mg/gm). All the extracts (hexane, ethyl acetate and hydroalcoholic) of selected medicinal plants at different concentrations (50µg, 100µg, 150µg and 200µg/cup) exhibited antibacterial activity along with standard drug (Rifampicin) against tested bacterial strains. Ethyl acetate and hydroalcoholic extract showed equal antibacterial activity on all bacterial strains.

Keywords: Bombex ceiba, Seeds, Phenolics, Alkaloids and Antibacterial activity.
2. Materials and Methods

2.1 Collection of plant material and preparation of extracts

The plant material seeds were collected at Araku valley, Visakhapatnam district, Andhra Pradesh, India and the plant was authenticated by taxonomist Prof. M. Venkaiah, Department of Botany, Andhra University. The collected seeds was shade dried and milled into powder. The powdered material was used for extraction with different solvents successively (Hexane, Ethyl acetate, and Hydro alcoholic) using maceration process. Then the extracts were used for further study.

2.2 Chemicals and test bacterial species

Muller Hinton agar media was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. The other chemicals were analytical grade. The microorganisms used for the experiments were procured from MTCC, IMTECH, Chandighar. Gram +ve organisms:

- Bacillus megaterium (B. m),
- Staphylococcus epidermidis (S. e), and
- Lactobacillus acedophillus (L. a).

Gram-ve organisms:

- Escherichia coli (E. c),
- Salmonella typhi (S. t) and
- Klebsiella pneumonia (K. p).

2.3 Qualitative and Quantitative Phytochemical Screening

Qualitative phytochemical screening was carried out using different standard phytochemical tests for different compounds (Kokate 1991; Prashant Tiwari et al., 2011) Quantitative estimation of phenols and alkaloids were carried out using Folin-Ciocalteau reagent (Mallikarjuna Rao et al., 2012) and Bromocresol Green solution (Fazal Sharma et al., 2008).

2.4 Culture Media for anti bacterial activity

The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. For Anti bacterial activity Muller-Hinton Agar media was used.

2.5 Standard and test solution preparation

The test compounds (dried extracts) at a concentration of 50, 100, 150 and 200µg/mL were dissolved in dimethylsulphoxide and used as stock solution. The reference standard (Rifampicin) as 0.6mg/mL concentration in HPLC grade water and finally added 100µL in each cup of Petri dish during antibacterial activity.

2.6 Evaluation of antibacterial activity

The antibacterial activity was assayed using cup/cylinder plate method. The method was based on capacity of different drugs by zone of inhibition (Size in mm) on microbial growth on Petri dish (Indian Pharmacopoeia., 1996; Ganga Rao et al., 2012). The tested microorganisms were spread on different plates using spread plate technique, on those plates 4 wells with 4mm diameter were placed using sterile borers. Accurately measured (100µl) solution of each concentration and reference standards were added to the cups with a micropipette and placed at 2-8°C for effective distribution of testing/standard compounds in wells. Later, they were incubated at 37°C for 24 hours, then Petri dishes were observed for presence or absence of definite zone of inhibition. If any zone of inhibition around the well (cup) indicate presence of antibacterial activity. At the same time, the vehicles (DMSO, HPLC grade water) were alson tested for antibacterial activity.

3. Results and Discussion

Qualitative phytochemical screening of B. ceiba extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, tannins, carbohydrates, oils and amino acids. The extracts gave negative results for the quinines and saponins. The all extracts of B. ceiba revealed the presence of phenols, alkaloids, carbohydrates, steroids, terpenoids and glycosides and gave negative result to saponins. The hydroalcoholic and ethyl acetate extracts revealed the presence of flavonoids and tannins but the hexane extract gave negative results. The hexane and ethyl acetate extracts reveals the presence of minute amount of oils but hydroalcoholic extracts gave negative results. All the extracts gave negative result to amino acids but
the hydroalcoholic extracts give minute result for the presence of amino acids. The results were shown in table 1.

Phenolic contents of B. ceiba extracts were ranging from 13.85±1.22 to 34.10±2.62 (mg/gm). The hydroalcoholic extract have more phenolic content i.e. 34.10±2.62 (mg/gm) than other extracts. As phenolic contents, alkaloid content was vary from 16.24±2.38 to 31.86±1.88 (mg/gm). The hydroalcoholic extract has more alkaloid content i.e. 31.86±1.88 (mg/gm) than other extracts. The results were shown in table 2.

All the extracts (hexane, ethyl acetate and hydroalcoholic) of selected medicinal plants at different concentrations (50µg, 100µg, 150µg and 200µg/cup) exhibited antibacterial activity along with standard drug (Rifampicin) against tested bacterial strains (Table 3). The antibacterial potency of different medicinal plants extracts depends on type of extraction and nature of components in them and sensitiveness of tested strains. Significantly higher antibacterial activity was observed with hydroalcoholic extract, whereas least activity was observed in case of hexane extract with intermediate values for ethyl acetate extract. Both gram positive and gram negative bacteria were susceptible to selected plants extracts which supports the earlier reports that plant extracts were most active against bacterial strains. Hexane extract of Bombax ceiba seeds showed zone of inhibition on B. megaterium, K. Pneumonia and S.pyphi at 50µg/cup. Ethyl acetate and hydroalcoholic extract showed equal antibacterial activity on all bacterial strains but hydroalcoholic extract showed better activity on K. Pneumonia (15mm) and B. megaterium (16mm) strains.

The current study outcome, demonstrates that Bombax ceiba seeds possess the significant and considerable antibacterial activity on different bacterial strains as currently using drugs and contain biological active compounds (Phenolics, Alkaloids, Steroids, Glycosides, Flavonoids and Terpenoids) which are effective in resisting the growth of the pathogenic bacteria (David et al., 2011; Rajeswari et al., 2014; Newman et al., 2003) and further studies are useful in isolation of drugs from these plants for many diseases.

**Table 1.** Nature of phytoconstituents presents in different extracts of Bombax ceiba.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Bombax ceiba</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane extract</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ ++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ ++</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
</tr>
<tr>
<td>Oils</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ ++</td>
</tr>
</tbody>
</table>

+,++,=Present, – = Absent (+=Less Intense; ++= More Intense)

**Table 2.** Total phenolic and alkaloid contents (mg/gm) of Bombax ceiba extracts.

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>Total Phenolic content (mg/gm)</th>
<th>Total alkaloid content (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>13.85±1.22</td>
<td>16.24±2.38</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>26.28±0.66</td>
<td>22.40±1.36</td>
</tr>
<tr>
<td>Hydro alcoholic</td>
<td>34.10±2.62</td>
<td>31.86±1.88</td>
</tr>
</tbody>
</table>

**Table 3.** Antibacterial activities of Bombax ceiba seeds extracts.

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>dose (µg/ cup)</th>
<th>Zone of inhibition* (in mm)</th>
<th>S.e</th>
<th>B.m</th>
<th>L.a</th>
<th>E.c</th>
<th>S.t</th>
<th>K.p</th>
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<tr>
<td>Hexane extract</td>
<td>50</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>200</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Hydro alcoholic extract</td>
<td>100</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>7</td>
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<tr>
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<td>150</td>
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<td>8</td>
<td>9</td>
<td>11</td>
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<td>10</td>
<td>9</td>
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<tr>
<td>Rifampicin</td>
<td>200</td>
<td>13</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td></td>
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</tr>
<tr>
<td>DMSO</td>
<td>100µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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</table>

*#Values Includes the cup diameter (4mm)

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4. Conclusion
The present study provide the scientific evidence to the Bombax ceiba plants traditional medicinal usage in the treatment of diseases.

Acknowledgements
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Conflicts of interest
Author has none to declare.

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