ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF THE PLANT BAUHINIA VARIEGATA
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ABSTRACT

Antibacterial activity of petroleum ether, chloroform, methanol and aqueous extracts of leaves of Bauhinia variegata was determined against gram positive and gram negative bacteria. Penicillin and gentamycin were used as reference standards for comparison of activity. The activities were found to be concentration dependent for all different samples tested.

It was found that aqueous extract has antibacterial activity against Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus while methanol extract has this activity against Pseudomonas aeruginosa and Staphylococcus aureus with minimum inhibitory concentration as compared with standard (Penicillin 10 µg/mL and gentamycin 80 µg/mL). The petroleum ether extract and chloroform extracts were active against Staphylococcus aureus and Escherichia coli. The minimum inhibitory concentration for aqueous extract was 10 µg/mL, 10 µg/mL, 50 µg/mL and 50 µg/mL, respectively against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa), for methanol extract was 500 µg/mL (Staphylococcus aureus) and 50 µg/mL (Pseudomonas aeruginosa), for petroleum ether extract was 100 µg/mL (Staphylococcus aureus) and 250 µg/mL (Escherichia coli), for chloroform extract was 250 µg/mL (Escherichia coli) and 500 µg/mL (Staphylococcus aureus).

Key word: Bauhinia variegata Linn, Antibacterial activity, Kanchanar

INTRODUCTION

Bauhinia variegata L. is also known as Kanchanar in Hindi and belongs to a family leguminosae. A medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India. Leaves are 10-15 cms long, as broad as or rather
broader than long, cleft ¼ to 1/3 of the way down into 2 obtuse lobes, pubescent beneath when young, the pubescence nerves persisting along2. The chemical constituents found are β-sitosterol, lupeol, kaempferol-3-glucoside and tannins3. Leaves of Bauhinia variegata extract contains carbohydrates, amides, reducing sugars, vitamin C3, crude protein, fibers4, calcium and phosphorus5. The crude protein content of leaves, collected from Izatnagar and Palampur, was reported to be 15.80 and 13.18%, respectively on dry matter basis6. Quercetin, rutin, quercitrin, apigenin and apigenin-7-O-glucoside were isolated from the leaves of Bauhinia variegata7. Two new long chain compounds heptatriacont-12,13-diol and dotetracont-15-en-9-ol were isolated from the leaves of Bauhinia variegata8. The leaves of the plant are widely used for stomatitis9 and also inhibit the activity of peptic enzymes10. The present study is therefore undertaken to study the antibacterial activity of various extracts of Bauhinia variegata L. leaves. Petroleum ether, chloroform, methanol and aqueous extracts were evaluated against Staphylococcus aureus, Bacillus subtilis (gram positive) and Pseudomonas aeruginosa, Escherichia coli (gram positive). Gentamycin and penicillin were used as the standard.

**EXPERIMENTAL**

**Plant material**

Bauhinia variegata L. leaves were obtained from Regional Research Institute, Bangalore and authenticated by Dr. Shiddamallyya N. (authentication reference No. RRI/BNG/SMP/Drug Authentication/2007-08/15 dated 09/05/2007). Voucher specimen was deposited at the Regional Research Institute, Bangalore (RRCBB account No.1630).

**Preparation of extract**

The powdered plant material was successively extracted by using Soxhlet extractor. Solvent was recovered, extract concentrated and percentage yield was calculated. The solvents were recovered by using simple distillation method. The charged drug from the central compartment was removed, dried, recharged and extracted by using next solvent of higher polarity than the first solvent. By using Soxhlet extractor, exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity are petroleum ether, chloroform, methanol and finally water. The percentage yield of each extracts was 4.2%, 0.71%, 13.72%, 17.64% w/w on dried plant material basis, respectively. Standard methods11,12 were used for preliminary phytochemical screening of the extract to know the nature of phytoconstituents present in them. From the phytochemical tests, it was
found that the extracts contain steroids, tannins, alkaloids, cardiac glycosides, flavonoids and sugars.

**Preparation of sample**

Different semi-solid fractions of extract were dissolved in corresponding solvent and different concentrations were expressed in terms of $\mu$g/mL of the solvent.

**Drugs used**

Penicillin (10 $\mu$g/mL) and gentamycin (80 $\mu$g/mL) were used as reference standard for antibacterial activity.

**Microorganisms used**

Four different organisms were used for the determination of Minimal Inhibitory Concentration (MIC) of the extracts. Four organism included two gram positive (*Staphylococcus aureus, Bacillus subtilis*) and two gram negative (*Pseudomonas aeruginosa Escherichia coli*). The organisms were procured from Department of Microbiology, The Oxford College of Science, Bangalore. All organisms were maintained at 4°C on slant of nutrient agar and in nutrient broth.

**Preparation of inoculum**

Stock cultures were maintained at 4°C on slant of nutrient agar. For the experiments, inoculum was prepared by subculturing each culture from slants to flask in sterile nutrient broth and incubated at 37°C for 24 hrs.

**Preparation of media**

Nutrient agar medium (NA) was used for preliminary antibacterial study. The medium was prepared by dissolving different ingredients of nutrient agar medium in distilled water and sterilized by autoclaving at 121°C for 15 minutes.

**Antibacterial activity**

**Determination of Minimal Inhibitory Concentration (MIC)**

The disc diffusion method$^{13}$ was used for the determination of minimum inhibitory concentration. *In vitro* antibacterial activity was screened by using nutrient agar (NA). The NA plates were prepared by pouring molten media into sterile petriplates. The plates were
allowed to solidify and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts of different concentrations were loaded on 3 mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter and the lowest concentration of each extract, which is showing inhibition of growth of bacteria, was determined. These studies were performed by using standard drugs (10 µg/disc penicillin and 80 µg/disc gentamycin).

RESULTS AND DISCUSSION

The results indicate that the all extracts possess the antibacterial activity. The MIC of the aqueous extract showed the effectiveness of the extract at low concentration against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* while methanol extract has this activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed minimum inhibitory concentration as compared with standard (Penicillin 10 µg/mL and gentamycin 80 µg/mL). The petroleum ether extract and chloroform extract were active against *Staphylococcus aureus* and *Escherichia coli*. Various extracts were compared for their antibacterial potential with the standard.

The objectives of the present paper were to study antibacterial activity of various extracts of the leaves of *Bauhinia variegata Linn*. The aqueous extract showed MIC against all the organisms at concentration (10 µg/mL, 50 µg/mL) as shown in Table 1. Methanol extract was effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC being 50 µg/mL and 500 µg/mL (Table 1).

**Table 1: MIC (µg/mL) values of different extracts of Bauhinia variegata**

<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Bacillus subtilis</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>100</td>
<td>250</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>500</td>
<td>250</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>
The petroleum ether extract (MIC being 100 µg/mL and 250 µg/mL, respectively) and chloroform extract (MIC being 500 µg/mL and 250 µg/mL, respectively) were active against *Staphylococcus aureus* and *Escherichia coli* and this activity may be due to presence of glycoside, alkaloid and tannins. From the zone of inhibition and MIC calculated for the extracts, it was observed that aqueous extract showed prominent antibacterial activity against all the four microorganisms.

<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Bacillus subtilis</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>6</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Water</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Penicillin (10 µg/mL)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Gentamycin (80 µg/mL)</td>
<td>16</td>
<td>16</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2: Zone of inhibition values (mm) in MIC of different extracts of *Bauhinia vaiegata*

![Fig. 1: E. Coli](image1.png) ![Fig. 2: Bacillus subtilis](image2.png)
ACKNOWLEDGEMENTS

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REFERENCES


Fig. 3: *P. aeruginosa*  
Fig. 4: *Staphylococcus aureus*


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