ABSTRACT

Dysoxylum binectariferum a member of Meliaceae was evaluated for its activity against bacteria and fungi. Methanolic extracts of leaves of this tree when tested by standard Kerby-Bauer disc diffusion methods against some human pathogenic bacteria like Gram positive (Staphylococcus aureus, Bacillus cereus) and Gram negative bacteria (Escherichia coli, Klebsiella pneumoniae) gave significant results. There was a consistent increase in the inhibition zone from 6.75 mm to 8.25 mm by St. aureus, 6.0 mm to 8.75 mm by Ba. cereus, 6.5 mm to 7.75 mm by E. coli and 6.5 mm- 8.5 mm by K. pneumoniae a clear dose specific activity. Similarly fungi (Mucor neithi and Saccharomyces cerevisiae) gave highly promising results in the range of 16.66 mm to 30 mm and 21.66 mm to 40 mm respectively.

KEYWORDS: Dysoxylum Binectariferum Hook. F., Antibacterial, Antifungal, Activity

INTRODUCTION

India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researchers as only 65% flora of the country have been surveyed so far (Jachak and Saklani, 2007). Exploring the possibilities of plant as bioactive viable through basic and applied studies will give us insight about sustainable management of bioresources. Dysoxylum binectariferum Hook. is a large tree endemic to Western Ghats of India belonging to family Meliaceae. Crude extracts of the tree were found to be highly effective against ovarian and breast cancer lines (Mohan kumar et al, 2010). Though limonoids and alkaloids from this tree showed pharmacological activity, its bioactivity against bacteria and fungi was unexplored. Hence this study was conducted to address the inhibitory activity of D. binectariferum against some pathogenic bacteria like Gram positive (Staphylococcus aureus and Bacillus cereus), Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae) and fungi (Mucor neithi and Saccharomyces cerevisiae).
MATERIALS AND METHODS

Leaf source: Fresh, mature, healthy leaves were collected from the tree of *D. binectariferum* growing in Ambegao village near Kolhapur, Maharashtra, India. The plant specimen was authenticated with Prof. S.R. Yadav from Shivaji University, Kolhapur.

Preparation of methanolic extract of *D. binectariferum* leaves:

Methanol extracts of leaves were obtained according to the method of Warthen et al. (1984). Initially, the leaves were crushed to a fine particle size and dried in shade at room temperature. Hundred grams of dried, crushed material was stirred in 1000 ml of methanol. The solution was left overnight and then filtered through Whatman number 40 filter paper. The procedure was repeated for solid filtration and two filtrates were combined. The solvent was evaporated at a temperature of 50 °C and a dark green residue from leaves was obtained. This crude extract was then used to prepare a stock solution. Crude extract was dissolved in methanol and the volume was made up to 500 ml. A drop of Tween -20 was added to the extract for complete solubility in the solution. The stock solution was then diluted to required percentage with water.

Test organisms:

The cultures of bacteria and fungi mentioned in this study were obtained from the Biotechnology Dept. of Parvatibai Chowgule College, Margao Goa.

Bacterial and fungal bioassay:

A standardized procedure for the disk diffusion susceptibility test, called the Kirby-Bauer disk diffusion test (Kerby and Bauer, 1966) was performed in order to test crude extract for Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and Fungi like (*Mucor neithi* and *Saccharomyces cerevisiae*). Though attempt is made to follow standard methods, the experiments were carried out to suit the lab facilities and infrastructure.

Preparation of culture plates: *Nutrient Agar* (Nutrient Hi Veg™ Agar Hi Media Laboratories Private Limited) medium was used for bacterial culture and *Sabouraud Dextrose Agar* (Hi Media Laboratories Private Limited medium) for fungal cultures.

McFarland standards:

Microbial test organisms were prepared as per McFarland standards. 0.5 McFarland standard 100 µl saline cultures were spread over the plate.
Disc preparation and inoculation:
Leaf extracts of known concentrations were immersed with sterilized 5 mm diameter filter paper discs, air-dried and were then placed in Petri dishes containing the spread of bacterial and fungal cultures. Methanol solution was used as control disc. Inoculated plates were incubated at 37\(^0\)c for 24 hours and observed for zone of inhibition, if any. Diameter of the zone was measured in mm. The bioassay was repeated three times and standard deviations were calculated.

Results and Discussion
Bacterial and fungal bioassay conducted showed moderate to high level of inhibitory activity. In case of bacteria though the activity is moderate, the consistent increase in the Inhibition zone diameter from 6.75 mm to 8.25 mm by S. aureus, 6.0 mm to 8.75 mm by B. cereus, 6.5 mm to 7.75 mm by E. coli and 6.5 mm- 8.5 mm by K. pneumoniae though the diameter of disc used being 5.0 mm suggests the dose specific activity. Similarly fungi M. neithi and S. cerevisiae gave highly promising results in the range of 16.66 mm to 30 mm and 21.66 mm to 40 mm respectively.(Table:1; Fig:1)

Though other species of Dysoxylum has been reported for various pharmacological activities D. binectariferum is bioassayed for the first time against bacteria and fungi. Similarly in previous unpublished research work, D. binectariferum methanolic leaf extract when bioassayed against the 3\(^{rd}\) to 4\(^{th}\) instars larvae of An. stephensi gave significant mortality. Thus results promised that D. binectariferum could be the one having broad spectrum bactericidal, fungicidal and mosquito larvicidal principle. Since all of them were sensitive to the plant extract it could be an important candidate for further work like isolation and characterization of bioactive principles.

Table 1: Determination of antibacterial and antifungal activity.
(Zone of inhibition area in ± standard deviation of three replicates.)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. in gms.</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>St.aureus</td>
<td>B. aureus</td>
<td>E.coli</td>
</tr>
<tr>
<td>Control</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>1</td>
<td>6.75±1.2990</td>
<td>6±0</td>
<td>6.5±0.5</td>
<td>6.5±1.1180</td>
</tr>
<tr>
<td>1.5</td>
<td>8±1.5811</td>
<td>8.5±0.5</td>
<td>8.5±0.8660</td>
<td>8±0</td>
</tr>
<tr>
<td>2</td>
<td>8.25±1.089</td>
<td>8.75±0.433</td>
<td>7.75±0.82915</td>
<td>8.5±0.5</td>
</tr>
</tbody>
</table>
Fig1: Antibacterial activity of leaf extracts of D. binectariferum against Gram-positive bacteria A- St. aureus B- B. aureus; Gram-negative bacteria C- E.coli D- K. pneumoniae; Antifungal activity against E- M. neithi F- S. cerevisiae

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References