ASSAY OF ANTIFUNGAL ACTIVITY OF LAWSONIA INERMS L. AND EUCALYPTUS CITRIODORA HOOK.

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ABSTRACT

Lawsonia inermis Linn. and Eucalyptus citriodora Hook. leaf extracts were evaluated against 10 plant pathogenic and 2 human pathogenic fungal species viz. Alterneria solani, Drechslera halodes (Helminthosporium halodes), Rhizoctonia solani (ITCC no. 4574), Fusarium solani, Curvularia lunata, Drechslera graminea, Fusarium moniliforme (ITCC no. 2927), Aspergillus flavus (Navjot 4 NS), A. parasiticus var. globous (MTCC No. 411), Trichophyton rubrum (MTCC 296), Aspergillus fumigatus (MTCC 2550) and Candida albicans (MTCC 227). The dried and powdered leaves were successively extracted with petroleum ether, benzene, chloroform, acetone, ethanol and water using soxhlet assembly. The antifungal activity assay was done by biofilm technique. Acetone extract of L. inermis leaves and Petroleum ether extract of E. citriodora leaves showed highest activity against all tested fungi. The inhibitory effect was significant and better than the synthetic fungicides used as most of the strains showed resistance against fluconazole and amphotericin B.

Key words: L. inermis, E. citriodora, plant pathogenic, human pathogenic, solvent extracts

INTRODUCTION

In recent year’s considerable research is prompted for development of novel antifungal agents with diverse mechanism of action due to continuing increase in incidences of life threatening and invasive fungal infections, limited number of antymycotics, their narrow activity spectrum, fungistatic rather than fungicidal activity and resistance development in fungal species. There are 4 groups of antifungal agents used (as still no licensed fungal vaccines are available) i.e. azoles derivatives (miconazole and fluconazole), polyene macrolides (amphotericin B, AmB), echinocandins (Caspofungin) and fluorinated pyrimidines (flucytosine) and each group has similar mode of action which raising the incidences of development of resistant species of pathogenic fungi\(^a\) for example Candida and Aspergillus species display resistance to azoles\(^b\). Therefore, the development of alternative antimicrobial drug from medicinal plants with broad fungicidal activity has become necessary. The herbal wealth of India and its medicinal properties have a long tradition as referred in ancient literature.

In the present investigation antifungal activity of Lawsonia inermis Linn and Eucalyptus citriodora Hook, two economically important genera of order Myrtales was studied. Lawsonia inermis Linn belongs to family Lythraceae and is commonly known as Heena or Mehndi. It is native plant of North Africa and south-west Asia. The various plant parts of L. inermis were used traditionally for different medicinal as well as cosmetic uses. The leaves and roots were used in ulcers, cough, bronchitis, diarrhoea, leucoderma, boils, anaemia, fever, falling of hair and greyness of hair\(^c\). The plant also has great medicinal importance due to its antibacterial activity\(^d\), antinflammatory and sediaative effects\(^e\) hepatoprotective activity\(^f\), cytoxic activity\(^g\), anti-inflammatory, antipyretic, and analgesic activities\(^h\), antibacterial activity\(^i\) and fungitoxic activity against different fungi\(^j\). Eucalyptus citriodora is an evergreen tree of 24-40 m in height with tall straight trunk belongs to family Myrtaceae. It is commonly known as lemon scented tree due to lemon type smell of aromatic substances in leaves and gum. Eucalyptus oil has great medicinal values due to its anti-inflammatory, antispasmodic, decongestant and antiseptic properties\(^k\). In addition, it also has anti diabetic activity\(^l\). Antibacterial and antifungal properties of Eucalyptus extract and oil are also reported by some authors\(^m,n\). Further screening of these medicinal plants may lead to discovery of novel antifungal compounds. In the present investigation different organic solvent extracts of L. inermis and E. citriodora were evaluated for their antifungal potential against various plant pathogenic and human pathogenic fungi.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of L. inermis and E. citriodora were collected from the campus of University College of Science, MLS University Udaipur in October 2008. The plants were identified by Dr. Roop Singh Rathore Incharge Herbarium, Department of Botany, University of Rajasthan, Jaipur, India and were given voucher specimen nos. RUBL 20427 (Lawsonia inermis) and RUBE 21256 (Eucalyptus citriodora) respectively.

Extraction of plant material

40 g dried leaf powder was successively extracted with 240 ml of each organic solvents in the following series, viz., petroleum ether benzene chloroform acetone ethanol water using soxhlet apparatus. Each time before extracting with next solvent the residual plant material was dried in an oven at 50 °C and used for extraction with next solvent. After complete extraction, respective solvents were evaporated under reduced pressure and the dried extracts thus obtained were stored at 4 °C in airtight bottlenecks for further studies. Percent extractive values were calculated by the following formula and are listed in table-1.

\[
\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100
\]

Table 1 Percent Extractive of Different Fractions of Lawsonia inermis Leaf and E. citriodora leaf extracts

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Percent Extractive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. inermis</td>
<td>E. citriodora</td>
</tr>
<tr>
<td>1.</td>
<td>PE fraction</td>
<td>6.9</td>
</tr>
<tr>
<td>2.</td>
<td>Benzene fraction</td>
<td>6.67</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform fraction</td>
<td>6.45</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone fraction</td>
<td>3.52</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol fraction</td>
<td>18.62</td>
</tr>
<tr>
<td>6.</td>
<td>Aqueous fraction</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Test Fungi

Plant pathogenic fungi namely Alterneria solani, Drechslera halodes (Helminthosporium halodes), Rhizoctonia solani (ITCC no. 4574), Fusarium solani, Curvularia lunata, Drechslera graminea, Fusarium moniliforme (ITCC no. 2927), Aspergillus flavus (Navjot 4 NS), A. parasiticus var. globous (MTCC No. 411) and three human pathogenic fungi i.e. Trichophyton rubrum (MTCC 296), Aspergillus fumigatus (MTCC 2550) and Candida albicans (MTCC 227) were used for experimental work.

Antifungal assay

The antifungal activity of prepared extracts was tested using poison food technique\(^n\). 100 mg of extract was dissolved in 10 ml acetone to prepare 10 mg/ml concentration of stock solution. 1 ml of stock solution was mixed with 9 ml molten sterile potato dextrose agar medium and this mixture was poured into pre-sterilized petri-plates (9 cm diameter) and allowed to solidify at room temperature. Thus prepared petri plates were inoculated aseptically with 5 mm disc of test fungi. The plates were incubated then 28±2°C for seven days. Antifungal activity was measured as a function of increase in growth of 5 mm disc of inoculum. For each fungal strain a negative control was maintained where acetone without extract was used. Standard antifungal agents such as fluconazole, amphotericin, bavistin and mancozeb were also maintained as positive control. The experiment was carried out three times and mean values are presented.

Results

In the present study L. inermis and E. citriodora leaf extracts prepared in various organic solvents were screened for antifungal activity. The inhibitory activity of the extract was compared with standard antifungals like fluconazole, amphotericin, bavistin and mancozeb. Results are presented in table no. 2 and 3.

The percent extractive values of partially purified leaf and seed extracts of L. inermis and E. citriodora were also obtained with different organic solvents. Results are given in table no. 1. Acetone extracts of L. inermis leaf gave maximum percent extractive value i.e. 18.62%, while methanol extracts of E. citriodora leaf gave maximum percent extractive value i.e. 15.85%. Minimum percent extractive value was obtained with benzene fractions which were 0.67% and 2.87 % for L. inermis and E. citriodora respectively.

Results of antifungal assay of L. inermis leaves extract (table no. 2, Fig. 1) suggests that of all the extracts assayed acetone extract was the most effective as it showed significant inhibition of all test fungi which was comparable with that of standards used. Methanol extract also showed significant activity against all plant pathogenic as well as human pathogenic fungi except A. flavus and A. parasiticus, against which petroleum ether and benzene extract was more effective. Significant inhibition of Drechslera graminea, Curvularia lunata, Aspergillus fumigatus and Candida albicans were observed with petroleum ether and...
Table 2 - Antifungal activity of leaf extracts of *C. inermis*

<table>
<thead>
<tr>
<th>Test Fungi</th>
<th>Growth zone (mm) after 7 days (extracts)</th>
<th>PE</th>
<th>BE</th>
<th>CH</th>
<th>AC</th>
<th>ME</th>
<th>A</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria solani</td>
<td>4.10 ± 0.10</td>
<td>17.0 ± 0.0</td>
<td>6.00 ± 0.01</td>
<td>8.40 ± 0.10</td>
<td>9.20 ± 0.07</td>
<td>11.20 ± 0.30</td>
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<tr>
<td>Drechslera halodes</td>
<td>4.10 ± 0.02</td>
<td>18.0 ± 0.5</td>
<td>11.78 ± 0.00</td>
<td>8.27 ± 0.10</td>
<td>9.19 ± 0.00</td>
<td>14.67 ± 0.21</td>
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<tr>
<td>Rhizoctonia solani</td>
<td>9.6 ± 0.15</td>
<td>14.1 ± 0.05</td>
<td>13.9 ± 0.04</td>
<td>15.0 ± 0.05</td>
<td>16.8 ± 0.05</td>
<td>16.61 ± 0.21</td>
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<tr>
<td>Fusarium solani</td>
<td>10.4 ± 0.10</td>
<td>13.5 ± 0.5</td>
<td>13.9 ± 0.41</td>
<td>16.78 ± 0.05</td>
<td>15.4 ± 0.07</td>
<td>26.62 ± 0.03</td>
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<tr>
<td>Curvularia lunata</td>
<td>10.42 ± 0.02</td>
<td>13.45 ± 0.6</td>
<td>11.12 ± 0.32</td>
<td>12.13 ± 0.06</td>
<td>12.23 ± 0.06</td>
<td>12.23 ± 0.04</td>
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</tr>
<tr>
<td>Dreschlera gramineae</td>
<td>6.65 ± 0.45</td>
<td>12.0 ± 0.05</td>
<td>7.23 ± 0.01</td>
<td>8.27 ± 0.03</td>
<td>9.20 ± 0.07</td>
<td>14.30 ± 0.23</td>
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</tr>
<tr>
<td>Fusarium moniliforme</td>
<td>8.45 ± 0.41</td>
<td>19.0 ± 0.2</td>
<td>15.66 ± 0.21</td>
<td>10.23 ± 0.22</td>
<td>9.13 ± 0.06</td>
<td>11.00 ± 0.21</td>
<td></td>
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</tr>
<tr>
<td>Aspergillus flavus</td>
<td>6.23 ± 0.25</td>
<td>11.2 ± 0.84</td>
<td>11.59 ± 0.09</td>
<td>11.55 ± 0.20</td>
<td>10.47 ± 0.28</td>
<td>12.54 ± 0.38</td>
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<tr>
<td>A. parasiticus</td>
<td>10.18 ± 0.04</td>
<td>16.5 ± 0.52</td>
<td>10.53 ± 0.27</td>
<td>15.25 ± 0.14</td>
<td>13.61 ± 0.34</td>
<td>14.09 ± 0.08</td>
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<tr>
<td>Trichophyton rubrum</td>
<td>11.25 ± 0.07</td>
<td>15.66 ± 0.02</td>
<td>12.00 ± 0.02</td>
<td>13.45 ± 0.03</td>
<td>11.80 ± 0.31</td>
<td>15.90 ± 0.03</td>
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<tr>
<td>Aspergillus fumigatus</td>
<td>10.22 ± 0.07</td>
<td>17.88 ± 0.03</td>
<td>13.89 ± 0.03</td>
<td>14.99 ± 0.02</td>
<td>13.23 ± 0.22</td>
<td>13.45 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>11.23 ± 0.04</td>
<td>22.53 ± 1.2</td>
<td>13.12 ± 0.06</td>
<td>14.23 ± 0.23</td>
<td>12.21 ± 0.11</td>
<td>13.12 ± 0.02</td>
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</table>

**DISCUSSION**

On the basis of the results obtained it can be suggested that *C. inermis* and *E. citriodora* have a broad spectrum antifungal activity against various plant pathogenic and human pathogenic fungi. Benzene and acetone fractions showed significant inhibition of all tested fungi. Benzene fraction showed comparable inhibition of *A. solani* and *D. gramineae*. Aqueous extract was ineffective against *F. moniliforme*. A reasonable inhibition of *A. solani* was observed with almost all extracts. Benzene extracts showed maximum activity. All water extracts were found to be significant inhibitors of human pathogenic fungi as compared to plant pathogenic fungi.

Results of antifungal potential of *E. citriodora* leaf are presented in Table no. 3 (Fig. 2). Petroleum ether fraction of leaves showed the maximum inhibition of all tested fungi. Benzene fraction showed comparable inhibition of *A. solani* and *D. gramineae*. Aqueous extract was ineffective against *F. solani*. A reasonable inhibition of *R. solani*, *C. lunata*, *A. flavus* and *C. albicans* was observed with almost all extracts. *E. citriodora* extracts showed more effective inhibition in comparison to *C. inermis* extracts.

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