ANTI CANDIDAL ACTIVITY OF PLANT EXTRACTS AGAINST BOVINE MASTITIS ISOLATES

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Mastitis is one of the major cause of economic loss to dairy industry. The association of fungi alone or along with bacteria makes milk not suitable for human consumption resulting in huge economic loss to the dairy industry. The continuous and indiscriminate use of antibiotics should be avoided in the treatment of mixed infections of yeast along with bacteria. (Blood and Radostitis,1989). Despite the existence of potent antifungal agents, no drug formulations are available in the market for fungal mastitis treatment. The traditional medicinal plants represent a reservoir of pharmacologically active substances or drugs (Rukayadi et al.,2008).

This study was aimed at preliminary screening of plant extracts against Candida spp isolated from cases of bovine mastitis. Selected plant extracts were screened for anti-Candidal activity against six bovine mastitis Candida isolates.

MATERIALS AND METHODS

Aqueous extracts of Terminalia chebula, Tinospora cordifolia obtained from Natural Remedies, Bangalore and alcoholic extract of Vitis vinifera seeds from Sabi labs Ltd, Bangalore, were used in this study. Aqueous extract of Tamarindus indica leaves was prepared following standard extraction procedure and utilized for screening against mastitis isolates. The fungal strains tested include six species of Candida isolated from Bovine mastitis in the Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai. The fungal strains were grown in Sabouraud dextrose agar and stock cultures were maintained in-20°C in soybean casein digest broth containing 15% glycerol. Basic biochemical test was done to identify the organisms. Two species each of Candida glabrata, Candida lipolytica and two identified only up to the species level were used in this study.

AntiCandidal assay

Antifungal susceptibility test was performed following the procedures described by Fontenelle et al., (2007) and Parekh and Chanda (2008). Fungal seeded plates with 100µl of 1 x 108 CFU/ml were allowed to solidify by keeping in room temperature for 20mins and then transferred to refrigerator. Wells of 5mm diameter were cut and the different extract concentrations of 150, 300, 500 mg/ml dissolved in 5% DMSO was incorporated into the wells, incubated at 37°C for 24-48 hrs and zones of inhibition was measured in millimeters using digital calipers. Each test was performed in duplicate. 5% DMSO solution and Amphotericin B (10?g/ml) were used as negative and drug control respectively. Plant extracts showing positive results by agar well diffusion assay was further subjected to minimum inhibitory concentration. MIC was assessed by microbroth dilution assay using Resazurin indicator (Sarkar et al. 2007).
RESULT AND DISCUSSION

AntiCandidal activity of four plant extracts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Canida lipolytica</th>
<th>Candida lipolytica</th>
<th>Candida glabrata</th>
<th>Candida spp. (1)</th>
<th>Candida spp. (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONC mg/ml</td>
<td>150</td>
<td>300</td>
<td>500</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Tamarindus indica</td>
<td>-</td>
<td>8.56</td>
<td>13.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T. chebula</td>
<td>13.21</td>
<td>16.58</td>
<td>26.88</td>
<td>12.82</td>
<td>16.77</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>17.15</td>
<td>17.64</td>
<td>13.91</td>
<td>14.41</td>
<td>15.66</td>
</tr>
<tr>
<td>Amphotericin B 10μg/ml</td>
<td>22.87</td>
<td>18.35</td>
<td>1.23</td>
<td>16.77</td>
<td>18.65</td>
</tr>
<tr>
<td>5% DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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The antifungal activity of the extracts tested is shown on Table 1. The test extracts inhibited the Candida species with varying degrees of sensitivity. Maximum zone of inhibition above 20mm was shown by T.chebula against Candida lipolytica and T. cordifolia against Candida spp (2) followed by Vitis vinifera seed extract above 17mm against Candida lipolytica and it inhibited 5 isolates out of six tested. Terminalia chebula was able to inhibit four out of six isolates tested in this study. Among the isolates tested Candida glabrata was not susceptible to any of the extracts and other isolate of C. glabrata was susceptible only to Vitis vinifera and T. cordifolia extracts only at higher concentrations tested. The diameter of the zone is concentration dependent as shown in the table and maximum zone was observed at higher concentration.

The MIC of Vitis vinifera seed extract, Terminalia chebula, Tinospora cordifolia and Tamarindus indica ranges from 0.625-0.078 mg/ml, 0.3125-0.625mg/ml, 2.5-5mg/ml and 0.156mg/ml respectively. MIC 50 of Amphotericin B was 625 µg/ml for all the isolates tested.

Antimicrobial efficacy of grape fruit seed against C. albicans was shown by Cvetnic et al., (2004). Antibacterial activity of Vitis vinifera seed extract was established against B. cereus, B. coagulans, B. subtilis, S. aureus, E. coli and P. aerugenosa by Jayaprakasha et al., (2003). The widest antibacterial activity of T.chebula was shown by Phadke and Kulkarni (1989); Ahmed et al.,(1998). Antibacterial activity of T. cordifolia against Gram positive cocci and rods was shown by Damodaran,(2004). The broad-spectrum antibacterial activity of T. indica was shown by Srinivasan et al.,(2001). Dougari (2006) established the antibacterial activity of T. indica leaves against S. aureus and E. coli but no antifungal activity was observed by them against C. albicans in their study. The leaf extracts showed lower activity against the test organism in their study but we could demonstrate antifungal activity of T. indica leaf extract against some Candida spp. Elizabeth (2005) showed T. chebula extract reducing the growth of C. albicans. Parekh and Chanda (2008) showed that C. glabrata was the most resistant strain against the extracts tested in their study. One isolate of C. glabrata was resistant to all the extracts screened and other isolate was inhibited by Vitis vinifera seed extract and T. cordifolia at higher concentration only. Yigit et al.,2009 showed that isolates of Candida spp tested in their study were found to be resistant to most plant extracts.

Barberan et al.,(1990) showed that presence of flavinoids, terpenes and certain degree of lipophilicity might determine the toxicity to fungi by interactions with the membrane constituents and their arrangement.

This study has revealed that the extracts tested possessed good antiCandidal activity against the isolates of Candida used in this study. The extracts of Vitis vinifera and T. chebula have been shown to possess greater activity and can form potential candidates for the treatment of bovine fungal mastitis.

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1.150 mg/ml; 2. 300 mg/ml; 3. 500 mg/ml  

Vitis vinifera seed extract on *C. lipolytica*

1.150 mg/ml; 2. 300 mg/ml; 3. 500 mg/ml  

AmphotericinB 10 µg/ml T. cordifolia extract on *C. glabrata*

1.150 mg/ml; 2. 300 mg/ml; 3. 500 mg/ml  

T. chebula extract on *Candida spp* (1)