Studies on Bioassay Directed Antifungal Activity of Medicinal Plants
_Calotropsis procera, Skimmia laureola, Peltophorum pterocarpum_ and two
pure Natural compounds ulopetrol and 4-methoxy-1-methyl-
3-(2'S-hydroxy-3'-ene butyl)-2-quinoline

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(Received 24th October, 2002, revised 28th July, 2003)

Summary: Our investigations on the antifungal activity of the crude ethanolic extracts of
different plants including _Calotropsis procera, Skimmia laureola and Peltophorum
pterocarpum_, have led to the determination of good results of these activities. We report the
evaluation of antifungal activity of crude extracts of _Calotropsis procera, Skimmia laureola_ and
_P. pterocarpum_. In addition, we also report the antifungal activity of ulopetrol (1) and 4-
methoxy-1-methyl-3-(2'S-hydroxy-3'-ene butyl)-2-quinoline (2) isolated from _Skimmia
laureola_ [1]. These plants were selected because of their reported medicinal importance in
indigenous system of medicines from ancient times and are abundantly available in various
parts of Pakistan [1,2].

Introduction

_Calotropsis procera_ (Ait) R. Br. (Asclepiadaceae), a xerophytic shrub widely distribu-
ted in the tropics of Asia and Africa is commonly known as "Akra" in India and Pakistan. In the traditional Indian
medicinal system, different parts of the plant have been advocated for a variety of diseases and have
also been considered as an antidote for snake poisoning [2]. It has been reputed in the Indian
traditional medicine for a variety of ailments including leprosy, ulcer and piles. Different parts of

_Skimmia laureola_ is found in the Northern areas of Pakistan and is used in the indigenous
system of medicine for the treatment of various ailments. The soot obtained from the burning of
leaves is inhaled for treatment of body pain, fever and influenza [5].

_Peltophorum pterocarpum_ is an ornamental tree grown in homes and gardens in India and
Pakistan [6], bearing fragrant yellow flowers, reported to posses antibacterial, anti-inflammatory
[7] and fungitoxic activity [8].

Results and Discussion

The crude extract of _Calotropsis procera_ exhibited antifungal activity [9] against Trichophytyn
longiformis, Candida albicans, Aspergillus flavus, Microsporium canis, Fusarium solani and Fusarium
moniliformis. The minimum inhibitory concentration (MIC) of crude extract against these fungi was used
as 400 µg/ml. The growth of Trichophytyn longiformis, Candida albicans and Fusarium
moniliformis was inhibited in 100% by the crude extract at a concentration of 400 µg/ml, while
standard fungicide Miconazole and Ketoconazole totally inhibited the growth at a concentration of 70
µg, 110.8 µg and 110.8 µg respectively. The growth of Microsporium canis was inhibited in 90% by the
crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the
growth at the concentration of 98.4 µg/ml. These results are summarized in Table-1.

The crude ethanolic extract of _S. laureola_ showed good activity against animal pathogen
_Microsporium canis_ and plant pathogen _Fusarium solani_ var. _lycopersici_ (Tomato) at a concentration of
400 µg/ml. Minimum inhibitory concentration (MIC) of the crude extract against _Microsporium canis_ was
used as 400 µg/ml. The growth of _Microsporium canis_ was inhibited in 67.7% by the crude extract at a
concentration of 400 µg/ml, while standard fungicide Miconazole and Ketoconazole totally inhibited the
growth of _Microsporium canis_ at a concentration of 72.10 and 62.25 µg/ml, respectively. The crude
extract of _S. laureola_ was found to be active against _Fusarium solani_ var. _lycopersici_ at a concentration of

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Table 1: Antifungal activity of crude extract of *C. procera*.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Sample</th>
<th>Control</th>
<th>% Inhibition</th>
<th>Standard Drugs</th>
<th>% Inhibition</th>
<th>MIC µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton longisporum</em></td>
<td>0</td>
<td>90</td>
<td>100</td>
<td>Miconazole</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>Ketoconazole</td>
<td>100</td>
<td>110.8</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>Ketoconazole</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>10</td>
<td>100</td>
<td>90</td>
<td>Miconazole</td>
<td>100</td>
<td>98.4</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>95</td>
<td>95</td>
<td>0</td>
<td>Ketoconazole</td>
<td>100</td>
<td>73.25</td>
</tr>
<tr>
<td><em>Fusarium moniliformis</em></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>Miconazole</td>
<td>100</td>
<td>110.8</td>
</tr>
</tbody>
</table>

Concentration of crude extract = 400 µg/ml.

Table 2: Antifungal activity of crude extract of *Skimmia laureola*.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Crude extract of <em>S. laureola</em></th>
<th>% Inhibition</th>
<th>Miconazole</th>
<th>Ketoconazole</th>
<th>Benlate</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microsporum canis</em></td>
<td>400 µg/ml</td>
<td>67.7</td>
<td>72.10</td>
<td>62.25 µg/ml</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>400 µg/ml</td>
<td>57.7</td>
<td>-</td>
<td>-</td>
<td>73.25 µg/ml</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Antifungal activity of ulopterol (1).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Compound (1)</th>
<th>% Inhibition</th>
<th>Miconazole</th>
<th>Ketoconazole</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Drechslera rostrata</em></td>
<td>200 µg/ml</td>
<td>50.8</td>
<td>25 µg/ml</td>
<td>25 µg/ml</td>
<td>100</td>
</tr>
<tr>
<td><em>Curvularia lunata</em></td>
<td>200 µg/ml</td>
<td>49.5</td>
<td>25 µg/ml</td>
<td>25 µg/ml</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Antifungal activity of 4-methoxy-1-methyl-3(2'S-hydroxy-3'-ene butyl)-2-quinolone (2).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Compound (2)</th>
<th>% Inhibition</th>
<th>Miconazole</th>
<th>Ketoconazole</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microsporum canis</em></td>
<td>200 µg/ml</td>
<td>68.7</td>
<td>72.10 µg/ml</td>
<td>62.25 µg/ml</td>
<td>100</td>
</tr>
<tr>
<td><em>Pseudoallescheria boydii</em></td>
<td>200 µg/ml</td>
<td>56.8</td>
<td>38.75 µg/ml</td>
<td>29.50 µg/ml</td>
<td>100</td>
</tr>
</tbody>
</table>

400 µg/ml, while standard fungicide Benlate completely inhibited the growth of *Fusarium solani* var. *lycopersici* at a concentration of 73.25 µg/ml. *Fusarium solani* var. *lycopersici* causes root rot, stem cankers associated with wounds, damping-off seedlings, destruction of spawn in beds of cultivated mushrooms and pea crop. The antifungal results are summarized in Table 2.

Compound (1) exhibited *in vitro* antifungal activity against *Drechslera rostrata* and *Curvularia lunata* with minimum inhibitory concentration of 200 µg/ml. Compound (1) also exhibited weak activity against *Curvularia lunata*. The fungicides Miconazole and Ketoconazole were used as standards. The results of antifungal assay results are summarized in Table 3.

Compound 2 exhibited some *in vitro* antifungal activity against *Microsporum canis* and *Pseudoallescheria boydii*. *Microsporum canis* causes infection of hair and skin in dogs and cats, while *Pseudoallescheria boydii* causes infection of skin, subcutaneous tissue, nasal sinuses and mycetoma. The minimum inhibitory concentration (MIC) of the compound 2, against *Microsporum canis* and *Pseudoallescheria boydii* was 200 µg/ml. The growth of *Microsporum canis* and *Pseudoallescheria boydii* was strongly inhibited (68.7 and 56.8%) by the compound 2, while standard fungicide Miconazole and Ketoconazole totally inhibited the growth of *Microsporum canis* and *Pseudoallescheria boydii* at 72.10 µg/ml, 62.25 µg/ml and 38.75 µg/ml, 29.50 µg/ml concentration respectively [1,10]. The results of antifungal assay are summarized in Table 4.

The crude extract of *P. pterocarpum* exhibited activity against a number of fungi. The in vitro antifungal activity was tested against Trichophyton longisporus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glabrata. Minimum inhibitory concentration of crude extract against *Microsporum canis* was used as 400 µg/ml. The growth of *Microsporum canis* was inhibited in 65% by the crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the growth of *Microsporum canis* at a concentration of 98.4 µg/ml. The crude extract of *P. pterocarpum* was found to be active against Trichophyton longisporus and Aspergillus flavus at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the growth of *Trichophyton longisporus* and *Aspergillus flavus* at a concentration of 70 µg/ml and 20 µg/ml respectively. The crude extract of *P. pterocarpum* exhibited no activity against Candida.
albicans, Fusarium solani and Candida glabrata. These results are summarized in Table-5.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Sample</th>
<th>Control</th>
<th>% Inhibition</th>
<th>Standard drugs</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton longisagus</td>
<td>70</td>
<td>95</td>
<td>26.3</td>
<td>Miconazole</td>
<td>70</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>Miconazole</td>
<td>110.8</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>90</td>
<td>95</td>
<td>15.7</td>
<td>Amphotericin B</td>
<td>20</td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>45</td>
<td>100</td>
<td>65</td>
<td>Miconazole</td>
<td>98.4</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>Miconazole</td>
<td>73.25</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>95</td>
<td>95</td>
<td>0</td>
<td>Miconazole</td>
<td>110.8</td>
</tr>
</tbody>
</table>

Concentration of sample = 400 µg/ml.

Sabouraud agar media were inoculated with 200 ml of compound pipetted from the stock solution. This gave a final concentration of 200 µg/ml of media. The tubes were then allowed to solidify in a slanted position at room temperature and the tubes were incubated at 27-29°C for 7-10 days and the visible growth observed. Minimum inhibitory concentrations (MIC) were expressed in µg/ml.

Acknowledgment

The authors fully acknowledge and highly appreciate the cooperation, support and kind help of Professor Dr. Muhammad Iqbal Choudhary for allowing us to conduct Bioassay studies of these medicinal plants at HEJ Research Institute of Chemistry, University of Karachi.

References