Antidermatophytic activity of *Pistia stratiotes*

Dermatophytes belonging to the three genera, *Trichophyton*, *Epidermophyton* and *Microsporum* affect the keratinous tissue of humans and of other vertebrates, causing superficial fungal infections.[1] The present study reports the *in vitro* antidermatophytic activity of methanolic leaf extract of *Pistia stratiotes* against a battery of dermatophytes. *P. stratiotes* (Araceae) is an aquatic, floating stoloniferous herb commonly found in ponds and streams. The leaves are obovate, light green in color with many prominent longitudinal veins surrounded at its base by a membranous sheath which is free-floating and spreads in the water.[2]

*P. stratiotes* leaves are used in traditional medicine for the treatment of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils, and wounds. The oil extract of *P. stratiotes* is used in the treatment of worm infestations, tuberculosis, asthma, and dysentery, and is applied externally to treat skin diseases, inflammation, piles, ulcers, syphilitic infections and burns.[3]

The leaves of *P. stratiotes* used in the present study were collected from the natural habitat in and around Chennai, Tamil Nadu. The Voucher specimen was compared with the Herbarium specimen (No.279) deposited at the Presidency College, Chennai. The collected leaves were cleaned, dried in the shade and ground into a fine powder from which 500 g were extracted repeatedly with 2 liters of methanol using soxhlet extractor at 50 ºC for 72 h. The extracts were filtered using Whatman filter paper (No.1) and concentrated in vacuum at 40 ºC using a rotary evaporator and the residues obtained were stored in a freezer at -80 ºC until further tests.

The American Type Culture Collection (ATCC) strains and clinical isolates of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Microsporum nanum* and *Epidermophyton floccosum* were obtained from the Department of Dermatology, Sri Ramachandra Medical College and Research Institute, Porur, Tamil Nadu. The fungal inoculum was prepared from a 21-day-old culture of dermatophytes by scraping with a sterile scalpel and macerating the scrape in 10 ml sterile distilled water. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values of the *P. stratiotes* extract were determined by microplate dilution method,[4] in which 96-well microtiter plates were prepared by dispensing into each well 95 μl of Sabouraud’s Dextrose Broth (SDB) and 5 μl of the fungal inoculum. A 100 μl of serially diluted *P. stratiotes* extract ranging from 500 μg/ml to 7.8 μg/ml was added in each well with the 1st well having 500 μg/ml concentrations and the 7th well having a concentration of 7.8 μg/ml. Negative control contained 200 μl of SDB without extract and inoculum. Miconazole in a concentration range of 96 to 1.5 μg/ml was prepared in SDB, containing fungal inoculum and used as reference standard drug (positive control). The final volume in all the wells was 200 μl. The contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at 28ºC for 10 days. MIC was determined by measuring the absorbance at 450 nm using the ELx 800 universal micro-plate reader (Biotek Instrument Inc.) and the MFC was determined by plating 5 μl of samples from the microtiter plate into Sabouraud’s Dextrose Agar (SDA) medium and incubating it at 28ºC for 10 days.

The results indicate that *P. stratiotes* methanolic extract was found to be the most active against the dermatophytes *T. rubrum*, *T. mentagrophytes* and *E. floccosum* with MIC and MFC values of 250 μg/ml, while against *M. gypseum* and *M. nanum*, the values were 125 μg/ml (Table 1). The values are

<table>
<thead>
<tr>
<th>Organism Isolate- Number</th>
<th>Methanolic extract (μg/ml)</th>
<th>Miconazole (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC</strong></td>
<td><strong>MFC</strong></td>
<td></td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em> ATCC 28188 10</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em> ATCC 9533 12</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td><em>Microsporum gypseum</em> ATCC 24102 5</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td><em>Microsporum nanum</em> ATCC 11832 5</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em> ATCC 52066 10</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

N = 15. The values are same for all the 15 replicate experiments. MIC—Minimum Inhibitory Concentration. MFC—Minimum Fungicidal Concentration. μg/ml—Microgram/milliliter. ATCC — American Type Culture Collection.
same for all the 15 replicate experiments. The results show that the trichophyton and epidermophyton species are more resistant to the extract and were inhibited at a higher dosage compared to the microsporum species. The MIC that inhibited the growth of fungi in the SDA medium indicated the fungicidal activity of the extract. The MIC and MFC of the methanolic extract of *P. stratiotes* were similar and this shows that MIC can be used as an indicator of fungicidal activity as mentioned in earlier studies on the effect of neem on dermatophytes. Miconazole at a concentration of 3 μg/ml inhibited all the clinical isolates and ATCC strains of tested dermatophytes. Determinations of the MIC of miconazole for all the reference strains were performed concomitantly to validate the methodology. The negative control showed no fungal growth. Previous reports on the chemical nature of *P. stratiotes* leaves showed the presence of alkanes, flavonoids and sterols. Antifungal activity of alkanes, flavonoids and sterols has been described by earlier workers. No data on the antimicrobial activity of *P. stratiotes* appears to have been published. The results of the present work indicate that *P. stratiotes* leaves possess antifungal properties, which explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections, and underline the importance of the ethnomedical approach for the selection of this plant in the discovery of new bioactive compounds. Further phytochemical research is needed to identify the active principles responsible for the antifungal activity of *P. stratiotes*.

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References


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