In vitro activity of some medicinal plants from Cachar district, Assam (India) against Candida albicans

Afjal Hussain Mazumder,1* Jayshree Das,1 Hemanta Kumar Gogoi,1 Pronobesh Chattopadhyay,1 Lokendra Singh2 and Satya Bhushan Paul3

1Defence Research Laboratory Tezpur, Post Bag No.-02, Assam-784001, India
2Directorate of Life Sciences, Ministry of Defence, Govt. of India, DRDO HQ New Delhi, India
3Department of Chemistry, Assam University, Silchar, Assam-788011, India

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ABSTRACT

Introduction: The current research has focused on the potentiality of medicinal plants for treatment of Candida albicans infections. Five plants viz. Clerodendron colebrookianum Walp. (Leaf), Gnetum gnemon L. (Leaf), Sarcochlamys pulcherrima (Roxb.) Gaud. (Leaf), Garcinia lancifolia (Don) Roxb. (Leaf) and Euryale ferox Salisb. (Seed), used as traditional medicines in Cachar district, Assam, India were selected to evaluate in vitro activity against C. albicans.

Methods: The plant samples were extracted with methanol. Agar well diffusion assay was used to test the activity of the plant extracts and broth microdilution method was used to determine the MIC.

Results: All extracts showed anticandidial activity with zones of inhibition ranging from 17 to 25 mm at 2 × 10^5 µg/ml extract. E. ferox and S. pulcherrima showed the highest activity with the MIC value of 1.25 × 10^4 µg/ml. The remaining extracts were comparatively less effective showing MIC value of 2.5 × 10^4 µg/ml.

Conclusions: Anticandidial activity of the plants extracts, observed in this study highlighted further in vivo investigation and identification of the active compounds for therapeutic uses. The anticandidial activity of S. pulcherrima and G. lancifolia is probably the first report to the best of our knowledge.

Keywords: Anticandidial activity, Euryale ferox, Garcinia lancifolia, Sarcochlamys pulcherrima.

INTRODUCTION

Candida species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to systemic candidiasis. Among the Candida species, the most predominant species is Candida albicans.[3] Clinico-mycological profile from the Northeast India also revealed that C. albicans was the common pathogen amongst the Candida species.[2] Emergence of multi drug resistant strains and failure of existing antifungal agents to cure Candida infections especially C. albicans is now a therapeutic challenge. Within the limited data available, an increased incidence of invasive candidiasis, aspergillosis and zygomycosis was reported by Chakrabarti et al. (2008) of which the invasive candidiasis is the most common opportunistic mycosis.[3]

Cachar district of Assam, India, an excellent reservoir of flora, together with the traditional information on their medicinal uses acts as a natural resource to target antimicrobial agent. Medicinal plants used traditionally could be one potent source for such antifungal agents for treatment of Candida infections, as evidenced from the results of antimicrobial screening of medicinal plants, reported from time to time. Selection of plants, based on ethnopharmacological perspective enhances the probability of success in new drug discovery efforts.[11,12] Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of new drugs.[13] Results of the extensive research on herbs, carried out throughout the world, unfurled the potentiality of developing antimicrobial agents from the plants.[4-10]

In the present study we have considered Clerodendron colebrookianum Walp. (Leaf), (Verbenaceae), Gnetum gnemon L. (Leaf), (Gnetaceae) Sarcochlamys pulcherrima (Roxb.) Gaud. (Leaf), (Urticaceae) Garcinia lancifolia (Don) Roxb (Leaf), (Clusiaceae) and Euryale ferox Salisb. (Seed),
(Nymphaeaceae) against Candida albicans. The plants were chosen on the basis of traditional information on medicinal uses by the tribal people of Cachar district of Assam. Moreover, the selection criteria included edibility of the plant parts and thus our research methodology will rise above the possibility of toxicity of the extracts to a maximum extent. Most of the parts of E. ferox are highly consumed by the people of Manipur, India and Manipuri people of Cachar District, Assam. While, tender leaves of other four plants are consumed by Khasi and Naga tribes of Machkhal, Binnakandi, Ramnagar and few other tribal pockets of Cachar district.

The fruits of E. ferox can be preserved for a longer period without any preservative agent, which may be attributed to the compounds with antimicrobial property. Chakma tribe in hill tracts districts of Bangladesh uses leaf paste of S. pulcherrima to treat boils and fever blisters and fresh leaf extract as eye drop. Garcinia species were reported to be good source of biologically active substances. Phytochemical screening indicated dominance of phenolic compounds in Garcinia species. However no information is available on G. lancifolia in respect to its antimicrobial activity as well as its chemical constituents. The leaf sap of Gnetum gnemon is used to cure an eye complication. Extracts from fruits and seeds of Gnetum possess antibacterial and antioxidant activity and also exert various physiological (pharmacological) activities and hence proposed to be used as an active ingredient of food, nutritional supplement, medicine, cosmetic etc. The ethnomedicinal informations are shown in Table 1. In spite of having several medicinal properties of these plants, so far anticanidial activity has not been reported. Hence, the present study was undertaken to test the efficacy of these plant species against Candida albicans.

### MATERIALS AND METHODS

#### Plant materials and extraction

Fresh plant samples were collected from Arun Punjee (Punjee means Tribal Village) of Machkhal, Cachar district, Assam during the month of April. The taxonomic identities of the plants were authenticated at Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, India. The herbaria were deposited at the herbarium repository of Defence Research Laboratory, Tezpur, Assam. Extract was prepared by soaking powdered air dried plant sample in methanol for 7 days. The solution was filtered, concentrated under reduced pressure at 40°C and lyophilized. The extractive values were recorded (Table 2) and kept in -20°C till further use.

**Table 1. Ethnomedicinal information of the test plants.**

<table>
<thead>
<tr>
<th>Species Family</th>
<th>Local name (Community)</th>
<th>Part used</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. colebrookianum</td>
<td>Jhurkhtang (khasi)</td>
<td>Leaves</td>
<td>High blood pressure</td>
</tr>
<tr>
<td>Verbenaceae</td>
<td></td>
<td></td>
<td>Athletes foot, eye complication</td>
</tr>
<tr>
<td>G. gnemon</td>
<td>Shyang (khasi)</td>
<td>Leaves</td>
<td>Boils and fever blisters, itching of eyes, to damage tape worm egg in pork</td>
</tr>
<tr>
<td>Gnetaceae</td>
<td></td>
<td></td>
<td>Headache</td>
</tr>
<tr>
<td>S. pulcherrima</td>
<td>Khajathshear (Khasi)</td>
<td>Leaves</td>
<td>Digestive disorders, diarrhoea etc. (personal interrogation)</td>
</tr>
<tr>
<td>Urticaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. lancifolia</td>
<td>Shasuit (Khasi)</td>
<td>Leaves</td>
<td></td>
</tr>
<tr>
<td>Clusiaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. ferox</td>
<td>Thangjing (Manipuri)</td>
<td>Seeds</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Anticanidial activity and total phenolic content (TPC) of plant extracts.**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>EV (%) w/w</th>
<th>Conc. (µg/ml)</th>
<th>TPC* (µg/ml)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 × 10²</td>
<td>1 × 10³</td>
<td>5 × 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZOI</td>
<td>I%</td>
<td>ZOI</td>
</tr>
<tr>
<td>C. colebrookianum</td>
<td>23.35</td>
<td>17</td>
<td>0.57</td>
<td>21.3</td>
</tr>
<tr>
<td>G. gnemon</td>
<td>23.02</td>
<td>18</td>
<td>0.60</td>
<td>22.5</td>
</tr>
<tr>
<td>S. pulcherrima</td>
<td>19.89</td>
<td>21</td>
<td>0.70</td>
<td>26.3</td>
</tr>
<tr>
<td>G. lancifolia</td>
<td>20.40</td>
<td>20</td>
<td>0.67</td>
<td>25.0</td>
</tr>
<tr>
<td>E. ferox</td>
<td>17.80</td>
<td>25</td>
<td>0.83</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Correlation coefficient: 0.899

Clotrimazole (100 µg/ml): ZOI: 17mm

DMSO (100%): No zone of inhibition

**EV:** Extractive value, **ZOI:** Inhibition zone (diameter in mm), **AI:** Activity index, **% I:** Inhibition percentage, **MIC:** Minimum inhibitory concentration.

* In mg Gallic Acid equivalent (GAE)/mg of dried extract.
prepared in dimethyl sulfoxide (DMSO). From the stock, the test extracts of desired concentrations were prepared in DMSO and sterilized using millipore filter (0.22 μm pore size).

_Candida albicans_ and inoculum preparation

_C. albicans_ (MTCC 3018) was cultured on sabouraud dextrose agar (SDA) slants at 28±2°C for 48 hours and stored at 4°C. Broth inoculum was prepared using sabouraud dextrose broth (SDB) and incubated at 28±2°C for 48 hours and final inoculum concentration of 1 × 10^8 CFU ml⁻¹ was prepared.[29]

Anticandidial assay

The activity of the plant extracts against the test organism was evaluated by agar well diffusion assay with some modification.[26] Inoculum of 150 μl was swabbed on a SDA plate, a well of 7 mm diameter was made in each agar plate and loaded with 200 μl of the respective test extracts (2 × 10^5, 1 × 10^5 and 5 × 10^4 μg/ml) The SDA plates were incubated at 28±2°C for 48 h. Anticandidial activity of each extract was expressed in terms of diameter of inhibition zone (mm) exhibited by the extracts. DMSO (100%) was used as negative control while clotrimazole (100 µg/ml) was used as positive control. Each experiment was replicated thrice and repeated twice. The activity index of the extracts and percent inhibition were calculated as follows.

Activity index = Zone of inhibition by extract
Zone of inhibition by standard antimicrobial agent

% Inhibition = Zone of inhibition(mm) × 100
Control

*Growth zone is equal to plate diameter i.e., 80 mm, as growth occurs all over the agar plate.

Determination of MIC

Minimum inhibitory concentration (MIC) values of the extracts were determined by broth microdilution method[28] with some modifications. Stock solution of each sample was serially diluted in 96-well microtiter plate with RPMI 1640 (Rosewell Park Memorial Institute, Himedia) to obtain a concentration ranging from 2.5 × 10^4 to 1.56 × 10^4 μg/ml. Inoculum density of 1 × 10^6 CFU/ml was approximately adjusted in each well and incubated at 28±2°C for 48 h. Clotrimazole was used as standard. The MIC of extract was interpreted as the lowest concentration, at which no visible growth was seen. Each experiment was performed in triplicate and repeated twice.

Statistical analysis

Statistical analysis was done with SPSS, version 17.0.

RESULTS AND DISCUSSION

Previous workers showed antifungal activity of _Zingiber officinalis_ and _Juglans cinera_ against a diverse group of human pathogenic fungi including strains that are highly resistant to amphotericin-B and ketoconazole and interestingly, in many cases, the medicinal plants were found more effective than the commercial antimicrobial drugs.[30]

Similarly, Sehgal et al. (2005) reported the higher inhibitory action of petroleum ether and methanol extract of latex from _Calotropis procera_ against _Candida albicans_, as compared to griseofulvin.[31] Even crude ethanol extracts of _Acacia nilotica_, _Cinnamomum zeylanicum_ and _Syzygium aromaticum_ showed good activity against multidrug resistant strains, isolated from nosocomial and community acquired infections, where modern antibiotic therapy has failed. The antimicrobial potency of these plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids.[32] Several other studies demonstrated that _Candida_ species are either highly resistant or less sensitive towards plant extracts as compared to moulds and other pathogenic microbes.[33–39]

Anticandidial activity and MIC of the plant extracts

In the initial screening, all the plants showed anticandidial activity, as evidenced by clear zone of inhibition ranged between 9 and 25 mm within the concentrations tested. In DMSO treated plate no zone was observed while clotrimazole (100 µg/ml) produced zone of inhibition of 17 mm. Activity of clotrimazole at very low concentration, as compared to griseofulvin. Even crude ethanol extracts of _Acacia nilotica_, _Cinnamomum zeylanicum_ and _Syzygium aromaticum_ showed good activity against multidrug resistant strains, isolated from nosocomial and community acquired infections, where modern antibiotic therapy has failed. The antimicrobial potency of these plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids.[33–39] Several other studies demonstrated that _Candida_ species are either highly resistant or less sensitive towards plant extracts as compared to moulds and other pathogenic microbes.[33–39]

In our previous study, varied total phenolic contents of the extracts (0.456–0.0429 mg GAE/mg dry weight of
extract) was recorded (Table 2), with highest phenolic content in *E. ferox* seed extract (0.456 mg GAE/mg DW) followed by *S. pulcherrima* leaf extract (0.33 mg GAE/mg DW).[22] The strong correlation of total phenolic content with antifungal activity might be due to phenolic compounds, which are responsible for antioxidant action. Significant antioxidant activity of *E. ferox* was reported earlier.[31] The observed antifungal activity of the plant extracts might be related to the high level of phenolic compounds along with other phytochemicals.[22,31] Earlier reports projected a positive correlation of phenolic compounds with antimicrobial activity.[19] However, the two parameters may not always correlate.[25] Total phenolic content estimated in the extracts in our earlier study showed strong negative correlation with the MIC of the extracts (correlation coefficient is −0.774, which signifies strong correlation between two variables).

The reported biological activity of the plants as well as their extensive use in traditional practice suggests that these plants have the potential to be a very useful antimicrobial agent particularly against *Candida albicans*. Although medicinal property of *E. ferox* and its traditional uses for treatment of various ailments have been reported earlier, information on scientific evaluation for its antifungal activity has not been done yet.[24,31] On the other hand, the significant activity of *S. pulcherrima* and *G. lancifolia* against *C. albicans* is probably the first report.

**CONCLUSIONS**

The results highlighted the possible use of *E. ferox*, *S. pulcherrima* and *G. lancifolia* as therapeutic agents against *Candida albicans*. However toxicity and clinical studies are required to validate the use of these medicinal plants in therapeutics. The antifungal activity of *S. pulcherrima* and *G. lancifolia* is the first time report, to the best of our knowledge. Further studies are going on *in vitro* evaluation on animal model, isolation and characterization of bioactive compounds from these plants.

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