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 Singh[2] and Satya Bhushan Paul[3]

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

***Corresponding author.**
Afjal Hussain Mazumder,
Defence Research Laboratory Tezpur, Post Bag No.-02, Assam-784001, India
E-mail: mdafjal123@rediffmail.com

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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.[4–6,8–10]

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[15] 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,[14] 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.[16] *Garcinia* species were reported to be good source of biologically active substances.[17,18] Phytochemical screening indicated dominance of phenolic compounds in *Garcinia* species.[19] 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.[20] 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.[21] The ethnomedicinal informations are shown in Table 1. In spite of having several medicinal properties of these plants, so far anticandidial 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.[23] 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. Stock solution of each extract (2×10^5 µg/ml, w/v) was

Table 1. Ethnomedicinal information of the test plants.

Species Family	Local name (Community)	Part used	Uses
C. colebrookianum Verbenaceae	*Jhurkhthang* (khasi)	Leaves	High blood pressure[34]
G. gnemon Gnetaceae	*Shayang* (khasi)	Leaves	Athletes foot[22], eye complication[20]
S. pulcherrima Urticaceae	*Khajathshear* (Khasi)	Leaves	Boils and fever blisters, itching of eyes[16], to damage tape worm egg in pork[22]
G. lancifolia Clusiaceae	*Shasuit* (Khasi)	Leaves	Headache[22]
E. ferox Nymphaeaceae	*Thangjing* (Manipuri)	Seeds	Digestive disotders, diarrheaetc.[14] Eye ailment (personal interrogation)

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

Plant species	EV (%) w/w	Conc. (µg/ml)									TPC[a]	MIC (µg/ml)
		2×10^5			1×10^5			5×10^4				
		ZOI	AI	I%	ZOI	AI	I%	ZOI	AI	I%		($\times 10^4$)
C. colebrookianum	23.35	17	0.57	21.3	11	0.36	13.8	9	0.30	11.3	0.0429	2.5
G. gnemon	23.02	18	0.60	22.5	12	0.40	15.0	10	0.33	12.5	0.0452	2.5
S. pulcherrima	19.89	21	0.70	26.3	16	0.53	20.0	13	0.43	16.3	0.33	1.25
G. lancifolia	20.40	20	0.67	25.0	14	0.47	17.5	12	0.40	15.0	0.0443	2.5
E. ferox	17.80	25	0.83	31.3	24	0.80	30.0	20	0.67	25.0	0.456	1.25
Correlation coefficient			0.899			0.908			0.887			–0.774
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.

[a] 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.[8]

Anticandidial assay

The activity of the plant extracts against the test organism was evaluated by agar well diffusion assay with some modification.[24] 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)[25] 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.

$$\text{Activity index} = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard antimicrobial agent}} \quad [26]$$

$$\% \text{ Inhibition} = \frac{\text{Zone of inhibition (mm)}}{*\text{Control}} \times 100 \quad [27]$$

*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 1×10^8 CFU/ml^{-1} approximately was 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 officinale* 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.[4] 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.[5] Even crude ethanol extracts of *Acacia nilotica, Cinnamum 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.[6] 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.[7–10]

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 the plant extracts may be attributed to its pure nature. On subsequent test for determination of MIC value, varying degree of sensitivity of the yeast towards each of the extracts (2.5×10^4 to 1.56×10^4 µg/ml) was recorded. In this study, *E. ferox* (seed) and *S. pulcherrima* (leaf) were found to be the most active with MIC value of 1.25×10^4 µg/ml. The MIC values of the remaining extracts were 2.5×10^4 µg/ml (Table 2). Since the crude extracts are mixture of multiple components, both active and non active, they are considered as effective even the MICs were high. Similar results were also recorded earlier.[29–30]

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. pulcherimma* leaf extract (0.33 mg GAE/mg DW).[22] The strong correlation of total phenolic content with anticandidial 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 anticandidial activity of the plant extracts might be related to the high level of phenolic compounds along with other phytochemicals.[22,32] Earlier reports projected a positive correlation of phenolic compounds with antimicrobial activity.[33] However, the two parameters may not always correlate.[23] 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.[14,31] 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 anticandidial activity of *S. pulcherrima* and *G. lancifolia* is the first time report, to the best of our knowledge. Further studies are going on *in vivo* evaluation on animal model, isolation and characterization of bioactive compounds from these plants.

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