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Anti-candidal effect of endophytic fungi isolated from *Calotropis gigantea*

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Abstract: *Candida albicans* is a most common cause of fungal infections in animals and birds. Understanding the increasing resistance of *C. albicans* to various antifungal therapeutic agents is important to discover new anti-candidal alternatives. The present study investigated the anti-candidal potential of five endophytic fungi extracts, isolated from the tropical ethnoveterinary plant, *Calotropis gigantea*. We firstly evaluated the *in vitro* antifungal activities of endophytic fungi extracts by the well diffusion method. Secondly, the cells of *C. albicans* were treated with the potent extract to observe significant ultrastructural changes. To further investigate the *in vivo* antifungal activity of the extract, some laboratory experiments with mice were undertaken, and posteriourly, the different organs were studied under the electron microscope for any deformities. *Phomopsis asparagi* showed the best anti-candidal activity with a minimum inhibitory concentration (MIC) of 46.9 µg/mL. The fungal test pathogen (*C. albicans*) exhibited various cell deformities when treated with the extract of *P. asparagi.* Histopathological studies of the vital organs of mice treated with the potent fungal extract did not show any significant pathological conditions when viewed under scanning electron microscope. Thus, *P. asparagi* can be a potential candidate for anti-candidal agents against *C. albicans.* Future studies will focus on the isolation of the bioactive components of the extract. Rev. Biol. Trop. 65 (4): 1437-1447. Epub 2017 December 01.

Key words: endophytic fungi, anti-candidal, *Candida albicans*, scanning electron microscope, ultrastructure.

Candida albicans is recognized to cause many important diseases in animals and birds, and represents an important veterinary pathogen. *C. albicans* mostly affects birds causing thrush affecting the oral mucosa, oesophagus and crop. Cattle, calves, sheep and foals are also susceptible to systemic candidiasis which occurs as a consequence of prolonged treatment with antibiotics and corticosteroids (Kuwamura, Ide, Yamate, Shiraishi, & Kotani, 2006). *C. albicans* infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungal, relapse of *Candida* infections and non-cost effective antifungal agents (Sasidharan, Zuraini, Latha, & Suryani, 2008). Therefore, there is an urgent need to design new drugs and to explore alternative sources of novel drugs.

Northeast (NE) India is considered one of the most important biodiversity hotspots of the globe with a wide range of physiographic and ecoclimatic conditions (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000). Northeast India in the Eastern Himalayan range is located between 20°35’37.2624”N - 78°57’46.368”E, and sprawling over 262 379 km^2 (Lyngwi, Koijam, Sharma, & Joshi, 2013). In tropical and subtropical countries, with hot and humid climates like India, with ideal conditions for fungal infections, turn them into a common problem among human and animal populations (Naglot et al., 2015). These areas, with hot,

humid and muggy weather, have favoured the growth of different fungal infections, and have worsened the symptoms of candida infections (Pannu, McCarthy, Martin, & Sutcliffe, 2009).

In Northeast India, the ethnoveterinary plant *Calotropis gigantea* is widely used by the local livestock farmers to increase appetite in animals. Furthermore, there are reports on the biological activities of this plant, to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhea (Srivastava, Singh, & Rawat, 2015). However, there are no reports on the biological activities of the endophytic fungi associated with these plants. Endophytes are harmless residents of the internal living tissues of plants (Bacon & White, 2000). These are known to produce some bioactive secondary metabolites which can protect their hosts from infectious agents and adverse conditions, and produce pharmacologically active compounds (Strobel, Daisy, Castillo, & Harper, 2004; Verma, Kharmar, & Strobel, 2009). In previous works, there have been some reports on the antimicrobial (Tanaka, Fukushima, Tsujiro, & Fujimori, 1997; Gong & Guo, 2009; Du, Li, Li, Shang, & Wang, 2012), antioxidant (Huang, Chen, Chang, Sheu, & Lin, 2007; Dhankhar, Kumar, Dhankhar, & Yadav, 2012; Nath, Raghunatha, & Joshi, 2012), anti-inflammatory (Deshmukh et al., 2009; Pretsch et al., 2014) activity of the endophytic fungi. Besides, some *in vivo* studies on the anti-cancer, anti-diabetic and other bioactivity (Konrath et al., 2012; Govindappa et al., 2015; Tan et al., 2015) have been evidenced, but *in vivo* antimicrobial studies on endophytic fungi are very scarce (Peláez et al., 2000; Nath & Joshi, 2016).In the present study, we aimed to identify *C. gigantea* fungal endophytes and to characterize their anti-candidal activity in an *in vivo* model.

MATERIALS AND METHODS

Collection of plant samples: Based on adequate information obtained from the local farmers of the tribal belts of Northeast India, the stem and leaves of the ethnoveterinary plant, *Calotropis gigantea* L. were collected in the month of April, 2013 (Vegetative form). The samples were then brought to the laboratory and were kept in 4 ºC for further processing.

Isolation of endophytic fungi: Plants were washed properly in running tap water and different parts of the plants (stem and leaves) were excised into segments of 0.5-1 mm in length. The segments were kept in 70 % ethanol (Lab Chemicals Ltd., India) for 1min followed by 4 % sodium hypochlorite solution for 3 min and again kept in 70 % ethanol for 1 min. The segments were rinsed thoroughly in sterile distilled water and then kept in laminar airflow for drying. Properly dried segments were inoculated into Potato Dextrose agar (PDA) supplemented with 100 µg/mL of streptomycin and incubated at 25 °C until fungal growth appeared from the segments (Petrini, 1986). Hyphal tips from the fungal growth were then subcultured to fresh PDA plates and preserved in sterile distilled water at room temperature.

Molecular characterization of endophytic fungi: Genomic DNA was isolated from the respective isolates using genomic DNA extraction kit (HiMedia, India). PCR amplification and sequencing of ITS rRNA gene was carried out in a 50 µL reaction mixture using primers ITS1 and ITS4 with the following conditions, denatured at 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, annealing at 52 °C for 30 sec, extension at 72 °C for 1 min, final step was carried out at 72 °C for 10 min (Nath & Joshi, 2016) using PCR Gene Amp 9700 (Applied Biosystems, USA). The amplified ITS rRNA gene was then purified using QIAquick Gel Extraction Spin Kit (QIAGEN, Germany). Sequencing of ITS rRNA gene were performed with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) using the same forward and reverse primers. The nucleotide sequences were then analyzed with the sequences obtained from NCBI database using BLAST and aligned by using the Clustal W program. Phylogenetic

tree was constructed using MEGA 4.1 software (Tamura et al., 2011). The sequences were deposited to the NCBI database and accession numbers were obtained.

Preparation of plant extract: The stem and leaves of *C. gigantea* were thoroughly washed in running water and then were finally rinsed in distilled water. The washed plants samples were then air dried in hot air oven at 50 °C for 72 h with forced air, after which the dried plants were grounded into smooth powder by using a clean pestle and mortar. Then, 100 g of the powdered plant sample were dissolved in ethanol (300 mL) for 4 days at room temperature with intermittent stirring. After four days, the mixture was filtered through Whatman No. 1 paper and was concentrated under vacuum using a rotary evaporator. The dried crude plant extract was then dissolved in dimethylsulfoxide (DMSO) to make a final concentration of 5 mg/mL, and then were sterilized by filtration using a 0.22 µm membrane for antimicrobial assay (Vijayarathna et al., 2012). The resultant extract was kept at 4 °C for further analysis.

Extraction of fungal crude extract: Fungal hyphae were cut into small pieces and were inoculated in a conical flask containing 100 mL of sterile potato dextrose broth (PDB). The flask was then incubated at 25 °C for 10-15 days. After obtaining proper growth, the broth was filtered through muslin cloth to separate the mycelia from the broth. The filtrate was then mixed with thrice the volume of 70 % ethyl alcohol and filtered through Whatman filter paper No.1. The solvent phase was evaporated under reduced pressure using rotary vaccum evaporator at 50 ºC (Nath & Joshi, 2013). The residues were re-dissolved in 50 % dimethyl sulphoxide (DMSO) to make a final concentration of 5 mg/mL for subsequent analysis.

***In vitro* antimicrobial activity by well diffusion method:** The antimicrobial activity of the ethanolic extract of endophytic fungi was tested against the *Candida albicans* (MTCC 183). All the tests were performed in triplicates. Test fungus was subcultured on Potato dextrose broth (PDB) for 48 h. The antifungal test was carried out on Mueller Hinton Agar (MHA) plates. Mueller Hinton agar plates were prepared and a well of 7 mm was made with sterile cork borer. One milliliter of broth containing the test micro-organisms was swapped on the agar plates. A volume of 50 µL of extracts was put in the wells and the plates were incubated at 37 °C for 24-48 h. The plates were then observed for inhibition zone (Nath, Chattopadhyay, & Joshi, 2015).

Determination of Minimum inhibitory concentration (MIC): MIC of the plant and fungal extracts along with the standard drug Fluconazole was determined by NCCLS Broth Microdilution (BM) method on sterile microtiter plate with 96 flat bottomed (Nascente et al., 2009) with slight modifications. The *C. albicans* isolate was suspended in saline solution with the turbidity adjusted to level 1 of the McFarland scale. A total of 100 µL of Potato dextrose Broth were dispensed in each well. The stock solution of the extracts was diluted to a concentration of 1.5 mg/mL and 50 µL were transferred into the first well, followed by serial dilution of the stock solution of extract to get the concentrations in the range of 1.5-1 500 µg/mL. Then 100 µL of saline solution containing fungal suspension was added to all the wells. The plates were then sealed properly to avoid dehydration of fungus and were incubated at 37 °C for 72 h. The readings were made visually, comparing the growth of the fungus in all the test wells with the well that had the positive control. MIC was taken as the lowest concentration that produced a significant inhibition (around 50 %) of the growth of the fungi with the positive control.

Ultrastructural analysis of pathogens by scanning electron microscopy (SEM): After treatment with the potent endophytic fungi, the test pathogens were analysed under scanning electron microscope (JSM-6360, Jeol) for their ultrastructural deformities. Test organisms were treated with 4x concentration of the MIC

of the fungal extracts and incubated overnight at 37 °C. The cells were pelleted after centrifugation at 8000 rpm for15 min. The pellets were then washed with PBS pH 7.3 several times to remove the debris and fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 4 hr. Glutaraldehyde was drained carefully and the pellets were washed again in PBS for several times to remove any traces of glutaraldehyde. The pellets were then smeared on small glass slide and brought to room temperature for drying. Finally, samples were sputter coated with a thin layer of gold–palladium and scanned under SEM.

Experimental animals: Male Swiss albino mice weighing between 25-30 g and aged between 5-6 weeks were procured from Pasteur's Institute, Shillong, Meghalaya. Mice in cages were placed in a room (temperature 25 ± 2 ºC) with controlled cycles of 12 h of light and 12 h of darkness. Water and food (Amrut, India) were provided to animals *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). The mice were acclimatized for one week before the experiments were carried out.

Acute toxicity studies: Acute oral toxicity test was performed as per OECD-423 guidelines (Ecobichon, 1992). Before the conduct of the experiment, the animals were starved for 24 h with free access to *ad libitum* water. Different animal groups were administered orally with the extracts at a dose level of 10 mg/kg body weight and were observed for 2 weeks. If among a group of six mice there was mortality seen in 2-3 mice, the dose was then considered as a toxic dose, and if only one animal died, then the same dose was repeated for the confirmation of toxic dose. If there was no mortality in the different animal groups (six in each group), then those animals were administered with extract doses of 100, 250, 500, 1 000 mg/kg body weight. Subsequently, the animals were observed for any toxicity/mortality for the next 14 days.

Study of antifungal activity in mice: The animals were divided into five groups consisting of five animals in each group: Control (Gp1) which received only sterile distilled water. *C. albicans* was grown for 72 hrs in PDB, centrifuged and washed in phosphate buffered saline and finally diluted in PBS to achieve ~$1x10^5$ CFU/mL. A volume of 0.1 mL of the fungal suspension was administered to the different groups (Gp 2-5) of animals through the intravenous route (Vijayarathna et al., 2012). After 1 h of post infection, the animal groups (Gp 3, 4) were administered orally with the fungal extract at doses of 100 and 250 mg/kg body wt, respectively for eight successive days. Fluconazole at a concentration of 10 mg/kg body weight was administered orally to the last group of animals (Gp 5). Different animal groups were checked for any change in body weight and body temperature every alternate day.

Assessment of fungal infection in vital organs: To assess the efficacy of fungal extract treatment on the establishment of infection in animals, two animals from each group were sacrificed on day 4 after infection and the rest of the animals after the end of the experiment i.e. on day 8. Liver and spleen samples from the sacrificed animals were collected aseptically, homogenized properly in sterile PBS and were swapped on Potato dextrose agar for the re-isolation of the organism to observe the number of viable fungi (Owais, Sharad, Shehbaz, & Saleemuddin, 2005).

Scanning electron microscope (SEM) observation of tissues: The liver and spleen tissues of mice were cut into small segments and were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 12 h. Glutaraldehyde was drained out carefully and washed thrice for 1 h in 0.1 M cacodylate buffer. The samples were dehydrated with acetone series (50, 70, 80, 90, 95 and 100 %) and drying was done with TMS (Trimethyl silane). Tissues were immersed twice in TMS for 10 min at 4 °C and were brought to room temperature

for drying. Finally, samples were sputter coated with a thin layer of gold-palladium and scanned under SEM.

The results of antifungal activity were expressed as mean ± SE values and data were tested by one-way analysis of variance. Differences were considered significant at $p<0.05$.

RESULTS

Isolation of endophytic fungi: In this study, a total of five endophytic fungi, three from the stems and two from the roots of the ethnoveterinary plant (*C. gigantea*), and were isolated based on morphological characteristics. The endophytic fungi were identified as *Phomopsis asparagi*, two strains of *Colletotrichum gloeosporioides*, *Calonectria eucalypti* and *Xylaria* sp. (Fig. 1). All the endophytic fungi were isolated from leaves, except *P. asparagi* which was isolated from the stem. The fungal isolates were further characterized based on the analysis of the phylogenetic relationship of the isolates using ITS region gene sequences. The ITS gene sequences of all the fungal isolates were submitted to NCBI GenBank and accession numbers were obtained (KF928280, KF928284, KM282291, KF928290 and KF928285).

Antifungal activity: Among the five endophytic fungi, only *P. asparagi* and *C. gloeosporioides*, showed antifungal activity against *C. albicans*. The ethanolic extract of *P. asparagi* showed considerable anti-candidal activity as it showed a highest inhibition zone. The plant extract of *C. gigantea* also showed antifungal activity with a narrow inhibition zone (Table 1). Considering their inhibition zone, results showed antifungal activity from the different endophytic fungi, and were significantly ($p<0.05$) different from each other.

Minimum inhibitory concentration (MIC): MICs of all tested fungal extracts ranged from 46.9-375 µg/mL (Table 1).

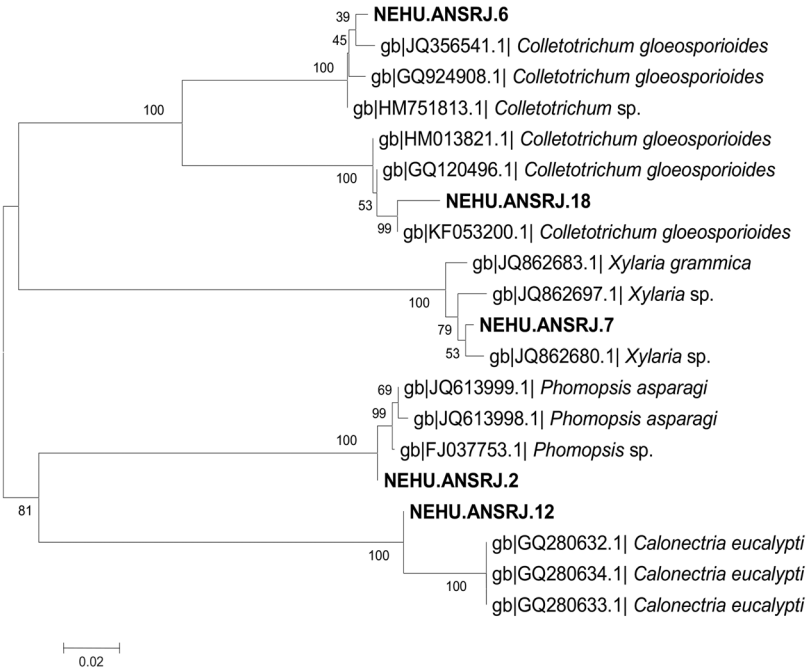

Fig. 1. Phylogenetic relationships generated for the five endophytic fungi isolated from *Calotropis gigantea* L. and the ITS sequences of closely related fungal strains retrieved (14 March, 2016) from NCBI GenBank.

TABLE 1
Inhibition zone and minimum inhibitory concentration observed for the ethanolic extracts of endophytic fungal and plant when compared to Fluconazole

	Ethanolic Extract (5 mg/mL)	Inhibition Zone (mm)	MIC (µg/mL)
Endophytic fungi	Phomopsis asparagi (NEHU. ANSRJ.2)	23 ± 0.67	46.9
	Colletotrichum gloeosporioides (NEHU. ANSRJ.6)	–	–
	Colletotrichum gloeosporioides (NEHU. ANSRJ.18)	16 ± 0.58	93.75
	Calonectria eucalypti (NEHU. ANSRJ.12)	–	–
	Xylaria sp. (NEHU. ANSRJ.7)	–	–
Plant	Calotropis gigantea	12 ± 0.58	375
Antibiotic	Fluconazole	28 ± 0.67	11.8
Negative Control	Sterile Water	–	–

The ethanolic extract of *P. asparagi* showed a significant anti-candidal activity with a MIC of 46.9 µg/mL. Besides, the Fluconazole positive control also showed significant anti-candidal activity (MIC 11.8 µg/mL) when compared to plant and fungal extracts.

SEM analysis of pathogens ultrastructure: The fungal test pathogen also revealed various cell deformities when treated with the extract of *Phomopsis asparagi*. Normal cells of *C. albicans* were spherical with smooth walls, while the treated fungal cells showed presence of deformities such as wrinkled and disintegrated structures. Further several small invaginations and convolutions appeared on *C. albicans* cell surfaces after the treatment (Fig. 2). Approximately, 70 % of the treated *C. albicans* cells were found to show structural deformities.

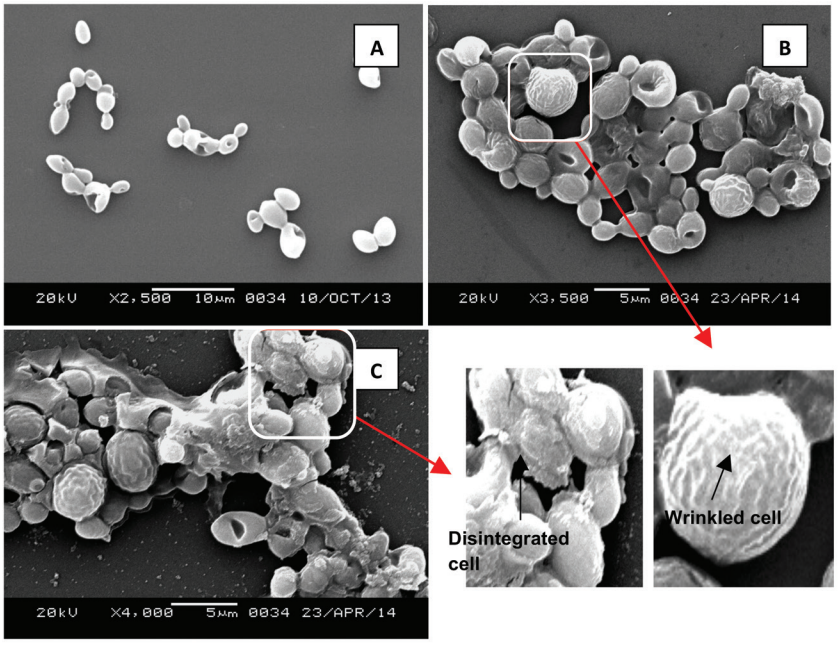

Fig. 2. (A) SEM micrographs of normal fungal cell of *C. albicans,* (B) Presence of wrinkled cells with a few normal cells of *C. albicans,* (C) Disintegrated cells of *C. albicans.*

TABLE 2
Candida albicans count (CFU/g of tissue) in vital organs of mice model treated with ethanolic extract of *Phomopsis asparagi* and antibiotics

Animal Groups	Fungal load in liver (CFU/g of tissue) post infection	
	4 days	8 days
Gp 1 (Control)	ND	ND
Gp 2 (Infected)	1.11×10^5	2.1×10^7
Gp 3 (Infected+ crude extract at 100 mg/kg body wt. of animal)	1.22×10^5	1.98×10^5
Gp 4 (Infected+ crude extract at 250 mg/kg body wt. of animal)	1.38×10^4	1.12×10^4
Gp 5 (Infected+ Fluconazole at 10 mg/kg body wt. of animal)	1.65×10^3	1.13×10^3

ND- Not Detected.

Acute toxicity test: The fungal isolate (*P. asparagi),* which showed significant anticandidal activity in terms of the highest zone of inhibition and MIC, were then evaluated *in vivo* by testing the extract in the mice model. No mortality was seen in the animals, however, animal groups administered with the extract doses of 500 and 1 000 mg/kg body wt showed tachycardia. Hence, the doses of 100 and 250 mg/kg body weight were determined as safe for the experiment.

Antifungal activity in animals: The animals of Gp 4 and Gp 5 treated with 250 mg/kg body wt. extract and 10 mg/kg body wt. fluconazole showed a high survival rate of 80 and 100 %, respectively. Gp 2 showed 80 % mortality rate within seven days of infection. Gp 3 with an extract dose of 100 mg/kg body wt., did not show therapeutic effect on the animals, and 40 % mortality was observed. The extract with a concentration of 250 mg/kg body wt. showed a good effect to lower mortality and 80 % survival rate, and its effect was near comparable to the commercial antibiotic fluconazole having 100 % survival rate. No significant changes were observed on the different group of animal's body temperature, but a gradual loss of body weight was observed in Gp 2 and Gp 3. A significant reduction in colony forming unit (CFU) was observed in the liver of members of Gp 4 and Gp 5, which received the extract at a dose of 250 mg/kg body wt. and fluconazole at a dose of 10 mg/kg body wt., when compared to control group (Table 2).

SEM analysis of vital organs: Tissues samples (liver and spleen) of the different groups of infected animals with *Candida albicans,* and those treated with the potent extract and the reference drug Fluconazole, were analyzed under SEM for any pathological changes. SEM analysis of the *C. albicans* cells treated with the potent extract (*P. asparagi*) showed ultrastructural deformities like wrinkled and disintegrated structures. Liver samples showed marked histopathological changes (Fig. 3) in the the affected animals, compared to the normal tissues of the healthy animals; but no changes could be found in spleen tissues.

DISCUSSION

The approaches used in this study attained the endophytes identification beyond the generic level. These were identified as *Phomopsis asparagi*, *Colletotrichum gloeosporioides*, *Calonectria eucalypti* and *Xylaria* sp. The phylogenetic analysis of their ITS rDNA sequences, resulted in a useful region for the molecular characterization and proper identification of fungal isolates (Bridge & Arora, 1998).

Among the five isolated endophytic fungi, only *P. asparagi* and *C. gloeosporioides* showed antifungal activity against *C. albicans*. Our data indicated that the fungal extracts

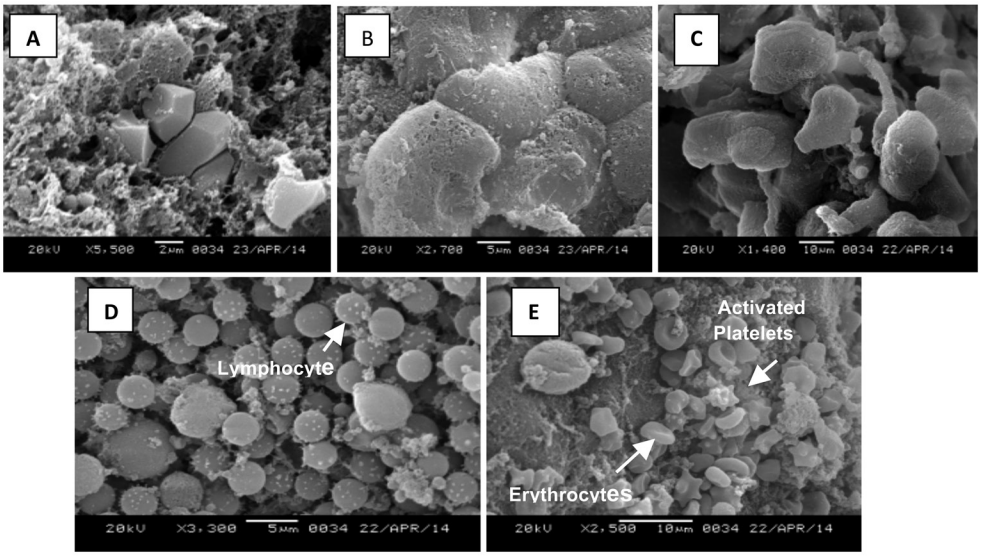

Fig. 3. (A) Scanning electron micrographs of liver cells of normal control mice showing smooth hepatocytes. (B) Mice infected with *C. albicans* showing rough and disintegrated cell surface of hepatocytes. (C) Treated group of mice (100 mg/kg body wt.) showing presence of both normal and disintegrated hepatocytes. (D) (E) Treated group of mice (250 mg/kg body wt.) showing presence of lymphocytes and activated platelets along with erythrocytes.

showed better anti-candidal activity than the plant extract. The potent anti-candidal activity was revealed by *P. asparagi* in terms of the highest zone of inhibition (23 mm), which is comparable to that of the standard Fluconazole (26 mm) with *P. asparagi,* showing the lowest MIC of 46.9 µg/mL against *C. albicans*. Isolation of *P. asparagi* from the medicinal plant has been reported earlier (Faria et al., 2016) but no report on anti-candidal activity of these endophytic fungi is available.

SEM study was used to view any surface alterations or general morphological changes of *C. albicans* cells after exposure to *P. asparagi* extract, which corroborated the *in vitro* antimicrobial results observed. Our SEM analysis of the *C. albicans* cells treated with the potent extract of *P. asparagi,* showed the ability of the extract to cause damage to the fungal pathogen. These results are in agreement with Vijayarathna et al. (2012) report, which also showed the destruction of cell membrane of *Candida albicans* cells.

In vivo activity of medicinal plants has been scarcely reported (Vijayarathna et al., 2012) and this represents one of the first reports available on the anti-candidal properties of endophytic fungi. In the present study, the most potent endophyte, *P. asparagi* was subjected to *in vivo* assays to assess its efficacy in an animal model, in cognizance to the artificial systemic infection of *C. albicans* in mice (Vijayarathna et al., 2012). The use of an *in vivo* model in mice was suggested by previous results of *in vivo* oral toxicity tests, in which the dose rate of 100 mg/kg body weight and 250 mg/kg body weight were considered safe for the experiment, since it did not show any mortality or abnormal physiological changes. In the animal model of this study, the fungal extract of *P. asparagi* at a dose of 250 mg/kg body weight, showed significant antifungal activity, in terms of low mortality, low fungal load in liver, and minor histopathological conditions in liver of the affected animal. Besides, SEM analysis of liver tissues, showed marked pathological changes in the affected and treated group of animals (wrinkled and disintegrated structures) in comparison to that of the normal tissues of healthy animals. The liver of the treated animals also

showed the presence of various immune cells like lymphocytes and platelets which play an important role in combating infections. The liver of the affected group showed the presence of disintegrated hepatocytes, whereas the treated group showed the presence of both disintegrated and normal hepatocytes which is in agreement with the histopathological findings of Khan et al. (2003) who reported the preservation of healthy architecture of the liver cells, which was attributed to liver regeneration. However, no such changes were observed in spleen tissues of the affected and treated animals. The results suggested that the *P. asparagi* extract possess both the fungicidal activity and the ability to potentiate the immune system as it can destroy *Candida* cells as well as activate various immune cells like lymphocytes and platelets.

During the last three decades, *C. albicans* has been the most prevalent pathogen in systemic fungal infections (Pfaller MA, 2004). Although the antifungal active principles are diverse and numerous, only a few classes of antifungal agents are currently available to treat yeast infections, due to the high toxicity of many of them (Spampinato & Leonardi, 2013). High morbidity and mortality rates due to opportunistic yeast infections represent the ineffectiveness of the current antifungal therapies.

The present study revealed the effective *in vivo* anti-candidal activity of the extract of *P. asparagi,* and thus can be recommended as a potential source for development of a safe natural anti-candidal product for commercial utilization. Nevertheless, the information available of these compounds is still poor, but offer potential sources of novel natural products, for exploitation in medicine, agriculture and the pharmaceutical industry (Strobel & Daisy, 2003). The potency of *P. asparagi* against this pathogen is attributed to the presence of effective antimicrobial agents in the endophytic fungal extract. However, the isolation of bioactive components and their characterization is underway for deeper insight into their functions.

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RESUMEN

Efecto anticandida de hongos endofíticos aislados de *Calotropis gigantea*. *Candida albicans* es la causa más común de infecciones fúngicas en animales y aves. La comprensión de la creciente resistencia de *C. albicans* a varios agentes terapéuticos antifúngicos es importante para descubrir nuevas alternativas anti-candida. El presente estudio investigó el potencial anti-candida de cinco extractos de hongos endofíticos, aislados de la planta etnoveterinaria tropical, *Calotropis gigantea*. En primer lugar se evaluaron las actividades antifúngicas in vitro de los extractos de hongos endofíticos por el método de difusión de pozos. En segundo lugar, las células de *C. albicans* se trataron con un potente extracto para observar cambios ultrastructurales significativos. Para investigar más a fondo la actividad antifúngica in vivo del extracto, se realizaron algunos experimentos de laboratorio con ratones, y posteriormente, los diferentes órganos se estudiaron bajo microscopio electrónico para cualquier deformidad. *Phomopsis asparagi* mostró la mejor actividad anti-candida con una concentración inhibitoria mínima (MIC) de 46.9 µg/mL. El patógeno de prueba fúngico (*C. albicans*) presentó diversas deformidades celulares al ser tratado con el extracto de *P. asparagi*. Los estudios histopatológicos de los órganos vitales de los ratones tratados con el potente extracto fúngico no mostraron ninguna condición patológica significativa cuando se observaron con un microscopio electrónico de barrido. Por lo tanto, *P. asparagi* puede ser un candidato potencial para agentes anti-candida contra *C. albicans*. Los estudios futuros se centrarán en el aislamiento de los componentes bioactivos del extracto.

Palabras clave: hongos endofíticos, anti-Candida, *Candida albicans*, microscopio electrónico de barrido, ultraestructura.

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