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Fern extracts potentiate fluconazole activity and inhibit morphological changes in *Candida* species

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ABSTRACT

Objective: To investigate the antifungal activity of the fern species *Lygodium venustum* (*L. venustum*) and *Pityrogramma calomelanos* (*P. calomelanos*) against *Candida albicans* and *Candida tropicalis* strains.

Methods: The microdilution method was used to evaluate the antifungal activity, as well as the modulating effects of ethanolic extracts of these plants in combination with fluconazole. The minimum inhibitory concentration (MIC), minimum fungicide concentration and morphological changes were also determined.

Results: The extract obtained from *L. venustum* presented a MIC > 8 192 μg/mL, while the extract obtained from and *P. calomelanos* presented a MIC = 8 192 μg/mL, indicating that they present weak antifungal activity. However, combination of the extracts with Fluconazole potentiated the antifungal activity of this drug. At different experimental conditions, such as concentration of the extract and type of strain, the extracts inhibited hyphae and pseudohyphae formation, indicating that these fern species can affect the morphology of the fungi.

Conclusions: The extracts obtained from the fern species *L. venustum* and *P. calomelanos* dose not present significant antifungal activity. However, *P. calomelanos* potentiates the activity of fluconazole and both extracts inhibits the morphological changes in *Candida* species, indicating that they have potential pharmacological activity as modulators of fungal biology. Therefore, novel studies are required to characterize the interference of these extracts in the virulence and pathogenicity of *Candida* species as well as the potential of fern species to treat fungal infections.

1. Introduction

Candida species are commensal microorganisms in healthy individuals. However, under certain conditions, such as immunosuppression, these fungi act as opportunistic pathogens, causing various infectious diseases. Thus, the infections caused by *Candida* spp. can range from simple colonization of the mucous membranes to systemic infections, which represent important public health problems due to the high morbidity and mortality rates [1].

Candida albicans (*C. albicans*) is one of the main causative agents of human infections [2]. As in most opportunistic infections, infections caused by this microorganism are favored by failures in the immune response of the host, as well as by the virulence mechanisms and morphological changes of the fungus [3]. *Candida tropicalis* (*C. tropicalis*) is

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an opportunistic pathogen that infects mainly neutropenic patients, causing diseases that are associated with suppression of the bacterial microbiota by uncontrolled antibiotic therapy [4].

Although the infections caused by *C. albicans* are most frequent, other species of the genus, such as *C. tropicalis, Candida glabrata, Candida krusei* and *Candida parapsilosis* have been highlighted as important infectious agents [5]. Therefore, the development of targeted therapies to overcome mechanisms of virulence, as well as to prevent and control fungal infections has gained increasing importance in the field of health research [6,7].

Antifungal agents may present a broad range of pharmacological activities and mechanisms of action by which they exert toxic effects to the microorganisms. Therefore, the discovery of drug targets in the microorganisms is crucial to the development of novel, effective and safe antifungal therapies [8]. In this context, the use of medicinal plants to treat fungal infections has been reported by several studies [9]. In fact, the knowledge of the use of medicinal plants has been handed down from generation to generation in many cultures and places [10], serving as a basis for the treatment of mycoses in traditional medicine [11].

Lygodium venustum (*L. venustum*) is a fern species that is traditionally used as an herbal remedy. The aerial parts of this plant are administrated topically or in the form of teas to treat numerous diseases, including infections and dermatosis [12]. However, the pharmacological activity of this plant against pathogenic microorganisms remains to be characterized [13–15].

The fern species *Pityrogramma calomelanos* (*P. calomelanos*), popularly known as 'feto-branco', 'avenca-branca' or 'avenca-preta', is used both in the decoration of environments and in medicine [16,17]. In folk medicine, this plant is used as astringent, painkiller, chest depurative and emmenagogue. Previous studies demonstrated that it presents antiviral, antihypertensive, antipyretic, antitussive and blood circulation stimulant properties, besides being indicated to treat kidney and bladder disorders [17–19].

Therefore, the aim of this work was to evaluate the antifungal activity of the ethanolic extracts obtained from *L. venustum* and *P. calomelanos* and investigate the modulating effect of these extracts on the morphology of *Candida* species.

2. Materials and methods

2.1. Plant material

The leaves of *L. venustum* and *P. calomelanos* were collected in Crato, Ceará State, Brazil. The plants were identified by Dr. Antonio Álamo Feitosa Saraiva and the samples were deposited in the herbarium (Herbário Caririense Dárdano de Andrade-Lima) of the Regional University of Cariri-URCA, with the following voucher numbers: 5569 and 5570, respectively.

2.2. Extract preparation

Fresh leaves of *P. calomelanos* (950 g) and *L. venustum* (211.18 g) were dried and kept at room temperature. The powder material of each plant was placed to soak in 1 L of 95% ethanol for 72 h at room temperature. Then, the plant materials were filtered and concentrated in a rotary evaporator at 60 °C at 760 mm/Hg pressure, yielding 26.3 of the ethanolic extract of *P. calomelanos* (EEPC) and 12.42 g of the ethanolic extract of *L. venustum* (EELV).

2.3. Microorganisms

The following *Candida* strains were used in the antifungal activity trial: *C. albicans*-CA INCQS 40006 and CA LM 77; *C. tropicalis*-CT INCQS 40042, CT LM 23. These strains were obtained from the Laboratory of Mycology of the Federal University of Paraíba.

2.4. Determination of minimum inhibitory concentration (MIC)

The MIC of the extracts were determined using the microdilution method in 96-well microtiter plates [20,21]. A solution containing 1350 μL of double concentrated sabouraud dextrose broth and 150 μL of the fungal suspension was prepared in a test tube before distribution to the microtiter plates. Each well was added with 100 μL of this solution and then, 100 μL of the extract solution was added in the first well to be serially diluted [22]. The effects of the extracts were analyzed by observing the turbidity produced by the fungal growth after incubation at 37 °C for 24 h. The MIC was defined as the lowest concentration that visually inhibited the fungal growth in comparison with the control group. Of note, both extracts were used in concentrations ranging from 1024 to 1 μg/mL.

2.5. Drug modulation test

In this test, the extracts were used at sub-inhibitory concentrations (MIC/16). Briefly, 100 μL of a solution containing sabouraud dextrose broth, fungal inoculum (at 10%) and the extracts was distributed in the microtiter plate. Then 100 μL of a fluconazole solution was added in the first well. This drug was serially diluted as previously described to achieve concentrations ranging from 512.0 to 0.5 μg/mL [23]. The plates were analyzed using a spectrometer (ELISA Termoplate®) and the readings were performed at 630 nm.

2.6. Determination of minimum fungicidal concentration

Petri dishes containing sabouraud dextrose agar were inoculated with 20 μL of solutions removed from each well in which there was no fungal growth. These plates were incubated at 35–37 °C for 24 h. The minimum fungicide concentration was defined as the lowest concentration seeded in sabouraud dextrose agar at which no growth was detected [24].

2.7. Evaluation of effects of L. venustum and P. calomelanos extracts on Candida micromorphology

The micromorphology assay was performed using a wet chamber to observe the morphological changes in *Candida* strains. The fungal strains were cultured in diluted (10×) potato dextrose agar medium [25,26]. The extracts were added to the potato dextrose agar medium using the concentrations determined by the MIC assay (MIC, MIC/2, MIC×2). Briefly, 2 mL of potato dextrose agar were placed in a glass slide with

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