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RESEARCH ARTICLE

# Evaluation of the Antifungal Activity of the Methanolic Extract of *Psidium Guineense* (Myrtaceae) Against Strains of the Genus *Candida*

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## ABSTRACT

**Candidiasis is the most common fungal infection of the oral cavity, and its etiological factor is the proliferation and growth of microorganisms of the genus *Candida*. *Candida* fungi make up the diverse microbiota of the oral cavity living in a harmless commensal relationship with the host and can colonise different habitats such as mucous membranes and skin. Several antifungal drugs have been used for the treatment of candidiasis, such as azole antifungals (ketoconazole, fluconazole) and polyene antifungals (nystatin, amphotericin B), however, several challenges have been observed regarding the effectiveness of drug treatment. Thus, the use of medicinal plants presents itself as a viable and promising alternative for the discovery of new phytopharmaceutical agents with great biological potential. Based on literature studies that show the physicochemical and ethnopharmacological characteristics of medicinal plant species, this research aimed to evaluate the antifungal activity of the methanolic extract of *Psidium guineense* (Myrtaceae). The study was carried out through *in vitro* assays where the methanolic extract of *Psidium guineense* was used as a test substance against the fungal species of *Candida albicans*, *Candida tropicalis* and *Candida krusei* previously identified and maintained in Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SSB) culture media. In addition, nystatin was used as a standard antifungal agent for the positive control. The broth microdilution technique was performed to determine the Minimum Inhibitory Concentration (MIC) of the methanolic extract of *Psidium guineense*, all analyses and tests were performed in duplicate. In view of this, the MIC value higher than 1024 µg/mL was obtained, indicating that the methanolic extract of *Psidium guineense* did not present antifungal activity through the methodology used against the strains tested.**

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## 1. Introduction

Candidosis is an opportunistic fungal disease caused by microorganisms of the genus *Candida*, which has about 200 distinct yeast species, the main agents of human involvement being the species *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*. The genus *Candida* is composed of cosmopolitan species that colonise the skin and mucous membranes of humans, where their main site of colonisation is the gastrointestinal tract, from the mouth to the rectum, and which can often be isolated from the urethra and vagina [1].

These fungi incorporated into a matrix of polymeric substances make up the biofilm, and this association favours inter- and intra-species metabolic and genetic exchanges. Thus, microorganisms in biofilm form are less susceptible to antimicrobials than in planktonic form. Furthermore,

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yeasts of the genus *Candida* can become pathogenic, showing the ability to adhere, colonise and form germ tubes in the host mucosa [2].

The transition of *Candida* strains from a harmless commensal state to a pathogenic state strongly depends on several predisposing factors. Thus, many debilitating conditions are involved in various clinical forms of candidiasis such as the increased use of immunosuppressive agents, blood dyscrasias, nutritional deficiencies and the acquired immunodeficiency syndrome (AIDS). Immunosuppressed individuals are more likely to be affected and low immunity, even after treatment, may present recurrences of the fungal disease [3].

Numerous systemic, local and genetic factors contribute to disorders in the homeostasis of the oral cavity. Consequently, excessive proliferation of *Candida* and changes in the expression of its virulence factors occur, and oral candidiasis is established as an opportunistic mycosis. The most common species present in the normal and infected oral mycobiota is *C. albicans*, representing about 80% of oral fungal isolates. Oral candidiasis, specifically, has various clinical manifestations ranging from superficial mucocutaneous disorders to an invasive infection affecting multiple organs [4], [5].

Being recognised as the most common oral infection in highly immunosuppressed individuals, oral candidiasis is widely associated with CD4+ T-cell count and its colonisation serves as a marker for infection progression and predictive of increased immunosuppression. In human immunodeficiency virus (HIV) infection, oral candidiasis can lead to more distressing secondary complications such as oesophageal candidiasis, and is considered an opportunistic mycosis defining acquired immunodeficiency syndrome (AIDS), although it can affect immunocompetent patients [6].

Several synthetic drugs have been developed to treat oral candidiasis, such as iodoquinol-based antiseptics, sulfamide derivatives, quinones and polyene antifungals (nystatin and amphotericin B). In addition to these, azole antifungals which are imidazole agents (ketoconazole and clotrimazole) and triazole agents (fluconazole and itraconazole) are also noteworthy. However, the treatment of fungal infections is difficult to be effective due to the high resistance of *Candida* species to the pharmacological action of conventional antifungals and underdosing that often leads to prolonged treatment and high rates of disease recurrence [7].

Indeed, the use of herbal medicine in its various forms of presentation such as teas, infusions, ointments, gels, and herbal medicines, is a viable alternative for health professionals for the prevention and treatment of various pathologies. Scientific studies investigating the chemical and pharmacological properties of medicinal plants allow their indication as a low-cost therapeutic option, with low toxicity and minimal side effects, when used or applied correctly [8], [9]. Among the various plants for medicinal use, the Myrtaceae family stands out, which comprises about 132 genera and 5671 species of trees and shrubs, distributed in all Brazilian phytogeographic domains. In this family is present the genus *Psidium* and the species *Psidium guineense*, popularly known as araçá, in which, biological studies carried out with this plant tool have already proven its pharmacological potential, including positive *in vitro* results for the antimycobacterial, antioxidant, anti-inflammatory and antiproliferative activities of the essential oil of *Psidium guineense* leaves. In addition, efficacy against gastrointestinal disorders (intestinal colic, diarrhoea, gastroenteritis and gastritis) and diseases of the urogenital tract [10]. Thus, this work analysed the possible antifungal activity of the methanolic extract of *Psidium guineense* against strains of the genus *Candida*.

## 2. Objectives

### 2.1. General

To evaluate the antifungal activity of the methanolic extract of *Psidium guineense* (Myrtaceae).

### 2.2. Specific

- To verify the antifungal capacity of *Psidium guineense* methanolic extract on *Candida albicans* strains;
- To investigate the inhibitory activity of *Psidium guineense* methanolic extract for *Candida tropicalis* strains;
- To analyze the antifungal potential of *Psidium guineense* methanolic extract against *Candida krusei* strains;
- To compare the antifungal effect of *Psidium guineense* methanolic extract against the different fungal strains tested.

## 3. Methodology

### 3.1. Test Substance

The methanolic extract of *Psidium guineense* leaves, provided by Prof. Dr. Yanna Carolina Ferreira Teles, from the Universidade Federal da Paraíba (UFPB), was used for the assay.

The extract was stored in amber glass bottles and kept under refrigeration. The emulsions of the extract at different concentrations were prepared at the time of the assay. In a sterile test tube, 60,000 µg of the extract, 0.15 mL of dimethyl sulfoxide (DMSO), 0.06 mL of Tween 80 (INLAB/Brazilian Industry), and enough for 3 mL of sterile distilled water were added. The mixture was stirred for 5 minutes in a Vortex device (Fanem), obtaining an emulsion with a concentration of 20,000 µg/mL of the extract, 5% DMSO and 2% Tween 80. And through dilutions in distilled water or in the culture medium itself, the desired concentration of the extract will be obtained. The project is following the rules of CGen–Conselho de Gestão do Patrimônio Genético, registered in the SISGEN platform under protocol number A81C329.

### 3.2. Fungal Species

Eight strains of *Candida albicans* (ATCC 76645, LM 05, LM 09, LM 52, LM 80, LM 92, LM 108 and LM 240), five strains of *Canida tropicalis* (ATCC 13803, LM 04, LM 11, LM 18 and LM 20) and three strains of *Candida krusei* (ATCC 6258, LM 13 and LM 656), available in

the Microbiology Laboratory of the Academic Unit of Biological Sciences (UACB)/CSTR/UFCG, were used.

All strains were maintained on Sabouraud dextrose agar (SDA) at 4 °C, and 24-hour replicates were used for the assays in SDA incubated at 35 °C. In the antimicrobial activity study, a fungal inoculum of approximately $1.5 \times 10^6$ CFU/mL was used standardized according to the turbidity of the 0.5 tube of the McFarland scale [10], [11].

### *3.3. Culture Medium*

Sabouraud dextrose agar (SDA) (Difco Lab., USA) and Sabouraud dextrose broth (SDB) (Difco Lab., USA) were used for the *in vitro* assays, prepared according to the manufacturer's instructions.

### *3.4. Antifungal Drug*

Nystatin powder (Pharma Nostra, Rio de Janeiro) was used as the standard antifungal (positive control). The solutions will be prepared at the moment of the tests execution, to reach the desired concentrations.

### *3.5. Determination of the Minimum Inhibitory Concentration (MIC)*

The Minimum Inhibitory Concentration of *Psidium guineense* extract will be determined by broth microdilution technique [10], [11]. Sterile, capped 96-hole plates were used. In each hole of the plate, 100 µL of the liquid Sabouraud dextrose double concentrated broth medium was added. Then, 100 µL of the extract emulsion at the initial concentration of 2048 µg/mL (also doubly concentrated), were dispensed into the wells of the first row of the plate. And by serial dilution in ratio of two, the concentrations of 1024, 512, 256, 128, 64, 32, 16, 8 and 4 µg/mL were obtained, so that in the first row of the plate is the highest concentration and in the last, the lowest concentration. Finally, 10 µL of the inoculum of approximately $1.5 \times 10^6$ CFU/mL of the fungal species was added to the cavities, where each column of the plate refers to a fungal strain, specifically.

In parallel, the same assay was performed with the antifungal nystatin at concentrations of 1024 µg/mL to 4 µg/mL. A microorganism control was performed by placing in the cavities 100 µL of the same doubly concentrated SDB, 100 µL of sterile distilled water, and 10 µL of the inoculum of each species. To verify the absence of interference in the results by the solvents used in the preparation of the emulsion, in this case DMSO (dimethyl sulfoxide) and Tween 80, a control was performed in which 100 µL of the doubly concentrated broth, 50 µL of DMSO (5%), 50 µL of Tween 80 (2%) and 10 µL of the fungal suspension were placed in the cavities. A sterility control of the medium was also performed by adding 200 µL of SDB to a hole without the fungal suspension.

The plates were aseptically closed and incubated at 35 °C for 24–48 hours to be read. The MIC of the extract and antifungal is defined as the lowest concentration able to visually inhibit the fungal growth seen in the holes when compared to control growth. The experiments were performed in duplicate.

## 4. Results

The antimicrobial activity of plant extracts is evaluated through the lowest concentration of the test substance necessary to inhibit the growth of the exposed microorganism, this value is known as Minimum Inhibitory Concentration (MIC). In this excerpt, the broth microdilution tests were performed and it was observed that the MIC of the methanolic extract of *Psidium guineense* (araçá) against the different strains of *C. albicans*, C. *tropicalis* and *C. krusei* was greater than 1024 µg/mL for all strains tested. The test results and MIC values obtained in this study for the antifungal activity of the methanolic extract of *P. guineense* against strains of the genus *Candida* are shown in Tables I, II and III.

## 5. Discussion

By analysing the tabulated data, it is possible to verify that all strains of the fungal species *Candida albicans*, *Candida tropicalis* and *Candida krusei*, used for the experiments, were resistant to the bioactive constituents present in the methanolic extract of *Psidium guineense* (araçá), with no inhibition of fungal growth at the concentrations tested by the technique in the present study.

Indeed, according to Sartoratto *et al.* [12], for the antimicrobial activity of products of natural origin to be considered strong, they must have an MIC of up to 500 µg/mL, moderate for MICs of 600 to 1500 µg/mL and weak for MICs above 1500 µg/mL. Therefore, the results acquired in this study by the broth microdilution technique show that the antifungal activity of the methanolic extract of araçá (*Psidium guineense*) presented a weak inhibition of

TABLE I: Minimum Inhibitory Concentration (MIC) in µg/mL of Psidium guineense Extract Against *C. Albicans* Strains

| | ATCC 76645 | LM 05 | LM 09 | LM 52 | LM 80 | LM 92 | LM 108 | LM 240 |
|---|---|---|---|---|---|---|---|---|
| 1024 µg/mL | – | – | – | – | – | – | – | – |
| 512 µg/mL | – | – | – | – | – | – | – | – |
| 256 µg/mL | – | – | – | – | – | – | – | – |
| 128 µg/mL | – | – | – | – | – | – | – | – |
| 64 µg/mL | – | – | – | – | – | – | – | – |
| 32 µg/mL | – | – | – | – | – | – | – | – |
| Negative control | – | – | – | – | – | – | – | – |
| Positive control | + | + | + | + | + | + | + | + |

Note: (+) Inhibited bacterial growth (–) No inhibition of bacterial growth. Source: Authors.

TABLE II: Minimum Inhibitory Concentration (MIC) in µg/mL of *Psidium guineense* Extract Against *C. Tropicalis* Strains

| | ATCC **13803** | LM **04** | LM **11** | LM **18** | LM **20** |
|---|---|---|---|---|---|
| 1024 µg/mL | – | – | – | – | – |
| 512 µg/mL | – | – | – | – | – |
| 256 µg/mL | – | – | – | – | – |
| 128 µg/mL | – | – | – | – | – |
| 64 µg/mL | – | – | – | – | – |
| 32 µg/mL | – | – | – | – | – |
| Negative control | – | – | – | – | – |
| Positive control | + | + | + | + | + |

Note: (+) Inhibited bacterial growth (–) No inhibition of bacterial growth. Source: Authors.

TABLE III: Minimum Inhibitory Concentration (MIC) in µg/mL of *Psidium guineense* Extract Against *C. Krusei* Strains

| | ATCC 6258 | LM 13 | LM 656 |
|---|---|---|---|
| 1024 µg/mL | – | – | – |
| 512 µg/mL | – | – | – |
| 256 µg/mL | – | – | – |
| 128 µg/mL | – | – | – |
| 64 µg/mL | – | – | – |
| 32 µg/mL | – | – | – |
| Negative control | – | – | – |
| Positive control | + | + | + |

Note: (+) Inhibited bacterial growth (–) No inhibition of bacterial growth. Source: Authors.

fungal growth against strains of the genus *Candida*, since it presents a MIC greater than 1024 µg/mL for all strains analysed.

In the tables it is also possible to observe that there was microbial growth in the negative control wells confirming the viability of the fungal strains used for the tests. In addition, in the wells of the plate referring to the positive control it was possible to verify the inhibition of growth demonstrating that the strains tested are sensitive to the control antifungal used, specifically nystatin.

Figueiredo *et al.* [13] found in their studies that the volatile composition of the plant species *Psidium guineense* ranged from 38 to 181 compounds, with a predominance of terpenes, mainly spatulenol (80.71%), limonene (47.4%), $\alpha$-pinene (35.6%) and caryophyllene (8.6%–24.4%). Indeed, Nascimento *et al.* [10] demonstrated the efficacy of *Psidium guineense* essential oil for antioxidant, anti-inflammatory and antimicrobial effects (MIC = 126.4 µg/mL) suggesting that such biological effects of this family come from the high predominance of the constituent spatulenol, corresponding to more than 80% of its composition.

Despite the negative antifungal effect against strains of the genus *Candida* shown in this study. Macedo *et al.* [14] analysed the antimicrobial activity of the *Psidium guineense* species against *Sthaphylococcus aureus* and *Klebsiella pneumoniae*, which are pathogens for humans and resistant to antibiotics, and it was shown that the aqueous and ethyl acetate extracts had a strong effect against strains of S. aureus (MIC = 250–500 µg/mL) and *K. pneumoniae* (MIC = 64 µg/mL) respectively. In addition, the association of the extract with cephalothin showed a lower Fractional Inhibitory Concentration Index (FICI) ranging from 0.125 to 0.5 µg/mL, showing that the natural product potentiated the effect of the synthetic drug.

## 6. Conclusion

It is concluded that the methanolic extract of the plant species *Psidium guineense* did not show antifungal activity by the technique used against the strains of *Candida albicans, Candida krusei* and *Candida tropicalis* tested. Thus, further studies are needed addressing different methodologies for determining the MIC and the antimycotic effect of the extract for further comparative analysis of the results found in this study, in order to provide an ethnopharmacological survey of the natural product studied.

## Conflict of Interest

Authors declare that they do not have any conflict of interest.

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