EVALUATION OF THE ANTIFUNGAL ACTIVITY OF HEXANIC AND METHANOLIC EXTRACTS OF PURPLE IPÊ (*HANDROANTHUS IMPETIGINOSUS*) AGAINST *CANDIDA ALBICANS* AND *ASPERGILLUS BRASILIENSIS*

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ABSTRACT

Purple Ipê (*Handroantus impetiginosus*) is widely known for its phytotherapeutic properties and for the treatment of various pathologies, despite the lack of clarity about its medicinal mechanisms. This study investigated the antifungal activity of purple Ipê extracts using the Minimum Inhibitory Concentration (MIC) technique *in vitro*. The present study aimed to gather information on the feasible and cost-effective potential of new antifungal activities derived from purple Ipê extracts against *Aspergillus* brasiliensis and *Candida albicans*. However, no significant results were obtained, so there was no motivation to proceed with *in vivo* research on larvae. It is concluded that further studies should be conducted to explore alternative effective extraction methods of purple Ipê in combination with bioactive compounds to harness its therapeutic potential in clinical contexts.

Keywords: purple ipê; Tenebrio molitor; antifungal activity.

INTRODUCTION

The incidence of invasive fungal infections has been growing, and in recent years, understanding of the epidemiology of fungal infections has improved. More than 1 billion people are affected, and 25 million patients are at imminent risk of serious organ damage or death due to fungal infection (OLIVER A. C. *et al.;* 2017). Quing Liu *et al.* (2017) report that antifungal resistance has become an emerging global problem, considering the diverse fungal infections and pre-existing antifungal treatment failures; therefore, new antifungal agents capable of overcoming this resistance need to be discovered. In this context, plants have been an important source of bioactive compounds for centuries. Natural compounds play a significant role in drug discovery and in the development of new therapeutic entities (MAJID SHARIFI-RAD, M et al.;

2020). Therefore, fungal infections by the C*. albicans* strain*,* especially in immunocompromised patients, and the propensity for antifungal resistance against azole-based drugs need to be addressed (PARAMESHWARI, K. K., & GIRIJA, C. S., 2024).

Purple ipê (*H. impetiginosus*) is a leafy and lush tree native to South America, most commonly found in the Brazilian cerrado. This plant has demonstrated effective fungicidal activity, especially against *C. albicans*. Substances extracted from the bark of purple ipê, such as a- and b-lapachone and xyloidone, are proven fungicides (BITTENCOURT & SILVA, 2020).

OBJECTIVES

General Objective

To evaluate the antifungal activity of hexane and methanol extracts of purple ipê (*H. impetiginosus*) on *C. albicans* and *A. brasiliensis*.

Specific Objectives:

1. Prepare hexane and methanol extracts using the inner bark of purple ipê (*H. impetiginosus*);
2. Determine the minimum inhibitory concentration (MIC) of the hexane and methanol extracts against C*. albicans* and A*. brasiliensis*.
3. Evaluate the antifungal activity of the hexane and methanolic extracts against *C. albicans* and *A. brasiliensis* species in in vivo experiments.

METHODOLOGY

A qualitative and quantitative experimental study was conducted in the laboratory using a comparative procedure.

Extracts

The chips from the inner bark of *H. impetiginosus* were dried at room temperature and then macerated in an industrial blender. The macerated material was stored in a beaker containing 300 ml of 99% methanol and another beaker containing

300 ml of 99% hexane, kept under refrigeration. Subsequently, this sample was filtered and dried using a rotary evaporator. The resulting extract was stored in an amber bottle protected from light at 4 °C (PACHECO *et al.*, 2015).

Culture and maintenance of microorganisms

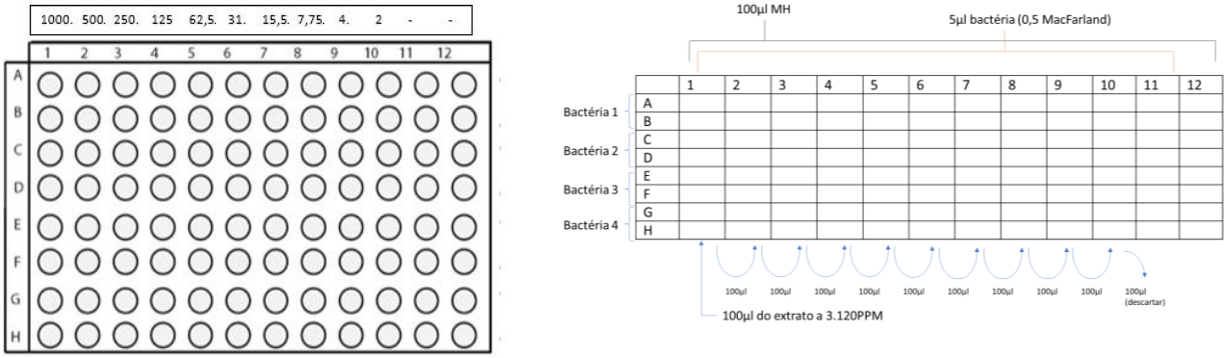
For the cultivation of fungi, strains of *C. albicans* ATCC (American Type Culture Collection) and *A. brasiliensis* (clinical isolate) were cultivated in Sabouraud Dextrose Agar (Peptone 10g/L; Dextrose 40g/L; Agar 15g/L), kept in an oven at 36 °C for 72 hours and subjected to experimentation (PACHECO *et al.,* 2015).

Determination of Minimum Inhibitory Concentration (MIC)

An extract solution was prepared with a test concentration of 3120 ppm with serial dilutions in base 2 performed up to column 10 (final concentration 6.0 ppm). The microdilution technique was then applied on a plate as described in CLSI M7 – A6 NCCLS, 2003 with adaptations. Fungal inocula from the above strains were tested, prepared containing $10^{(4)}$cells/ml. The microorganisms were inoculated in duplicate in wells 1 to 11, with 12 being the negative control well on the test plate. The plates were incubated in an oven at 37°C for 12 to 18 hours, and then the colorimetric method with 0.01% resazurin sodium was used, applying 15 µL of the solution in each well and waiting three hours to perform the visual reading of the results, where blue indicates fungal inactivity and pink indicates activity.

Figure 1. **Example of diagram (A) and map (B) of the experimental plate**

A) B)

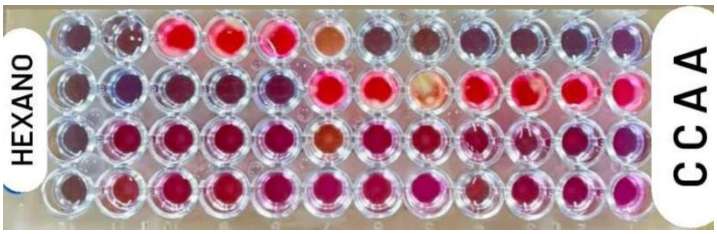


Source: Author's own work.

RESULTS

During the *in vitro* process, the fungus *A. brasiliensis* showed slight inhibition of its growth by the hexane extract. In relation to the fungus *C. albicans*, both lower rows remained pink, indicating that there was no inhibition of its growth, as seen in **Figure 2**.

Figure 2. Result of the test plate



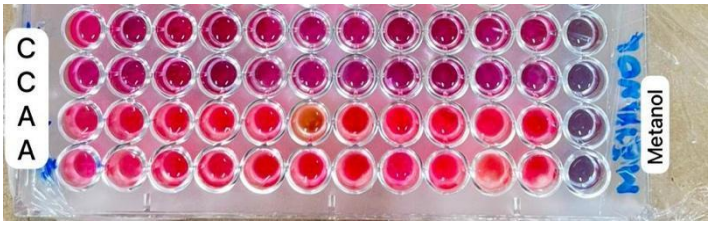
A – Aspergillus brasiliensis

C – Candida albicans

Source: Author's own work.

The methanolic extract, similar to the previous one, was unable to inhibit the growth of *C. albicans* or *A. brasiliensis*, as shown in **Figure 3.**

Figure 3. Results of the test plate



A – Aspergillus brasiliensis

C – Candida albicans

Source: Author's own work

CONCLUSION

Therefore, given the slight inhibition of the *A. brasiliensis* strain by the hexane extract and the absence of inhibition of the *C. albicans* strain for both hexane and methanol extracts, it was decided not to continue with *in vivo* research on larvae, since the absence of antifungal activity *in vitro* compromises the relevance and justification for conducting additional studies in live models.

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