Antifungal Activity of Peruvian Banana Peel (*Musa paradisiaca* L.) on *Candida albicans*: An *In Vitro* Study

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ABSTRACT

Aim: The purpose of this study was to compare the antifungal activity of three concentrations of a hydroethanolic extract of the *Musa × paradisiaca* peel against *Candida albicans* strain ATCC 10231.

Materials and methods: The agar diffusion method was used, and the culture medium used was Sabouraud agar. Petri dishes were prepared with concentrations of 10, 30, and 50% of hydroethanolic extract of the *M. × paradisiaca* peel; nystatin was used as a positive control, and 96% ethanol was used as a negative control. After 24 hours of incubation, each plate was examined, and the diameters (mm) of the growth inhibition halos were measured around each well using a digital vernier caliper.

Results: The results showed that the antifungal activity of the extract varied, depending on the concentration, as shown using analysis of variance (ANOVA; $p < 0.05$). When comparing the different concentrations, it was found by Duncan test that the greatest activity was obtained at 50%.

Conclusion: It was concluded that the hydroethanolic extract of *M. × paradisiaca* at 50% exerted a greater antifungal effect on the strain of *C. albicans* than did the extract at lower concentrations.

Clinical significance: By knowing the antimicrobial effect of *M. × paradisiaca*, this substance can be effectively used in products aimed to cure candidiasis infection.

Keywords: Antifungal, Banana, *Candida albicans*, Hydroethanolic extract, *Musa × paradisiaca*, Oral candidiasis.

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INTRODUCTION

Oral candidiasis is a major health problem worldwide. This fungal pathogen is very important for humans, because of its clinical significance and for scientific research.¹

Candidiasis in the oral cavity is an infection caused by fungi, and the most frequently isolated pathogen is *Candida*. This fungus is usually a saprophytic colonizing agent of the gastrointestinal and genitourinary systems, but it is also found in infections involved in the modification of the oral microflora, as well as in systemic diseases, and has been shown to weaken the normal functioning of the immune system. The most frequently implicated and most damaging species of this genus is *Candida albicans*.²

Today, the treatment of candidiasis is based on antimicrobials in different pharmaceutical forms. However, an optimal therapeutic result is not always obtained.^{3–5} At the same time, the use of phytodrugs can be considered a useful alternative offered by traditional and natural medicine.^{6,7}

The banana peel accounts for approximately 30% of the weight of the fruit. Potential applications for banana peel depend on its chemical composition. The banana peel is rich in dietary fiber, proteins, essential amino acids, polyunsaturated fatty acids, and potassium; between efforts to use the shell protein, methanol, ethanol, pectins, and enzymes coal has been obtained vegetable, an alternative fuel source for cooking. Also we consider it can be a potential source of antioxidants and antimicrobials, as well as phytochemical compounds with activity against free radicals.^{8,9} Synthetic drugs available cause adverse effects and lead to fungal resistance; their application is also associated with higher costs. Thus, natural product alternatives, such as *Musa × paradisiaca*, which contains flavonoids, associated with antifungal activity, are a therapeutic option for the treatment of this problem.

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Given all of the above, the purpose of this study was to compare the antifungal activity of three concentrations of a hydroethanolic extract of the *M. × paradisiaca* peel against *C. albicans* strain ATCC 10231.

MATERIALS AND METHODS

Collection and Taxonomic Identification of the Plant Sample

In May 2017 1 kg of stems and leaves of *M. × paradisiaca* was collected, which included the flower of the plant species and taken to the Herbarium Truxillense of the National University of Trujillo, Peru, for the identification and taxonomic determination.

A total of 50 bananas were collected from the district of Piura, Province of Sullana, Region of Piura, Peru, and placed in a cardboard

box with holes. The box was taken to the School of Pharmacy and Biochemistry of the National University of Trujillo for further processing

Preparation of the Plant Sample

Selection and Washing

Bananas in good condition were selected, i.e., not showing attacks by fungi and insects or discoloration. The fruits were washed with distilled water, followed by disinfection with 0.5% sodium hypochlorite. Subsequently, the bananas were dried for 48 hours. The peel was cut into pieces of approximately 1 cm × 1 cm and then placed in Kraft paper and dried in an oven at 40°C (Figs 1A to C).

Homogenization and Sieving

The peels were pulverized with a mortar and pestle into a powder and then passed through a set of sieves to obtain particles of homogeneous size (Figs 1D to F).

Storage

The obtained powder was stored in a wide-mouth amber glass jar.

Preparation of the Hydroethanolic Extract

Alcoholic Maceration

A total of 100 g of the banana peel powder was placed in a wide-mouth amber glass jar. Then 70% ethanol was added to cover the sample by more than 2 cm and mixed well, considering that the mixture should occupy a maximum of three fourths of the container. The container was covered, and the sample was macerated for 7 days, with shaking for 15 minutes twice a day.

Preparation of a Dry Extract

Afterward the macerate was filtered through Whatman No. 1 filter paper using a vacuum pump. The filtrate was called a hydroethanolic extract. Subsequently, the hydroethanolic extract was concentrated

in a rotavapor until a syrupy mass was obtained, which was dried in an oven at 40°C. The resulting product was called a dry extract.

Preparation of Concentrations

These extracts were prepared at concentrations of 10%, 30%, and 50% in 70 Gay Lussac (GL) ethanol. The hydroethanolic extracts of each plant sample were stored in amber glass bottles at 4–8°C until use.

Microbial Species

The strain of *C. albicans* ATCC 10231 was obtained from the GenLab Laboratory of Peru SAC-Lima and preserved in the Microbiology Laboratory of the Faculty of Medicine of the National University of Trujillo. Once the strain was obtained, it was reactivated in test tubes with screw cap using Sabouraud dextrose agar medium 4% at 25°C for 48 hours for *C. albicans* ATCC 10231 (Fig. 2A).

Inoculum Preparation

For the preparation of a suspension, *C. albicans* was grown for 48 hours and then the cells were suspended in a sterile 0.85% saline solution. The turbidity was adjusted to the equivalent of 0.5 McFarland standard for *C. albicans*. Inocula were prepared by taking one colony of the reactivated strain of *C. albicans* with a Kohler loop under sterile conditions and then suspending the biomass in 5 mL of 0.9% physiological saline in a test tube until reaching an equivalent concentration of 0.5 on the McFarland scale by measuring the optical density using a nephelometer.

Sample Inoculation

Sabouraud agar was used as the culture medium for the agar diffusion test. To each plate, 50 µL of the standardized sample was added with a micropipette and spread with a sterile swab over the surface of the culture medium, allowing 3–5 minutes for drying. Ten petri dishes were prepared with the strain of *C. albicans*. Each plate was labeled with a permanent marker and corresponded

Figs 1A to F: Preparation of the plant sample: (A) Bananas were selected in good condition; (B) The fruit and peel were dried; (C) The peel was cut into pieces of approximately 1 cm × 1 cm; (D–F) Homogenization and sieving: The peels were pulverized with a mortar and pestle into a powder and then passed through a set of sieves to obtain particles of a homogeneous size

Figs 2A and B: Antimicrobial test: (A) Strain of *Candida albicans* ATCC 10231; (B) Plates with hydroethanolic extract of *Musa x paradisiaca* (banana) at concentrations of 10%, 30%, and 50%, the positive control (nystatin) and the negative control (96% ethanol) (Figs 2B and C).

to the hydroethanolic extract of *M. x paradisiaca* (banana) at concentrations of 10%, 30%, and 50%, the positive control (nystatin), and the negative control (96% ethanol) (Figs 2B and C).

Antimicrobial Test

Subsequently, using a sterile punch of 6 mm in diameter, five wells (6 mm deep) were made in each plate. To each well, 50 μ L of the hydroethanolic extract at each concentration and controls were added. The plates were left for an hour to allow sample diffusion into the agar, followed by incubation at 37°C for 24 hours.

Reading the Results

After 24 hours of incubation, each plate was examined, and the diameter (mm) of the growth inhibition halos around each well was measured using a digital vernier caliper. Ten replicates of each assay were performed.

Data Analysis

Experimental data were entered into the database in IBM SPSS Statistics version 23 and later worked with the ANOVA statistical test and Duncan test on the assumption of normal distribution.

RESULTS

Average Sizes of Inhibition Halos for the Hydroethanolic Extract (E.E.) of the *Musa x paradisiaca* Peel against *C. albicans* strain ATCC 10231

The 10% hydroethanolic extract of the *M. x paradisiaca* peel produced an inhibition halo of 8.2 mm, with a standard deviation of 1.03 mm; at 30%, the mean diameter and the standard deviation were 10.5 and 1.74 mm, respectively; at 50%, the mean diameter was 12.5 mm and the standard deviation was 1.86 mm, while the average diameter in the nystatin group was 8.9 mm (Table 1).

Analysis of Variance of the Diameters of Inhibition Halos (mm)

When the data were subjected to the *F* test of ANOVA, comparing the means of each study group, a value of $F = 18.229$ was obtained, with a significant difference ($p < 0.001$), i.e., the different concentrations produced different mean effects (Table 2).

Comparison of the Diameters of Inhibition Halos (mm) According to the Treatment Group

In this multiple comparison test, the Duncan test was applied, and it was observed that the hydroethanolic extract of *M. x paradisiaca*

Table 1: Average sizes of inhibition halos for the hydroethanolic extract (E.E.) of the *Musa x paradisiaca* peel against *Candida albicans* strain ATCC 10231

Treatment groups	n	Average (mm)	Standard dev.
E.E. <i>Musa x paradisiaca</i> 10%	10	8.2	1.03
E.E. <i>Musa x paradisiaca</i> 30%	10	10.5	1.74
E.E. <i>Musa x paradisiaca</i> 50%	10	12.5	1.86
Nystatin (control +)	10	8.9	0.43

at 10% and nystatin showed no significant difference ($p > 0.05$). However, there were differences at the other concentrations of the extract (30% and 50%), which indicated different effects of the concentrations in terms of the strain susceptibility, which is expressed as a diameter of the inhibition halo (Table 3).

DISCUSSION

In the present study, positive results were obtained for antifungal activity of three different extract concentrations (10%, 30%, and 50%) and a positive control (nystatin) against a strain of *C. albicans* using the agar diffusion method. The results confirmed the findings of other authors, such as Fugaban⁹ and Karadi,¹⁰ who demonstrated the antifungal activity of *M. x paradisiaca* against *C. albicans*.

According to Nagalingam,¹¹ this medicinal plant could be used in the treatment of diseases caused by organisms such as *C. albicans*.

The antifungal activity of the 10% hydroethanolic extract against the strain of *C. albicans* was shown to result in an inhibition halo of 8.2 mm, which was comparable to that of nystatin, which produced an inhibition halo of 8.9 mm, showing no significant difference.

Regarding the antifungal activity of the 30% hydroethanolic extract against the strain of *C. albicans*, a 10.5-mm inhibition halo was observed, demonstrating a greater antifungal activity than that of nystatin.

The study by Karadi¹⁰ showed that *M. x paradisiaca* exerted a potent inhibitory effect against fungal strains, with the antifungal activity as strong as fluconazole. In this study, it was demonstrated that *M. x paradisiaca* showed a greater inhibitory activity than that of nystatin.

Table 2: Analysis of variance of the diameters of inhibition halos (mm)

SV	SS	df	MS	F	p
Treatment	105.682	3	35.227	18.229	0.0000
Error	69.569	36	1.932		
Total	175.251	39			

SV, source of variation; SS, sum of squares; DF, degree of freedom; MS, mean square; F, comparison of several variables; p, probability

Table 3: Comparison of the diameters of inhibition halos (mm) according to the treatment group

Treatment groups	n	Subset for $\alpha = 0.05$		
		1	2	3
E.E. <i>Musa</i> × <i>paradisica</i> 10%	10	8.2		
Nystatin (control +)	10	8.9		
E.E. <i>Musa</i> × <i>paradisica</i> 30%	10		10.5	
E.E. <i>Musa</i> × <i>paradisica</i> 50%	10			12.5

Finally, the antifungal activity of the 50% hydroethanolic extract against the *C. albicans* strain resulted in an inhibition halo of 12.5 mm, which was the highest activity among the three concentrations of the extract, along with the positive control, in agreement with the data of other studies, such as that by Fugaban,⁹ in which the 50% concentration of an *M. × paradisica* extract exhibited the highest antifungal activity against *C. albicans*.

Kapadia¹² demonstrated that the extract used in their study produced a larger inhibition zone (24 mm in diameter) against *C. albicans* than against all other bacteria and fungi studied.

In this study, among all concentrations, the 50% concentration of the *M. × paradisica* extract exhibited the highest antifungal activity against *C. albicans*. It was observed that compared with the other concentrations, the 50% concentration of the hydroethanolic extract of *M. × paradisica* produced the largest average inhibition halo, with a diameter of 12.5 mm, which exceeded that of 6.625 nystatin (diameter = 8.9 mm).

Similar results were reported in the study by Fugaban,⁹ which compared the effects of an *M. × paradisica* extract at concentrations of 100%, 50%, and 30% against *C. albicans*. The results indicated that the extract at a concentration of 50% was more effective against *C. albicans*. Thus, the results were similar to ours and provided baseline information on the antifungal properties of *M. × paradisica* against *C. albicans*.

Likewise, our data were compared with those obtained in the study by Egbunu² who concluded that bananas possessed antifungal properties against *C. albicans*.

The antimicrobial activity of hydroethanolic extracts of *M. × paradisica* depends on several factors,^{11,13–16} including the harvest season, geographic location, extraction method, fruit maturity index, variety, and chemical structure of the components of the oil and its concentration.^{17,18} The storage time and conditions also influence the antimicrobial activity because hydroethanolic extracts are very sensitive to light and high temperatures.^{19–21} Thus, these factors can affect the inhibitory power of the extract. In this study, these factors were controlled to preserve the composition of the hydroethanolic extract of *M. × paradisica* and its antifungal power.^{10,22,23}

Currently around 3,000 natural substances were studied, of which only 10% have a positive impact on the pharmaceutical

industry.^{24–27} The components of these products are secondary metabolites of plants that can be physically separated from the membranous tissue.^{28,29} Thus, for example, traditionally, essential oils have been used as bactericides, fungicides, antiparasitics, analgesics, and spasmolytics.^{30,31} However, the lipophilic nature of essential oils makes its administration difficult to be used as antimicrobial agents.^{14,32,33} The literature has shown that monoterpenes present in many of these substances such as that of *M. × paradisica* have shown high fungicidal activity against *C. albicans*, similar to the fungistatic of nystatin.^{9,12,22,34–36} However, in spite of the objectives set out in this study, it is necessary to know more deeply the mechanisms of action of this natural product, analyzing its composition and synergistic effect.^{37–40} The literature on *M. × paradisica* as such is less, which can be considered a limitation for this study in the statistical as well as theoretical part. However, the high frequency of edentulous patients and patients carrying prostheses in conjunction with the results found invite new studies to be carried out in this same line, seeking to find the appropriate antifungal that in addition to controlling fungal infection prevents any alteration to the mouth structures where it makes contact.

The result obtained in this study clearly demonstrates that banana fruit peel has a broad spectrum of biological activities and could be used as a good source of antifungal agent. However, to continue with the search for new antibacterial agents, it is necessary to test the great variety of this species that are potential source of bioactive agents and also to evaluate its pharmaceutical properties, such as its pharmacodynamics, pharmacokinetics, and toxicity, among others

CONCLUSION

The hydroethanolic extract of the *M. × paradisica* peel showed a greater antifungal effect against a strain of *C. albicans*. With the results of the executed *in vitro* study, it converts *M. × paradisica* into a therapeutic alternative for candidiasis, reducing the frequency of side effects caused by chlorhexidine and other chemical substances.

AUTHOR CONTRIBUTIONS

All authors conceived, designed, written, revised and improved the study.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analyzed during the current study are available from the corresponding author on request.

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