Original Article

Antifungal Activity of *Morinda citrifolia* Methanolic Extract against *Candida albicans:* An *In Vitro* Study

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ABSTRACT

Aim: Natural medicine used as an alternative and/or complementary treatment to counteract diseases is of great importance in public health. Therefore, the purpose of the present study was to assess the *in vitro* antifungal activity of *Morinda citrifolia* methanolic extract of peel, pulp, and seed against *Candida albicans*. **Materials and Methods:** The present study was experimental *in vitro* and cross-sectional. Eight replicates were prepared in *Sabouraud dextrose* agar with five wells each, where 0.12% chlorhexidine, distilled water, and methanolic extract of seed, peel, and pulp of *Morinda citrifolia* fruit were placed at concentrations of 10,690, 8,270, and 6,430 mg/mL, respectively, to evaluate sensitivity according to Duraffourd's scale. In addition, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined by dilution and agar seeding method. Statistical analysis was performed by analysis of variance (ANOVA) and Tukey's *post hoc* test, considering a significance level of $P < 0.05$. **Results:** The inhibition halos of *Morinda citrifolia* methanolic extract of seed, peel, and pulp against *Candida albicans* measured on average 15.94, 11.94, and 11.56 mm, respectively. The MIC of seed, peel, and pulp extract were 1366.25, 2067.5, and 1607.5 mg/mL respectively, whereas the MFC for seed, peel, and pulp extract were 2672.50, 2067.5, and 3215 mg/mL, respectively. Moreover, seed extract presented significantly higher antifungal activity than peel and pulp ($P < 0.001$). **Conclusions:** *Morinda citrifolia* methanolic extract of peel, pulp, and seed showed fungistatic and fungicidal effect against *Candida albicans*, being this very sensitive to seed extract with a MIC of 1366.25 mg/mL and a MFC of 2672.5 mg/mL, which allows recommending the development of effective pharmacological formulations for the control of candidiasis.

Keywords: *Candida albicans, chlorhexidine, fungus drug sensitivity tests, inhibition halos, microbial sensitivity test, minimum fungicidal concentration, minimum inhibitory concentration, Morinda citrifolia, Noni*

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Introduction

According to a 2016 research paper about global burden of disease, oral pathologies affect 3580 million people, approximately half of the world's population.[1,2] Among the most common diseases affecting the oral cavity of humans is candidiasis, a multifaceted fungal disease that includes mucosal and skin infections caused by yeasts of the genus *Candida*.[3]

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Candida albicans naturally inhabits the oral cavity, and up to 80% of general population are asymptomatic carriers,[4] as there is a balance between the individual's defense mechanisms and the invasive potential of this yeast. However, it has been reported that some factors can provoke the ecological imbalance of oral environment, causing the transition of the fungus from commensal to pathogenic.[5-8] Currently, the clinical use of antifungal agents has increased, leading to drug resistance. Therefore, although fungi can be intrinsically resistant to these drugs (primary resistance), they can also develop resistance in response to drug exposure during treatment (secondary resistance), because of indiscriminate use or self-medication.[9] Because of this, the population is forced to seek other alternatives to treat fungal infections, such as phytotherapy.[10]

Medicines of plant origin have an enormous therapeutic potential due mostly to secondary metabolites such as alkaloids, steroids, tannins and phenolic compounds, flavonoids, resins, fatty acids, among others.[11,12] Among these natural products is *Morinda citrifolia*, also known as Noni, which is widely used in alternative and complementary medicine in Latin America, because its parts (roots, leaves, fruits, and seeds) are used for disease control; likewise, in dental field, it has proven to be important in periodontal tissue regeneration.[13-20]

Some studies attribute the antimicrobial properties of *Morinda citrifolia* to flavonoids, coumarins, quinones, and iridoids.[19,20] Ruiz *et al.* conducted a study to make the preliminary identification of aqueous and ethanolic extracts of *Morinda citrifolia* fruit and leaves, identifying that the fruit extract had a percentage of 0.032 of total flavonoids, whereas the leave extract had a percentage of 0.191, being evident that these compounds had different proportions in different parts of the plant.[21] Deng *et al.* found that iridoids were present in various parts of the plant, with fruit being the main source. In addition, there was a large variation in iridoid content depending on the geographical area where fruit was grown,[22] which could mean that *Morinda citrifolia* presents variation in its chemical composition depending on the place where it is cultivated, constituting a relevant factor to consider when evaluating its antifungal action.

Several studies have reported good antifungal activity of *Morinda citrifolia* fruit extract at various concentrations against *Candida albicans*. It was found that the higher the concentration of the extract, the higher the antifungal activity.[20,23,24] Other studies[19,25] reported discrepant results regarding the antifungal activity of *Morinda citrifolia* extract against *Candida albicans*. Most of the research evaluating the antifungal action of *Morinda citrifolia* has been carried out in Asia,[23,24,26] whereas in Peru, there have not yet been solid studies assessing its antifungal action against *Candida albicans*, considering its minimum inhibitory and fungicidal concentration. Therefore, the present study aimed to assess the *in vitro* antifungal activity of *Morinda citrifolia* (Noni) methanolic extract of seeds, peel, and pulp on *Candida albicans* strains.

Materials and Methods

Type and delimitation of the study

This *in vitro*, cross-sectional and analytical experimental study was approved by a research committee of the Universidad Nacional Federico Villarreal in Peru, with resolution RR-2900-2018-UNFV and approval letter OGGE-059-2019. The experimental part was performed in Molecular Biology Laboratory of the Universidad Peruana de Ciencias Aplicadas (UPC) and the Instituto de Investigación Nutricional between April and August 2021.

Collection and elaboration of methanolic extract

In this experimental research, all methods were carried out in accordance with the relevant guidelines set out in the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The recollection of *Morinda citrifolia* and its taxonomic recognition was carried out in the herbarium of the Natural History Museum of the Universidad Nacional Mayor de San Marcos (No. 60-USM-2020). Subsequently, the fruits were selected, removing other biological elements of the plant, and then meticulously separated into seeds (262 g), peel (153 g), and pulp (180 g). Each was then macerated with pure methanol (1:1 weight/volume) for 7 days at room temperature and under constant agitation. After this period, the extracts were filtered and the solvent was evaporated, obtaining a final concentration of 10,690, 8270, and 6430 mg/mL for the seed, peel, and pulp, respectively.[27] Then, to evaluate the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of *Morinda citrifolia* methanolic extract, serial dilutions of factor 2 from 1:2 to 1:128 were carried out.

Procedure to evaluate antifungal sensitivity

The fungus was cultured on *Sabouraud dextrose* agar under controlled anaerobic conditions at 37°C for 72 h. After culture time, seven to eight colonies were isolated and inoculated in 3 mL of *brain heart infusion* broth culture and then placed in an anaerobiosis jar (BBL GasPak, BD, Franklin Lakes, NJ, USA) for 72 h at 37°C using a Memmert (Schwabach, Germany) incubator, together with Anaerocult C (Darmstadt,

Germany). Subsequently, for growth inhibition test, the well diffusion (agar punching) methodology was used. For this, *Sabouraud dextrose* agar was prepared according to manufacturer's instructions (in autoclave at 121°C for 15 min). The agar was then allowed to cool to approximately 55°C and poured into 94 × 16 mm petri dishes. The plates were allowed to cool and solidify for approximately 2 h; then the fungal suspension was inoculated homogeneously. The whole procedure was performed under a type II biosafety cabinet to avoid contamination. Then, with a punch, five 8 mm diameter perforations were made in the agar. Next, a volume of 150 µL of *Morinda citrifolia* methanolic extract from seed (10690 mg/mL), peel (8270 mg/mL), and pulp (6430 mg/mL) was added to each well. 0.12% chlorhexidine was used as a positive control and distilled water was used as a negative control. The petri dishes were incubated in controlled anaerobic conditions at 37°C for 24 h. After incubation time, the diameters of inhibition halos were measured in millimeters (mm) using a digital Vernier caliper (Vogel, Kevelaer, Germany), and the data were entered in a Microsoft Excel 2019 spreadsheet.

To reduce measurement bias as much as possible, the double-blind technique was used (both the person who measured the inhibition halos and the person who performed the statistical analysis were unaware of the group assignment according to the product used). Likewise, the calibration of the measurement of inhibitory halos of the principal investigator was performed by both intraexaminer (SM) and interexaminer (SM and CC), obtaining a Pearson's R correlation coefficient of 0.96 (confidence interval [CI]: 0.89–1.00) and 0.91 (CI: 0.87–0.94) respectively, demonstrating very good concordance.

Finally, the Duraffourd scale[28,29] was used to evaluate the antifungal sensitivity of *Morinda citrifolia* methanolic extract in its different concentrations for seed, peel, and pulp, according to their inhibition halos and compared with 0.12% chlorhexidine (control) [Table 1].

Table 1: Duraffourd scale to determine antifungal sensitivity according to the diameter of inhibition halos

Duraffourd scale		
Classification	**Representation**	**Diameter (mm)**
Null		<8
Sensitive	+	8–14
Very sensitive	++	14–20
Highly sensitive	+++	>20

mm = millimeters

Sample size calculation and sampling for comparison of inhibition halos

The sample size consisted of eight replicates ($n = 8$) and was calculated from a pilot study by mean comparison formula, considering an $\alpha = 0.05$ and a statistical power of $1-\beta = 0.80$ with variances $S_1^2 = 0.56$ and $S_2^2 = 0.81$ and a mean difference of 1.2 mm. In addition, the study units were selected by simple random sampling without replacement. The groups were formed as follows:

- Eight replicates with *Morinda citrifolia* seed methanolic extract (stock: 10690 mg/mL)
- Eight replicates with *Morinda citrifolia* peel methanolic extract (stock: 8270 mg/mL)
- Eight replicates with *Morinda citrifolia* pulp methanolic extract (stock: 6430 mg/mL)
- Eight replicates with 0.12% chlorhexidine [control (+)]
- Eight replicates with distilled water [control (-)].

Determination of minimum inhibitory concentration and minimum fungicide concentration

Candida albicans strains (ATCC 10231) grown in the Molecular Biology Laboratory of the UPC were used.

MFC is considered as the minimum concentration of an antibiotic that in a predetermined period of time is capable of inducing *in vitro* death of 99.9% of a fungal population. MIC is the minimum concentration of extract where there was no visible growth after incubation period.[30]

Sabouraud dextrose agar plates were prepared 48–72 h in advance. In sterile 1.5 mL microcentrifuge tubes, 100 µL of the extract was added for each dilution. Absorbance was measured using a DR 6000 UV-VIS spectrophotometer (Hach, Dusseldorf, Germany) at 1 cm distance. Absorbance at 600 nm gave a value between 0.08 and 0.10. This was followed by preparation of the inoculum suspension adjusted to 1.5×10^8 colony forming units (CFU)/mL, according to McFarland's 0.5 bd bbl turbidity standard.

A volume of 5 µL of *Candida albicans* was added to each microcentrifuge tube. It was homogenized by vortexing (Thermolyne, Ramsey, MN, USA), and turbidity absorbance of the stock solution and its dilutions was measured with spectrophotometer. The tubes were then incubated at 37°C for 24 h under anaerobic conditions. Subsequently, 100 µL of the extract was inoculated and spread on *Sabouraud dextrose* agar plates using sterile handles. The plates were incubated at 37°C for 24 h under anaerobic conditions, and visual inspection for colony growth was performed. According to these results, it was possible to determine MFC.[31]

Table 2: Descriptive values of inhibition halos (mm) according to antifungal activity of the stock solution of *Morinda citrifolia* methanolic extract for each fruit part

Solution	n	Mean (mm)	SD	SE	95% CI		Minimum	Maximum	Sensitivity*
					LL	UL			
Peel stock	8	11.94	0.73	0.26	11.33	12.55	11.00	13.00	+
Seed stock	8	15.94	0.78	0.27	15.29	16.59	15.00	17.00	++
Pulp stock	8	11.56	0.90	0.32	10.81	12.32	10.00	13.00	+
0.12% CHX	8	22.13	1.25	0.44	21.08	23.17	20.00	24.00	+++
Total	32	15.39	4.41	0.78	13.80	16.98	10.00	24.00	

LL = lower limit, n = replicates (sample size), SD = standard deviation, SE = standard error of mean, UL = upper limit, 95% CI = 95% confidence interval

*Based on Duraffourd scale: sensitive (+), very sensitive (+++), and highly sensitive (+++++), CHX: 0.12% chlorhexidine digluconate

Statistical analysis

Data were collected on *ad hoc* form and entered into a Microsoft Excel 2019 spreadsheet. Subsequently, data were exported and processed with SPSS version 28 statistical package. For descriptive analysis, measures of central tendency and dispersion such as mean and standard deviation were used. Shapiro-Wilk normality test and Levene's homoscedasticity test were performed, both demonstrating that data met the requirements for applying parametric tests. The inhibition halos were compared with analysis of variance (ANOVA) test and Tukey's *post hoc* adjustment, considering a 95% significance level and type I error.

Results

Candida albicans showed sensitivity to stock solutions of *Morinda citrifolia* methanolic extract from pulp (6430 mg/mL), seed (10690 mg/mL), and peel (8270 mg/mL), being its antifungal activity, according to Duraffourd scale, sensitive for pulp and peel, and very sensitive for seed [Table 2].

Morinda citrifolia pulp methanolic extract presented the lower antifungal activity with average inhibition halos of 11.56 mm (95% CI: 10.81–12.32) against *Candida albicans*, whereas its seed methanolic extract presented higher antifungal activity with average inhibition halos of 15.94 mm (95% CI: 15.29–16.59) against *Candida albicans*. However, the highest average antifungal activity was obtained by 0.12% chlorhexidine (control) with 22.13 mm (CI: 21.08–23.17) [Table 2 and Figure 1].

When comparing stock solution of *Morinda citrifolia* peel, seed, and pulp methanolic extract with 0.12% chlorhexidine (control), significant differences in antifungal activity could be observed ($P < 0.001$) [Table 3]. Thus, when pairwise comparisons were made, *Morinda citrifolia* seed methanolic extract presented significantly higher antifungal activity than peel ($P < 0.001$) and pulp ($P < 0.001$), respectively. However, the seed could not match the efficacy of 0.12% chlorhexidine, as it presented significantly higher

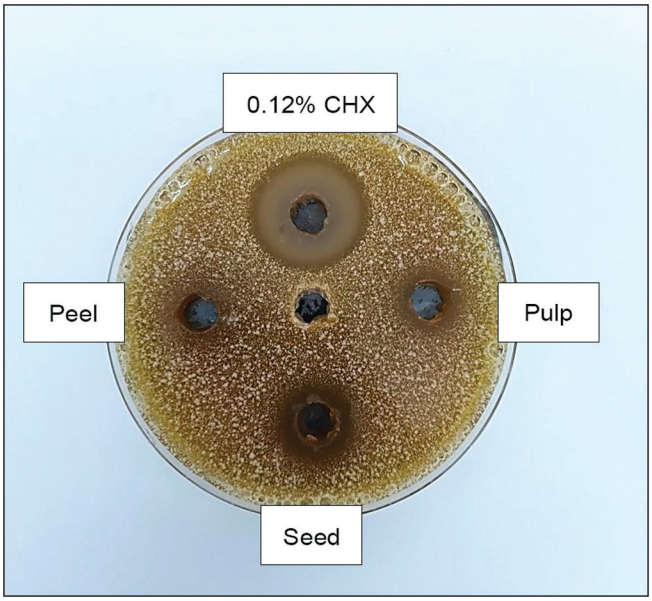

Figure 1: Inhibition ranges of *Morinda citrifolia* methanolic extract stock solution according to fruit parts. CHX: 0.12% chlorhexidine digluconate (positive control). Center well corresponds to distilled water (negative control)

antifungal effectiveness than the methanolic extract from all fruit parts ($P < 0.001$). In addition, *Morinda citrifolia* fruit pulp and peel methanolic extract showed similar antifungal activity ($P = 0.853$) [Table 4 and Figure 2].

Regarding *Morinda citrifolia* peel methanolic extract, it was observed that the MIC against *Candida albicans* at 24 h was the second dilution (2067.5 mg/mL), according to turbidity absorbance in tube. In addition, when visualizing the colonies growth on petri dish, it could be observed that this second dilution was also the MFC [Table 5].

Regarding *Morinda citrifolia* seed methanolic extract, it could be observed that the MIC against *Candida albicans* at 24 h was the third dilution (1366.25 mg/mL), according to turbidity absorbance in tube. In addition, when visualizing colony growth on petri dish,

Table 3: Comparison of inhibition halos according to antimicrobial activity of *Morinda citrifolia* methanolic extract from each part of the fruit

Solution	Mean (mm)	SD	SE	F	*P* value
Peel stock	11.94	0.73	0.26	219.85	<0.001*
Seed stock	15.94	0.78	0.27		
Pulp stock	11.56	0.90	0.32		
0.12% CHX	22.13	1.25	0.44		

F = intersubject one-way analysis of variance (ANOVA) test statistic, SD = standard deviation, SE = standard error of mean

**P* value < 0.05 (significant difference)

Table 4: Multiple comparisons of antimicrobial activity of *Morinda citrifolia* methanolic extract according to fruit parts

Solution	Seed stock	Pulp stock	0.12% CHX
Peel stock	*P* < 0.001*	*P* = 0.853	*P* < 0.001*
Seed stock		*P* < 0.001*	*P* < 0.001*
Pulp stock	*P* < 0.001*		*P* < 0.001*

**P* < 0.05 (significant differences) based on Tukey's *post hoc* test; 0.12% CHX: 0.12% chlorhexidine

it was observed that the MFC was the second dilution (2672.5 mg/mL) [Table 6].

Regarding *Morinda citrifolia* pulp methanolic extract, it could be observed that the MIC against *Candida albicans* at 24 h was the second dilution (1607.5 mg/mL), according to turbidity absorbance in the tube. In addition, when visualizing colony growth on plate, it was observed that the MFC was the first dilution (3215 mg/mL) [Table 7].

Discussion

Natural medicine used as an alternative and/or complementary treatment to counteract diseases is of great importance in public health. Therefore, the aim of the present study was to assess the *in vitro* antifungal activity of *Morinda citrifolia* methanolic extract of peel, pulp, and seed against *Candida albicans* strains.

In the present study, the results showed that *Morinda citrifolia* seed (10690 mg/mL), pulp (6430 mg/mL), and peel (8270 mg/mL) methanolic extract had antifungal activity, with the maximum effect observed in the seed methanolic extract. In addition, MIC and MFC of seed (1366.25 mg/mL), peel (2067.5 mg/mL), and pulp (1607.5 mg/mL) extracts were determined, with values of 2672.5, 2067.5, and 3215 mg/mL, respectively.

It is likely that the greater antifungal effect of *Morinda citrifolia* seed methanolic extract is due to higher concentration of coumarins, flavonoids, and/or quinones in this part of the fruit,[19] since these compounds can act in the fungus as potent inhibitors of the electronic transport chain, uncouplers of oxidative phosphorylation, intercalating agents of the DNA double helix, agents of biomolecules reductive alkylation, and as producers of free radicals.[31,32] In addition, flavonoids present in *Morinda citrifolia* in

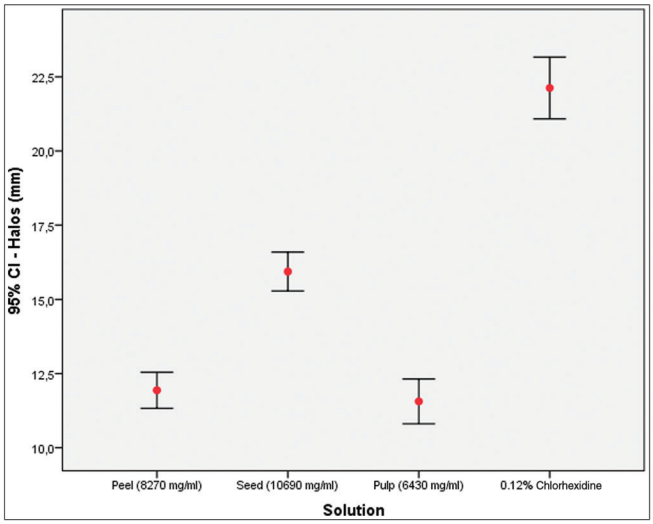

Figure 2: Means comparison at 95% confidence of inhibition halos according to *Morinda citrifolia* fruit parts at maximum concentration

the form of glycosides give it a high solubility in water and polar solvents, so when dissolved in methanol, this could increase its pharmacological action as an antimicrobial agent.[33]

Other components of *Morinda citrifolia* to which antifungal action would be attributed are iridoids, especially two: asperulosidic acid and deacetylasperulosidic acid, because these are found in the fruit at higher concentrations, which may vary according to geographical area where it grows.[20,22] In addition, other reports mention that the content of iridoids in *Morinda citrifolia* would be much higher than the content of flavonoids and coumarins.[34-36] Although the pharmacological activity of iridoids is not fully clarified, there is consistent evidence that it is an essential component of this plant.[20,22,34-36]

Table 5: Quantitative method for *Candida albicans* growth in serial dilutions of *Morinda citrifolia* peel methanolic extract

Growth	Stock, 8270 mg/mL	4135 mg/mL	2067.5 mg/mL	1033.75 mg/mL	516.88 mg/mL	258.43 mg/mL	129.22 mg/mL	64.61 mg/mL	PC	NC
In tube	-	-	-	+	+	+	+	+	+	-
On plate	-	-	-	+	+	+	+	+	+	-

NC = negative control (Sabouraud dextrose agar with pure *Morinda citrifolia* methanolic extract without microbes), PC = positive control (Sabouraud dextrose agar with *Candida albicans* inoculation without *Morinda citrifolia* methanolic extract), with growth (+), no growth (-)

Table 6: Quantitative method for *Candida albicans* growth in serial dilutions of *Morinda citrifolia* seed methanolic extract

Growth	Stock, 10690 mg/mL	5345 mg/mL	2672.5 mg/mL	1366.25 mg/mL	668.13 mg/mL	334.06 mg/mL	167.03 mg/mL	83.52 mg/mL	PC	NC
In tube	-	-	-	-	+	+	+	+	+	-
On plate	-	-	-	+	+	+	+	+	+	-

NC = negative control (Sabouraud dextrose agar with pure *Morinda citrifolia* methanolic extract without microbes), PC = positive control (Sabouraud dextrose agar with *Candida albicans* inoculation without *Morinda citrifolia* methanolic extract), with growth (+), no growth (-)

Table 7: Quantitative method for *Candida albicans* growth in serial dilutions of *Morinda citrifolia* pulp methanolic extract

Growth	Stock, 6430 mg/mL	3215 mg/mL	1607.5 mg/mL	803.75 mg/mL	401.88 mg/mL	200.94 mg/mL	100.47 mg/mL	50.23 mg/mL	PC	NC
In tube	-	-	-	+	+	+	+	+	+	-
On plate	-	-	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	-

NC = negative control (Sabouraud dextrose agar with pure *Morinda citrifolia* methanolic extract without microbes), PC = positive control (Sabouraud dextrose agar with *Candida albicans* inoculation without *Morinda citrifolia* methanolic extract), with growth (+), no growth (-)

There is a compound found exclusively in *Morinda citrifolia* fruit, which is eugenol,[37] and it has demonstrated proven antifungal properties against *Candida albicans*, significantly reducing the number of colony-forming units and histologically reducing the focal areas occupied by hyphae.[38] Another compound found in *Morinda citrifolia* fruit is scopoletin,[39] which is a coumarin that demonstrated antifungal properties against *Candida* fungi, inhibiting the growth rate of preformed biofilms by up to 68.2% and significantly decreasing the extent of biofilms growing on the surface of coverslips, preventing the formation of hyphae. Its mechanism of action is related to the lysis of the cell wall, also affecting the sterols of the plasma membrane.[40]

In the present study, *Morinda citrifolia* seed methanolic extract, at a concentration of 10,690 mg/mL, presented greater antimicrobial activity against *Candida albicans* at 24 h compared to other parts of the fruit, with an inhibitory halo of 15.94 mm, being this very sensitive according to Duraffourd scale. These results differ from those reported by Castillo *et al.*,[19] because *Morinda citrifolia* seeds in dry extract formed inhibition halos of 12 mm, and with hexane, chloroform, and ethyl acetate, they formed inhibition halos ranging from 8 to 12 mm. These differences could be due to fact that Castillo *et al.*[19] used hexane, chloroform, and ethyl acetate as solvents, whereas in the present study, methanol was used as solvent, which according to a study reported by Muenmuang *et al.*,[41] the methanolic extract provides the highest extraction yield of *Morinda citrifolia* fruit.

Morinda citrifolia pulp extract at a concentration of 6430 mg/mL presented the lowest antifungal activity with average inhibition halos of 11.56 mm. These results are similar to those obtained by Jayaraman *et al.*[25] However, these results disagree with those reported by Barani *et al.*,[23] who used the disk diffusion method, and unlike the present study, they used the lyophilized fruit juice extract at a concentration of 1 mg/mL, effectively inhibiting the growth of *Candida albicans* and giving inhibition halos of 16.6 ± 0.3 mm, increasing the inhibition pattern as the extract concentration increased.

In the present study, *Candida albicans* showed little sensitivity to *Morinda citrifolia* pulp methanolic extract despite applying the maximum concentration (6430 mg/

mL) for 24 h, which is in agreement with that obtained by Jayaraman *et al.*[25] However, this differs from that reported by West *et al.*,[20] because they isolated iridoids from *Morinda citrifolia* pulp and obtained that all *Candida albicans* growth was stopped at an iridoid concentration of 0.8 mg/mL. Furthermore, in the present study, it was obtained that the MIC of *Morinda citrifolia* pulp methanolic extract was 1607.5 mg/mL at an incubation period of 24 h, being this discrepant with that reported by Jainkittivong *et al.*,[24] who used lyophilized extract of pulp juice and observed that at 50 mg/mL concentration, no growth of *Candida albicans* was detected in an incubation time of 30 min, and at 60 mg/mL, no growth was detected in an incubation time of 15 min. These differences are probably due to fact that Jainkittivong *et al.*[24] obtained their extracts by lyophilization, which allowed greater preservation of their volatile or thermosensitive components, mixing freezing and dehydration.[11,42,43]

Most studies agree that antifungal effect of *Morinda citrifolia* extract would have positive dependence on its concentration, i.e., the higher the concentration, the better the antifungal effect,[20,23,24] which is consistent with findings obtained in the present study, because peel, seed, and pulp showed the best antifungal activity against *Candida albicans* when they were used at their maximum methanolic concentration.

Currently, natural products derived from plants for disease management and control have gained importance because of their low-cost effectiveness.[44] Therefore, the importance of the present study lies in the fact that it provides relevant information on antifungal properties of *Morinda citrifolia* against *Candida albicans*, because this yeast proved to be very sensitive to *Morinda citrifolia* seed methanolic extract. This lays the foundation for future research that can enrich knowledge and encourage the creation of products made from *Morinda citrifolia* seeds such as mouthwashes, toothpastes, or cleaning agents for dentures to be used in randomized clinical trials to prevent and combat candidiasis, because *Morinda citrifolia* is a highly marketed, popular, inexpensive product that does not generate adverse reactions.

Among the limitations of the present study, no studies were found in which the antifungal activity of *Morinda citrifolia* fruit peel was assessed, so the results obtained could not be compared. However, this is of little relevance because the fruit part that presented best antifungal activity against *Candida albicans* was the seed. In addition, it is recognized that findings obtained in this *in vitro* study cannot be extrapolated to clinical area. Therefore, it is important to continue this *Morinda citrifolia* research line and evaluate its antifungal effect on biofilm from clinical samples. In addition, studies are needed to assess *in vitro* the minimum concentration of cytotoxicity in *Morinda citrifolia* methanolic extract of peel, pulp, and seed using cell lines with the LC_{50} method, to know at what concentration, it could cause cell damage (hepatic or renal).[45]

It is recommended to conduct studies on the antifungal effectiveness of *Morinda citrifolia*, most representative chemical compounds such as iridoids, eugenol, and scopoletin against *Candida albicans*, because it is not yet fully elucidated, which is the main chemical compound responsible for the antifungal action of *Morinda citrifolia* or if this is due to a joint action of all its components. In addition, further studies are recommended to evaluate the antifungal potential of other parts of *Morinda citrifolia*, such as root, flower, stem, and leaf. On the other hand, it would be important to evaluate the combination of *Morinda citrifolia* and synthetic antifungals to verify if there is significant synergy, with the aim of creating a new mouthwash that can be used in conventional therapy or as a complimentary therapy, favoring the study, use, and preservation of native species biodiversity.

Conclusions

In summary, assuming the limitations of this *in vitro* study, it can be concluded that *Morinda citrifolia* methanolic extract of peel, pulp, and seed showed fungistatic and fungicidal effect against *Candida albicans*, being this very sensitive to seed extract with MIC of 1366.25 mg/mL and MFC of 2672.5 mg/mL, which allows to recommend the development of effective pharmacological formulations for control of candidiasis.

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Conflicts of interest

There are no conflicts of interest.

Authors contributions

SMMC conceived the research idea; SMMC, CFCR, and LACG prepared the article; CFCR, SMMC, and CLG collected and tabulated the information; LACG, GGL, and MILC carried out the bibliographic search; CFCR and ACP interpreted the results statisticians; SMMC, CFCR, and ACP helped in the development of the discussion; and CFCR, SMMC, CLG, MILC, and LACG carried out the critical revision of the article. All authors approved the final version of the article.

Ethical policy and institutional review board statement

This project is exempted from ethical approval because it was an experimental *in vitro* study. However, its execution was approved by a research committee of the Universidad Nacional Federico Villarreal, with resolution RR-2900-2018-UNFV and approval letter OGGE-059-2019.

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Ms. Sara Medrano-Colmenares, e-mail: saskimc@gmail.com) on request.

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