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Antifungal effect of *Crescentia cujete* fruit extracts on clinical strains of *Candida albicans*

Efecto antifúngico de extractos del fruto de *Crescentia cujete* en cepas clínicas de *Cándida albicans*

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ABSTRACT

Introduction: Candidiasis is a widespread fungal infection ranging from superficial to severe and potentially life-threatening. **Objective**: To evaluate the antifungal effects of ethanol and N-hexane extracts from *Crescentia cujete* (*C. cujete*) fruit on *Candida albicans* (*C. albicans*) strains. **Method**: *C. cujete* fruit pulp was dried and extracted using ethanol and N-hexane with Soxhlet extraction. Two reference strains of *C. albicans* (ATCC 10231 and 90028) and 12 clinical isolates from vaginal smears were cultured under different conditions: without extract, with serial dilutions of the ethanolic or N-hexane extract, and with ethanol and N-hexane at equivalent concentrations. Growth was measured by absorbance using MicroELISA equipment. **Results**: The N-hexane extract significantly reduced the growth of 9/12 clinical strains, while the ethanolic extract reduced 4/12. Both extracts were effective against the ATCC 90028 and ATCC 10231 strains. A direct correlation was found between growth inhibition and extract concentration, with N-hexane showing a more potent effect (Spearman's Rho=0.61, p-value <0.05) compared to the ethanolic extract (Spearman's Rho=0.39, p-value<0.05). **Conclusions**: This study demonstrates the antifungal effects of *C. cujete* extracts on *C. albicans*, including strains resistant to common antifungals. The findings suggest the presence of low-polarity compounds in the fruit with significant inhibitory activity.

Keywords: *Crescentia cujete*; Antifungal agents; Plant extracts; *Candida albicans*; Drug resistance.

RESUMEN

Introducción: candidiasis es una infección fúngica común que puede variar desde superficial hasta invasiva y potencialmente mortal. **Objetivo**: evaluar el efecto antifúngico de los extractos de etanol y N-hexano del fruto de *Crescentia cujete* (*C. cujete*) sobre cepas de *Candida albicans* (*C. albicans*). **Método**: la pulpa del fruto de *C. cujete* fue secada y extraída con etanol y N-hexano mediante extracción Soxhlet. Se utilizaron dos cepas de referencia de *C. albicans* (ATCC 10231 y 90028) y 12 aislados clínicos de muestras vaginales. Las cepas fueron cultivadas bajo diversas condiciones: sin extracto, con diluciones seriadas de los extractos etanólico o N-hexano, y con etanol y N-hexano en concentraciones equivalentes. Se midió el crecimiento mediante absorbancia con equipo MicroELISA. **Resultados**: el extracto de N-hexano redujo significativamente el crecimiento de 9/12 cepas clínicas,

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mientras que el extracto etanólico redujo 4/12. Ambos extractos fueron efectivos contra las cepas ATCC 90028 y ATCC 10231. Se observó una correlación directa entre la inhibición del crecimiento y la concentración de los extractos, siendo más notable con N-hexano (Spearman's Rho=0.61, p<0.05) que con el etanólico (Spearman's Rho=0.39, p<0.05). **Conclusiones**: se demuestra por primera vez la actividad antifúngica de los extractos de *C. cujete* contra *C. albicans*, con la inclusión de cepas resistentes a antifúngicos comunes.

Palabras clave: *Crescentia cujete*; agentes antifúngicos; extractos vegetales; *Candida albicans*; resistencia medicamentos.

INTRODUCTION

Candidiasis is one of the most common fungal infections worldwide and has the potential to trigger from superficial infections to invasive infections that can lead to death.[1–3] It is caused by Candida spp, the fourth causal agent of systemic infections in humans, even concerning prevalent bacterial pathogens.[4] Candida spp is also a common cause of skin, oral cavity, and genitourinary tract infections and is primarily known to cause what is known as vulvovaginal candidiasis. It has been reported that 50% to 75% of women worldwide have had at least one event of vulvovaginitis during their adulthood and that approximately 75 million have four or more events each year.[1,5] There are about 20 species of Candida that can cause infections in humans. However, 85 to 95% of the yeasts isolated from vaginal secretions of adult women correspond to the species *Candida albicans* (*C. Albicans*).[2,5]

Treatments against candidiasis, including polyenes, pyrimidine analogs, echinocandins, allylamines, and triazoles, are currently available.[6,7] However, therapeutic failures with these active ingredients are frequent, essentially due to the emergence of strains-resistant *C. albicans*. Therefore, one of the main challenges in antifungal therapy against *C. albicans* is the exploration of new pharmacological alternatives.

There is evidence of the antimicrobial potential of *Crescentia cujete* (*C. cujete*), a plant of the Bignoniaceae family native to the Americas and widely distributed in Asian and African countries. The traditional use of the fruit of *C. cujete* to treat vaginal infections in a Colombian subpopulation has recently been documented.[7,8] Additionally, it has been reported that extracts of different polarities prepared from the leaves or stems of *C. cujete* have antifungal activity against *C. albicans*, commonly reference strains.[9] However, there is limited information on the behavior of clinical isolates against extracts of different polarities of *C. cujete*, especially those prepared from the fruit of this plant.

Natural isolates of *C. albicans* exhibit significant genetic, chromosomal, and epigenetic differences that determine a broad spectrum of phenotypic properties. Substantial differences regarding virulence, ability to form biofilm, and adaptability to different environments (changes in cellular states in response to environmental signals) have been reported.[10] Likewise, this variability is reflected in the differential responses observed in the presence of antifungals. It has been reported that such differences from natural isolates are even more marked concerning reference strains, which are used more frequently to evaluate the antifungal effect of various substances. Hence, it is essential to evaluate the response of clinical strains of *C. albicans* against new molecules or compounds with therapeutic potential.

The present study examined the antifungal effect of the ethanolic and N-hexane extracts of the *C. cujete* fruit on two reference strains and 12 clinical isolates of *C. albicans*.

METHOD

Type of study

An experimental study was conducted.

Study area and population

The green fruits of the *C. cujete* plant were collected in the Galapa department of Atlántico, located in the Colombian Caribbean Region, at coordinates 10°53'54.1"N, 74°53'10.9"W. The municipality of Galapa is situated at an altitude of 83 meters above sea level, with average temperatures of 28°C. The fruits were harvested in October 2021 between 7:00 a.m. and 10:00 a.m.

The assays were performed on clinical strains of *C. albicans* isolated from vaginal secretion samples from patients hospitalized in a health care institution (IPS) in Barranquilla, Atlántico.

Participants and Instruments

Fruits of trees with approximate ages of two years and sizes of two meters, without plague or deformity, were chosen. The plant material was identified by the "Armando Dugand Gnecco" Herbarium National Collection Registry No. 83 index Herbariorum: "DUGAND" of the Universidad del Atlántico (Barranquilla/Colombia), where the voucher is filed with registration number 3350.

Assays were performed on 12 clinical strains and the reference strains ATCC 90028 (susceptible) and ATCC 10231 (resistant). The clinical strains were isolated from vaginal secretion samples from patients hospitalized in a health care institution (IPS) in Barranquilla/Atlántico, previously identified for diagnosis. The antifungal effect of the ethanolic extract and N-Hexane was evaluated, taking into account the recommendations of document M27 A3 for the study of sensitivity to antifungals, standardized by the Clinical and Laboratory Standards Institute (CLSI)**.**[11]

Procedure and data collection

Plant extract preparation: The fruit of *C. cujete* was washed with distilled water to remove unwanted contaminating material. The fruit pulp was extracted for drying in an oven with air circulation at 40°C for 24 hours. Five hundred grams of dry samples were obtained, finely pulverized, and stored in a sterile container until processed. To obtain the plant extract, 150 g of powdered dried fruit were taken and subjected to a Soxhlet extraction method in reagent-grade ethanol or n-hexane, respectively. The resulting extracts were concentrated under reduced pressure with a flash-type rotary evaporator (Buchí Heating Bath B-490). The crude extract was collected and dried at room temperature under sterile conditions.

Testing microorganisms: Assays were performed on 12 clinical strains and the reference strains ATCC 90028 (susceptible) and ATCC 10231 (resistant). The clinical strains were isolated from vaginal secretion samples from patients hospitalized in a health care institution (IPS) in Barranquilla/Atlántico, previously identified for diagnosis. For confirmation purposes, the strains were replicated in CHROMagar Candida (CAC) medium and subsequently preserved in Sabouraud dextrose agar supplemented with chloramphenicol, with periodic replications until the susceptibility study with the ethanolic extract and N-Hexane was carried out following the recommendations of the CLSI.[11]

Antifungal effect test: The dilution method was used in 96-well round-bottom plates, taking into account the recommendations of document M27 A3 for the study of sensitivity to antifungals, standardized by the CLSI.[11] The clinical strains of *C. albicans* were seeded in triplicate at a McFarlan scale of 0.5 in RMPI 1640 medium (Sigma-Aldrich) with Glutamine, without Sodium Bicarbonate, buffered with Acid 3- morpholino-4-yl-propane-1-sulfonic acid (MOPS) at 0.164 M (Sigma), pH 7±0.1, and 0.2% glucose, in the presence of different concentrations of ethanolic extract and n-hexane. Extract concentrations were obtained by serial twofold additive dilutions from 70 mg/mL of the respective crude extract. The following controls were included in

each plate in triplicate: 1) culture medium sterility control for each dilution of the respective extract. 2) Growth control of each strain without ethanolic extract and n-hexane. 3) Growth control of each strain in the presence of ethanol and n-hexane in the same concentrations of the extract by dilution to rule out an inhibitory effect of traces of solvent that the dilutions of the extracts may have.

The plates were incubated for 48 hours in a humidity chamber at room temperature. The absorbance reading was performed using the respective blanks and Micro Elisa equipment (STAR FAX-2100) from Awareness Technoloyinc in a wavelength range of 592 to 630 nm.

Statistic analysis

Data analysis was performed using SPSS software (Statistical Package for Social Studies, version 25.0). The treatment results on the strains are shown as a percentage of growth inhibition against the control, calculated as described in previous studies.[12] The Rho Spearman coefficient was calculated to examine the correlation between the growth inhibition of the strains and the concentration of each extract. The U. Mann-Whitney test was applied to compare the median percentage of inhibition between the extracts for each concentration. Additionally, the average inhibition percentages of the individual strains were compared with the growth control using the Student's T-test to determine if the extract's effect on each strain's growth was significant. All analyses were performed with a confidence level of 95%, and a value of $p < 0.05$ was considered statistically significant.

Declaration on the ethical aspect

This research has the approval of the Ethics Committee of the Universidad Metropolitana, Colombia, Act No 668 (code: BMD2023.001), guaranteeing compliance with the provisions of the Declaration of Helsinki of 1975 and Resolution 8430 of 1993 if the Ministery of Health of Colombian for research with Human beings. The informed consent was obtained by the IPS both verbally and in writing, under medical supervision, at the time of collecting the routine vaginal swab sample for the diagnosis of vaginal infection in hospitalized women. Once the IPS laboratory confirmed the infection with *C. albicans*, the strains and susceptibility reports of the patients who gave their consent were provided anonymously to be included in this research.

RESULTS

The N-Hexane extract of *C. cujete* fruit has demonstrated a significantly stronger antifungal effect than the ethanolic extract against *C. albicans*. Most strains (78.6%) were sensitive to the N-Hexane extract, while only a small proportion (42.9%) were sensitive to the ethanolic extract. In the strains sensitive to both extracts, the maximum inhibitory effect was achieved with lower concentrations of the N-Hexane extract than the ethanolic extract.

The antifungal effect of the ethanolic extract and N-Hexane of the *C. cujete* fruit was evaluated on 12 clinical isolates and two reference strains of *C. albicans*. The general analysis of the 14 strains showed a strong correlation between the concentration of the N-Hexane extract and the percentage of inhibition (Figure 1. A), while less correlation was observed with the ethanolic extract (Figure 1. B). Additionally, the median percentage of inhibition showed significant differences ($p<0.05$) between the two extracts. The N-Hexane extract exhibited a more significant inhibitory effect in all the concentrations evaluated than the ethanolic extract (Table 1).

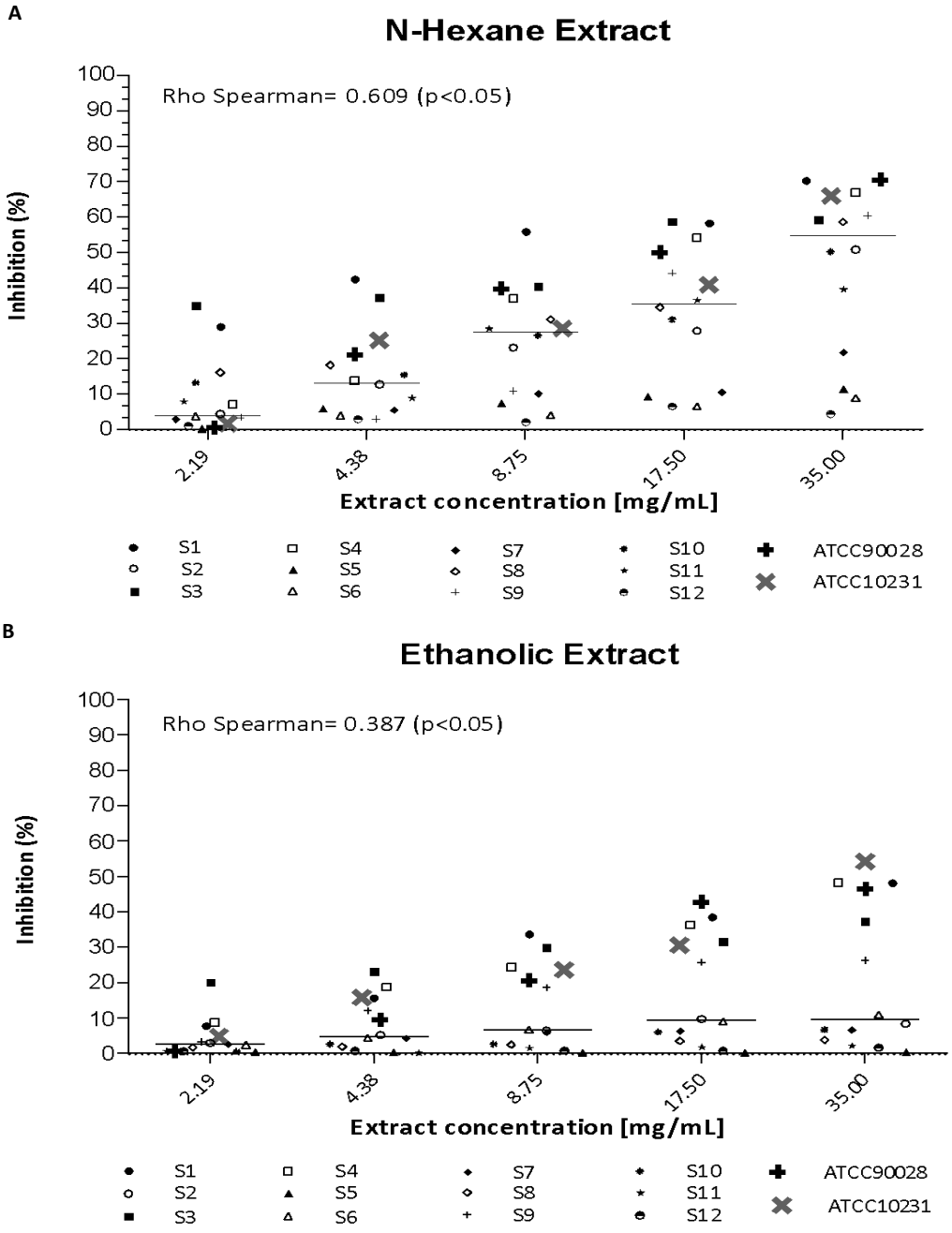

Figure 1. Antifungal effect of the ethanolic extract and N-hexane of the *C. cujete* fruit on *C. albicans* strains. Each character in the figure represents the average percentage of inhibition of three replicates for each strain studied. S1-S12: clinical isolates.

The inhibition averages between each strain and its respective growth control was compared. At all concentrations, it was observed that the N-Hexane extract significantly reduced growth in a higher proportion of strains than the ethanolic extract (Figure 2). Most strains (11/14) showed a significant reduction in growth when treated with the N-Hexane extract, while a small number (6/14) exhibited inhibition with the ethanolic extract. However, both extracts affected the growth of the reference strains ATCC90028 and ATCC10231. Additionally, it was observed that those strains that did not show significant inhibition with the N-Hexane extract (S5, S6, and S12) did not show this effect with the ethanolic extract either. Likewise, in all the strains

affected by the ethanolic extract (S1, S3, S4, S9, and reference strains), the minimum concentration required to reduce growth significantly was higher for the ethanolic extract than the N-Hexane extract. (Table 2).

Table 1. Comparison of *C. cujete* extracts according to the global inhibitory effect on *C. albicans* strains.

Extract concentration (mg/mL)	N-Hexane extract		Ethanol extract		P-value
	Median[a]	**IQR[b]**	**Median[a]**	**IQR[b]**	
2.19	4.21	11.56	2.48	4.47	0.018
4.38	12.72	16.13	5.25	12.69	0.002
8.75	26.01	25.56	7.03	21.71	<0.001
17.50	35.14	37.54	9.52	27.84	<0.001
35.00	50.18	44.77	9.46	36.42	<0.001

[a]Median and [b]Interquartile Range (IQR) of the % growth inhibition of the set of strains evaluated (12 clinical strains and two reference strains ATCC90028 and ATCC10231).

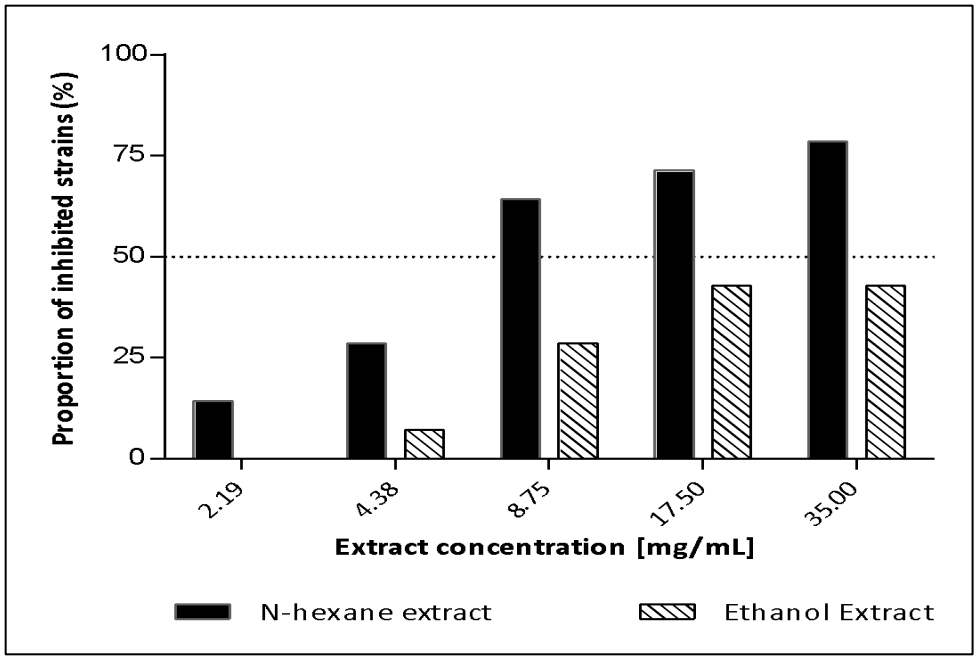

Figure 2. Proportion of C. albicans strains inhibited according to the concentration of the Ethanol and N-hexane extracts of *C. cujete*. The bars represent the percentage of strains compared to the total (N=14), showing a significant reduction in growth compared to the respective control without treatment ($p<0.05$).

Table 2. Summary of results: effect of the ethanolic extract and N-Hexane of *C. cujete* on the growth of clinical strains of *C. albicans*.

Strain	N-Hexane Extract			Ethanol Extract		
	Inhibitory effect[a]	**Concentration [b] mg/mL**	**MIE[c] %±SD**	**Inhibitory effect[a]**	**Concentration[b] mg/mL**	**MIE[c] %±SD**
S1	+	≥2.19	70.40±12.5	+	≥8.75	48.30±9.20
S2	+	≥8.75	50.80±9.00	-	----	----
S3	+	≥2.19	59.30±10.60	+	≥4.38	37.30±7.11
S4	+	≥8.75	67.10±11.90	+	≥8.75	48.40±7.20
S5	-	----	----	-	----	----
S6	-	----	----	-	----	----

S7	+	35	21.90±3.90	-	----	----
S8	+	≥8.75	58.70±10.50	-	----	----
S9	+	≥17.5	60.50±10.80	+	≥17.50	26.30±5.00
S10	+	≥8.75	50.2±8.9	-	----	----
S11	+	≥8.75	39.7±7.1	-	----	----
S12	-	----	----	-	----	----
ATCC90028	+	≥4.38	70.6±12.6	+	≥17.5	46.5±8.9
ATCC10231	+	≥4.38	66.1±11.8.00	+	≥8.75	54.3±10.4.00

[a]Inhibitory effect concerning the growth control statistically non-significant (-) or significant (+). [b]Minimum concentration, at which a significant inhibitory effect was observed. [c]**MIE (%)**, the maximum inhibitory effect observed. SD, standard deviation.

DISCUSSION

Due to widespread candidiasis worldwide and the progressive problem of resistance to antifungals, there is a growing demand for new antifungal therapies to treat infections by Candida spp. In this sense, the present study makes a new contribution by exploring the antifungal effect of extracts from the fruit of *C. cujete* on strains of *C. albicans*. The inhibitory response to treatments with ethanolic extracts and N-hexane observed in clinical isolates and reference strains stands out among the results.

In general, it was observed that both extracts evaluated caused a significant reduction in the growth of all the reference strains and several clinical isolates. However, the effects were more notable against the N-hexane extract, both in terms of the magnitude of the effect and the number of variants affected. In all dilutions, the median growth inhibition percentage was significantly higher with the n-hexane extract than with the ethanolic extract (Figure 1). A similar trend was observed in the Rho Spearman correlation coefficient (Table 1). Of particular interest is that all the strains that exhibited significant growth reduction with the ethanolic extract treatment also showed this effect with the N-Hexane extract. Likewise, when comparing the responses to both treatments on the individual strains, all the minimum concentrations of the ethanolic extract required to evidence this effect were more significant than or equal to those of the N-Hexane extract (Table 2). Additional studies are required to establish whether a single compound is present in higher concentrations in the N-hexane extract or if different compounds contribute to the results observed with the different extracts.

In *C. cujete*, various phytochemical compounds that exhibit antifungal properties have been identified. Among these, terpenoids have been documented in the fruit of *C. cujete*, identified predominantly in the N-Hexane extract.[13] Additionally, Zore et al.[14] demonstrated that terpenoids can inhibit the growth of *C. albicans* mediated by membrane alteration and the cell cycle's arrest.[14] According to previous studies, The fatty acids present mainly in N-Hexane fractions of *C. cujete* could also inhibit *C. albicans*. Other metabolites such as flavonoids, naphthoquinones, and saponins have also shown anti-*Cándida albicans* activity, and their presence has also been reported in *C. cujete*.[9,13,15,16] However, their concentrations in ethanolic and N-hexane extracts of the fruit are not well known.

After the individual analysis, it was established that both the N-hexane extract and the ethanolic extract affected the growth of the reference strains ATCC 90028 and ATCC 10231 similarly and significantly. The relevance of this finding lies in the fact that these strains are typically distinguished by the response to fluconazole; specifically, ATCC 90028 exhibits sensitivity, while ATCC 10231 exhibits resistance to

treatment. [17,18] Similarly, Navarro et al.,[19] also reported the observation of an inhibitory effect on the growth of the strain ATCC 10231 when they used an N-Hexane extract of Crescentia alata, a species belonging to the same family of *C. cujete* (Bignoniaceae). It is well known that fluconazole is one of the most frequently used antimycotics in the primary therapy of *C. Albicans* infections. Hence, the treatment of candidiasis is considered increasingly problematic due to the accumulated resistance of *C. Albicans* isolates, especially against fluconazole.[20] In this sense, our findings add clues that deserve additional studies intending to favor efforts for the antimicrobial control of *C. Albicans*.

Along the same lines, it was highlighted that the N-Hexane extract significantly inhibited the growth of most of the clinical strains evaluated (9/12). However, essential fluctuations were observed between the different strains regarding the magnitude of the effect and the minimum concentrations required to achieve a significant response against the control. Multiple factors influence the variability of response to antifungal treatments. Intraspecies analyses have established that genetic, chromosomal, and epigenetic characteristics are widely variable among natural isolates of *C. albicans* and, to the same extent, condition their phenotypic traits, including antifungal susceptibility profile.[10,21] This result again represents an input to propose and examine new hypotheses about the usefulness of *C. cujete* in searching for new candidiasis treatments.

CONCLUSIONS

This research presents preliminary evidence on the antifungal potential of ethanolic and N-hexane extracts from the fruit of *C. cujete* on clinical and reference strains of *C. albicans*. The results show promising effects, suggesting that bioactive substances present in the fruit of C. can be used to control infections by natural variants of *C. albicans*, including those resistant to fluconazole. The comparative evaluation of the effects of total extracts with solvents of opposite polarity provides a starting point for further development of more specific studies.

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STATEMENT ON CONFLICTS OF INTEREST

The authors of this research declare that we have no conflict of interest.

AUTHORS' CONTRIBUTION

MDW participated in the conception of the research idea, study design, interpretation of the results, writing, and approval of the final manuscript.

MSX conducted the study design, statistical analysis, writing of the results, and approval of the final manuscript

RTLM participated in the experimental study, data acquisition, draft writing, and final manuscript approval.

JGA participated in experimental studies, data acquisition, draft writing, and final manuscript approval.

CLYE monitored project execution, administrative procedures, and logistics in data collection and reviewed the manuscript's final approval.

OPP participated in the study design, statistical analysis, writing of the results, and approval of the final manuscript

RSA participated in the study design, statistical analysis, writing of the results, and approval of the final manuscript

GTYP monitored project execution, administrative procedures, and logistics during data collection, review, and final approval of the manuscript.

BMAC participated in the conception of the research idea, study design, interpretation of the results, writing, and approval of the final manuscript.

REFERENCES

1. Parambath S, Dao A, Kim HY, Zawahir S, Izquierdo AA, Tacconelli E, et al. *Candida albicans*-A systematic review to inform the World Health Organization Fungal Priority Pathogens List. Med Mycol. 2024;27;62:1-25. https://doi.org/10.1093/mmy/myae045

2. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012;36:288-305. https://doi.org/10.1111/j.1574-6976.2011.00278.x

3. Gunasekara T, Radhika N, Ragunathan K, Gunathilaka D, Weerasekera M, Hewageegana H, et al. Determination of antimicrobial potential of five herbs used in Ayurveda practices against *Candida albicans*, *Candida parapsilosis* and Methicillin Resistant *Staphylococcus aureus*. Anc Sci Life. 2017;36:187-90. https://doi.org/10.4103/asl.ASL_179_16

4. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309-17. https://doi.org/10.1086/421946

5. Sobel JD. Vulvovaginal candidosis. Lancet. 2007;369:1961-71. https://doi.org/10.1016/S0140-6736(07)60917-9

6. White TC, Holleman S, Dy F, Mirels LF, Stevens DA. Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrob Agents Chemother. 2002;46:1704-13. https://doi.org/10.1128/AAC.46.6.1704-1713.2002

7. Sanglard D, Odds FC. Resistance of Candida species to antifungal agents: Molecular mechanisms and clinical consequences. Lancet Infect Dis. 2002;2:73-85. https://doi.org/10.1016/s1473-3099(02)00181-0

8. Castillo CL. Plantas medicinales utilizadas en el tratamiento de enfermedades ginecológicas en Leticia y Puerto Nariño (Amazonas, Colombia). Etnobiologia. 2015;13:53-72.

9. Chukwunonye UCE, Obioma DE, Gaza ASP, Obisike CV. Pharmacognostic studies and antimicrobial activity of *Crescentia cujete linnaeus* stem bark (Bignoniaceae). Indo Am J Pharm Sci. 2017;4:484-90. https://doi.org/10.5281/zenodo.377021

10. Ene IV, Lohse MB, Vladu AV, Morschhäuser J, Johnson AD, Bennett RJ. Phenotypic profiling reveals that *Candida albicans* opaque cells represent a metabolically specialized cell state compared to default white cells. mBio. 2016;7:e01269-16. https://doi.org/10.1128/mBio.01269-16

11. Cantón E, Martin Mazuelos E, Espinel-Ingroff A. Métodos estandarizados por el CLSI para el estudio de la sensibilidad a los antifúngicos (documentos M27-A3, M38-A y M44-A). Rev Iberoam Micol. 2007:15a;1-17.

12. Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, John B, et al. Antifungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. Asian Pac J Trop Med. 2013;6:673-81. https://doi.org/10.1016/S1995-7645(13)60117-0

13. Billacura M, Caezar G, Laciapag R. Phytochemical screening, cytotoxicity, antioxidant, and anthelmintic property of the various extracts from Crescentia cujete Linn. FRUIT. Sci Int Lahore. 2017;29:31-5.

14. Zore GB, Thakre AD, Jadhav S, Karuppayil SM. Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of cell cycle. Phytomedicine. 2011;18:1181-90. https://doi.org/10.1016/j.phymed.2011.03.008

15. Seleem D, Pardi V, Murata RM. Review of flavonoids: A diverse group of natural compounds with anti-*Candida albicans* activity in vitro. Arch Oral Biol. 2017;76:76-83. https://doi.org/10.1016/j.archoralbio.2016.08.030.

16. Parente FGG, Oliveira AP, Rodrigues CMS, Júnior RG, Mir I, Paulo a M, et al. Phytochemical screening and antioxidant activity of methanolic fraction from the leaves of Crescentia cujete L. (Bignoniaceae). J Chem Pharm Res. 2016;8:231-6.

17. Espinel-Ingroff A, Rodríguez-Tudela JL, Martínez-Suárez JV. Comparison of two alternative microdilution procedures with the National Committee for Clinical Laboratory Standards reference macrodilution method M27-P for in vitro testing of fluconazole-resistant and -susceptible isolates of *Candida albicans*. J Clin Microbiol. 1995;33:3154-8. https://doi.org/10.1128/jcm.33.12.3154-3158.1995

18. Mertas A, Garbusińska A, Szliszka E, Jureczko A, Kowalska M, Król W. The influence of tea tree oil (Melaleuca alternifolia) on fluconazole activity against fluconazole-resistant *Candida albicans* Strains. BioMed Res Int. 2015;2015:590470. https://doi.org/10.1155/2015/590470

19. Navarro V, Villarreal MaL, Rojas G, Lozoya X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharmacol. 1996;53:143-7. https://doi.org/10.1016/0378-8741(96)01429-8

20. Zhang W, Song X, Wu H, Zheng R. Epidemiology, risk factors and outcomes of *Candida albicans* vs. non-albicans candidaemia in adult patients in Northeast China. Epidemiol Infect. 2019;147:e277. https://doi.org/10.1017/S0950268819001638

21. Hirakawa MP, Martinez DA, Sakthikumar S, Anderson MZ, Berlin A, Gujja S, et al. Genetic and phenotypic intra-species variation in *Candida albicans*. Genome Res. 2015;25:413-25. https://doi.org/10.1101/gr.174623.114