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Comparative Antifungal Activity of *Curcuma longa* Ethanol Extract Alone and in Combination with *Lactobacillus* Metabolites on Clinical Isolates of *Candida albicans*

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ABSTRACT

Background: *Candida albicans* is a major opportunistic fungal pathogen whose management is increasingly challenged by antifungal resistance. The search for alternative antifungal agents has stimulated interest in medicinal plants and probiotics because of their antimicrobial properties. This study evaluated the antifungal activity of ethanol extract of *Curcuma longa* (turmeric) alone and in combination with metabolites of *Lactobacillus bulgaricus* against clinical isolates of *Candida albicans*.

Methods: Ethanol extract of *C. longa* rhizomes were prepared by maceration, yielding 3.43% crude extract. Cell-free metabolites of *L. bulgaricus* were obtained from cultured isolates. Five clinical isolates obtained from wound swab, high vaginal swab, ear swab, catheter tip, and urine were tested using agar well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. Data were analyzed using descriptive statistics and SPSS version 27.

Results: Turmeric extract demonstrated concentration-dependent antifungal activity, no inhibition was observed at concentrations below 200 mg/ml, while susceptibility increased from 20% at 200 mg/ml to 60% at 400mg/ml. The combination of *C. longa* extract and *L. bulgaricus* metabolites showed improved activity, inhibiting 40%, 80%, and 100% 0f isolates at 100 mg/ml, 200 mg/ml, and 400 mg/ml, respectively. MIC values for the extract alone ranged from 25 mg/ml to > 100 mg/ml, whereas the combination treatment reduced MIC values to 12.5-50 mg/ml, MFC values for the extract alone ranged from 50-200 mg/ml. High vaginal swab isolate exhibited the greatest susceptibility, while wound and catheter tip isolates were less susceptible. Ketoconazole demonstrated superior antifungal activity compared with both treatments.

Conclusion: Ethanol *Curcuma longa* extract possesses antifungal activity against clinical isolates of *Candida albicans*, and its activity appears to be enhanced when combined with *Lactobacillus bulgaricus* metabolites. These findings suggest that plant-probiotic combinations may have potential as complementary antifungal approaches and warrant further investigation using larger sample sizes and advanced interaction studies to validate the therapeutic potential of this plant-probiotic combination.

Keywords: *Candida albicans; Curcuma longa; Lactobacillus bulgaricus;* Antifungal activity; Candidiasis.

INTRODUCTION

Candidiasis is a fungal infection primarily caused by *Candida albicans*, affecting mucous membranes, skin, gastrointestinal and urinary tracts, and in severe cases the bloodstream, where it may become life-threatening if untreated (1). Although *C. albicans* forms part of the normal human microbiota, it can become pathogenic under conditions such as immune suppression. Its pathogenicity is associated with several virulence factors including adhesins, morphogenesis, phenotypic switching, and the ability to transition between yeast and hyphal forms, which contributes significantly to disease severity (1).

The increasing prevalence of candidiasis, particularly infections involving virulent and drug-resistant strains, poses a major public health challenge. Conventional antifungal agents such as azoles, polyenes, 5-flucytosine, and echinocandins are becoming less effective because of emerging resistance, especially to azoles and echinocandins, thereby reducing available treatment options (2,3). This growing resistance has intensified the search for alternative antifungal agents with improved efficacy.

Medicinal plants have long been recognized as valuable sources of therapeutic compounds, particularly in Africa and Asia, where they remain widely used in tradition medicine (4,5). Several plant-derived bioactive compounds exhibit anti-*Candida* activity by disrupting fungal cell wall integrity, membrane function, metabolism, adherence, morphogenesis, and biofilm formation (4). *Curcuma longa* (turmeric), a rhizomatous

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plant belonging to the family of *Zingiberaceae*, possesses diverse pharmacological properties, including antimicrobial activity (6). Curcumin, the major bioactive constituent of turmeric, has demonstrated inhibitory effects against *C. albicans* and other pathogenic fungi (7).

Probiotics, are live microorganisms that confer health benefits when administered in adequate amounts and are also known for their antimicrobial properties. Species of *Lactobacillus* inhibit *C. albicans* through mechanisms such as competitive exclusion and the production of lactic acid, hydrogen peroxide, and antimicrobial compounds including bacteriocins (8,9). These bacteria are naturally present in the gastrointestinal and vaginal microbiota and are commonly associated with fermented dairy products (10).

Although previous studies have independently demonstrated the antifungal activity of *Curcuma longa* extracts against *Candida albicans* and the inhibitory effects of *Lactobacillus* species on *Candida* infections (5,7-10). However, limited information exists regarding the combined antifungal effects of ethanol extract of *Curcuma longa* and *Lactobacillus bulgaricus* metabolites against clinical isolates of *C. albicans.* This knowledge gap limits understanding of whether combining these natural agents may enhance antifungal activity beyond that observed when each is used individually. Therefore, this study evaluated the comparative antifungal activity of ethanol *Curcuma longa* extract alone and in combination with *Lactobacillus* metabolites against clinical isolates of *C. albicans,* in order to determine their potential as natural antifungal agents.

MATERIALS AND METHODS

Study Area

This study was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria.

Sample Collection

Five archived Clinical isolates of *Candida albicans*, each representing a different clinical source, were obtained from the Medical Microbiology Laboratory the University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City, Edo State Nigeria. UBTH is a tertiary healthcare and academic institution affiliated with the University of Benin and serves as a major referral center in the South-South region of Nigeria.

The isolates were recovered from wound swab, high vaginal swab, ear swab, catheter tip, and urine specimens collected from patients with suspected candidiasis. Only previously identified and stored isolates were used in this study, and all patient identifiers were removed prior to laboratory analysis to ensure confidentiality. All specimens had originally been collected using standard aseptic procedures to minimize contamination. Confirmation of *C. albicans* was carried out using standard mycological techniques, including colonial morphology, Gram staining and germ tube test.

Data Collection

Data were collected through laboratory-based in vitro experimental procedures. The antifungal activity of ethanol *Curcuma longa* extract alone and in combination with *Lactobacillus bulgaricus* metabolites was evaluated using agar well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. The isolates obtained were subcultured, identified, and standardized prior to antifungal susceptibility testing. Antifungal susceptibility testing procedures were performed in accordance with standard microbiological methods and CLSI-recommended laboratory practices where applicable. Measurements obtained included zones of inhibition (mm), MIC values, and MFC values at different extract concentrations. Sterile distilled water was used as the negative control, while ketoconazole served as the positive control for antifungal activity. All experiments were performed in triplicate to improve reliability and reproducibility of results. Data generated from repeated experimental observations were recorded and used for statistical analysis.

Sample Processing

The obtained isolates were sub-cultured on Sabouraud Dextrose Agar (SDA) and incubated aerobically at 37 °C for 24 – 48 hours to obtain pure colonies. Identification of *C. albicans* isolates were confirmed using standard mycological techniques, including colonial morphology, Gram staining, and germ tube test. Confirmed isolates were maintained in Sabouraud Dextrose Broth and stored under refrigerated conditions until use. For assays, well-isolated colonies were aseptically transferred into sterile normal saline, and the inoculum turbidity was adjusted to match the 0.5 Mcfarland standard, corresponding approximately to 1×10^6 CFU/ml. Standardization of the inoculum was performed to ensure uniform fungal density across all experimental procedures. All procedures were carried out using standard microbiological techniques and biosafety precautions to minimize contamination and ensure reproducibility of results.

Preparation of Ethanol *Curcuma Longa* Extract and *Lactobacillus* Metabolites

Fresh rhizomes of *Curcuma longa* were washed thoroughly with distilled water, peeled, sliced into smaller pieces and air-dried at room temperature until a constant weight was obtained. The dried rhizomes were then pulverized into fine powder using a laboratory grinder. Seven hundred grams (700 g) of the powdered rhizome were macerated in 2 L of ethanol for 72 hours with intermittent stirring. The mixture was filtered

using Whatman No. 1filter paper, and the filtrate was concentrated using a water bath maintained at 40 – 50°C to obtain a crude ethanol extract. The resulting extract weighed 24 g, corresponding to a percentage yield of 3.43%. The crude extract was stored in sterile airtight containers until use. For antifungal susceptibility testing, the extract was reconstituted in sterile distilled water to prepare concentrations of 25, 50, 100, 200, and 400 mg/ml. A volume of 250 µl of each concentration was dispensed into the agar wells during antifungal assays.

A probiotic strain of *Lactobacillus bulgaricus* was isolated from commercially available yoghurt and identified using Gram staining and catalase testing. The isolate was cultured on de Man, Rogosa and Sharpe (MRS) agar incubated under anaerobic conditions at 37 °C for 24–48 hours. A pure colony was subsequently inoculated into MRS broth and incubated to allow the production of secondary metabolites. Following incubation, the broth culture was centrifuged at 4000 revolution per minute (rpm) for 15 minutes, and the supernatant was collected. The cell-free supernatant was sterilized by filtration through a 0.22 µm membrane filter and stored at 4 °C until use. The pH of the supernatant was measured prior to antifungal testing and recorded. The sterile cell-free supernatant served as the source of *Lactobacillus* metabolites used in the study. For combination testing, ethanol *Curcuma longa* extract and *Lactobacillus bulgaricus* metabolites were mixed in a 1:1 ratio immediately before use. This ratio was selected to allow equal representation of broth components during antifungal susceptibility testing.

Test for Antifungal Activity

The antifungal activity of ethanol *Curcuma longa* extract alone and in combination with *Lactobacillus bulgaricus* metabolites was evaluated using agar well diffusion, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays. All test were performed in triplicate. Standardized *Candida albicans* inocula adjusted to 0.5 McFarland standard were used throughout the study.

For the agar well diffusion assay, Sabouraud Dextrose agar (SDA) plates were inoculated with the test organism, and 10mm wells were bored into the agar. A volume of 250µl of ethanol *Curcuma longa* extract at concentrations of 25, 50, 100, 200 and 400 mg/ml were dispensed into the respective wells. For combination testing, equal volumes of the extract and *Lactobacillus bulgaricus* cell-free supernatant were mixed in a 1:1 ratio before application. Ketoconazole was used as the positive control. The plates were incubated at 37ºC for 24 hours, after which inhibition zones were measured in millimeters (mm), and the mean values were calculated from three independent determinations.

The minimum inhibitory concentrations (MIC) of the extract alone and in combination with *Lactobacillus* supernatant was determined using the agar dilution method with two-fold serial dilutions from 12.5–200 mg/ml, where standardized test organisms were inoculated onto extract-containing agar plates and incubated for 24 hours. The lowest concentration inhibiting visible growth was recorded as the MIC.

The minimum fungicidal concentration (MFC) of each treatment group was determined by sub-culturing from MIC plates onto extract-free agar and incubated for 24 hours; the lowest concentration showing no growth was considered the MFC.

Statistical Analysis

Data obtained from the study were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 27. Descriptive statistics, including frequencies, percentages, and mean values, were used to summarize and compare the antifungal activities of ethanol *Curcuma longa* extract alone and in combination with *Lactobacillus bulgaricus* supernatant against clinical isolates of *Candida albicans*. Results were presented in tables.

RESULTS

The physical characteristics and percentage yield of the ethanol extract of *Curcuma longa* rhizome are presented in Table 1. The extract was characterized by a spicy aromatic odour, smooth texture, orange-brown colour, and thick consistency. Ethanol was used as the extraction solvent. The percentage yield obtained from the extraction process was 3.43%, indicating that a relatively small proportion of the plant material was recovered as crude extract.

The antifungal activity of ethanol extract of *Curcuma longa* against clinical isolates of *Candida albicans* as measured by zone of inhibition is presented in Table 2. No inhibitory activity was observed at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml against all isolates tested. At 200 mg/ml, only the urine isolate (CA5) exhibited susceptibility, producing a zone of inhibition of 11 mm. At 400 mg/ml, inhibitory activity was observed against the high vaginal swab isolate (CA2), ear swab isolate (CA3), and urine isolate (CA5), with zones of inhibition of 11 mm, 11 mm, and 13 mm, respectively. However, no inhibition was recorded against the wound swab isolate (CA1) and catheter tip isolate (CA4) at any concentration tested. The positive control, ketoconazole, exhibited inhibitory activity against all isolates, with zones of inhibition ranging from 14 mm to 20 mm. These findings indicate that the ethanol extract possesses antifungal activity which increases with concentration. The susceptibility profile of clinical isolates of *Candida albicans* to ethanol *Curcuma longa* extract showed (Table 3) that none of the isolates was susceptible at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml. At 200 mg/ml, only one isolate representing 20% of the total isolates was susceptible. Susceptibility increased to three isolates, representing 60%, at 400 mg/ml. The results

indicate that the antifungal activity of the extract increased with increasing concentration.

The antifungal activity of ethanol extract of *Curcuma longa* combined with *Lactobacillus bulgaricus* supernatant against clinical isolates of *Candida albicans* is showed in Table 4. No inhibitory activity was observed at concentrations of 25 mg/ml and 50 mg/ml. At 100 mg/ml, inhibition zones of 11 mm were observed against the high vaginal swab isolate (CA2) and urine isolate (CA5). At 200 mg/ml, inhibitory activity was observed against four isolates, with zones of inhibition ranging from 11 mm to 13 mm. At 400 mg/ml, all isolates were inhibited, producing zones of inhibition ranging from 12 mm to 15 mm. These findings demonstrate that the combination treatment exhibited greater antifungal activity than the ethanol extract alone. The susceptibility profile of clinical isolates of *Candida albicans* to ethanol extract of *Curcuma longa* combined with *Lactobacillus bulgaricus* supernatant is presented in Table 5. No isolate was susceptible at concentrations of 25 mg/ml and 50 mg/ml. At 100 mg/ml, two isolates representing 40% susceptibility were inhibited. Susceptibility further increased to 80% at 200 mg/ml and reached 100% at 400 mg/ml. This result indicates that the combination treatment exhibited enhanced antifungal activity against the clinical isolates.

The comparative susceptibility of clinical isolates of *Candida albicans* to ethanol extract of *Curcuma longa* alone and in combination with *Lactobacillus bulgaricus* is presented in Table 6. While neither treatment demonstrated inhibitory activity at concentrations of 25 mg/ml and 50 mg/ml, the combination treatment showed greater susceptibility rates at higher concentrations. At 100 mg/ml, the combination treatment inhibited 40% of the isolates compared to 0% for the extract alone. At 200 mg/ml, susceptibility increased from 20% for the extract alone to 80% for the combination treatment. At 400 mg/ml, all isolates (100%) were susceptible to the combination treatment, whereas only 60% were susceptible to the extract alone. These findings show a synergistic antifungal effect between the ethanol extract and *Lactobacillus bulgaricus* supernatant.

The minimum inhibitory concentration (MIC) of ethanol extract *Curcuma longa* against clinical isolates of *Candida albicans* is presented in Table 7. The MIC values varied among the isolates, indicating differences in susceptibility. The lowest MIC value of 25 mg/ml was observed for the high vaginal swab isolate (CA2) and catheter tip isolate (CA4), while the ear swab isolate (CA3) exhibited an MIC of 50 mg/ml. The urine isolate (CA5) required a concentration of 100 mg/ml for growth inhibition, whereas the wound swab isolate (CA1) remained resistant at all concentrations tested, indicating an MIC greater than 100 mg.

The minimum inhibitory concentration (MIC) of ethanol extract *Curcuma longa* combined with *Lactobacillus bulgaricus* supernatant against clinical isolates of *Candida albicans* is presented in Table 8. The lowest MIC value of 12.5 mg/ml was observed for the wound swab isolate (CA1), while MIC values of 25 mg/ml and 50 mg/ml were recorded for the remaining isolates. Compared with the ethanol extract alone, the combination treatment generally exhibited lower MIC values, indicating enhanced inhibitory activity against *Candida albicans*.

The minimum fungicidal concentration (MFC) of ethanol extract *Curcuma longa* against clinical isolates of *Candida albicans* is presented in Table 9. The lowest MFC value of 50 mg/ml was observed for the high vaginal swab isolate (CA2), while the ear swab isolate (CA3) exhibited an MFC value of 100 mg/ml. The wound swab isolate (CA1), catheter tip isolate (CA4), and urine isolate (CA5) required a concentration of 200 mg/ml to achieve complete fungicidal activity.

The minimum fungicidal concentration (MFC) of ethanol extract of *Curcuma longa* combined with *Lactobacillus bulgaricus* metabolites against clinical isolates of *Candida albicans* as shown in Table 10 reveal most isolates (CA1, CA2, CA4, and CA5) had an MFC of 100 mg/ml, while the ear swab isolates (CA3) showed a lower MFC of 50 mg/ml, indicating greater susceptibility. The absence of growth at these concentrations confirms the fungicidal effect of the combined treatment.

Table 1: Physical Properties and Percentage Yield of Crude Ethanol Extract of *Curcuma longa* Rhizome.

Property	Ethanol Extract
Odour	A Spicy aromatic smell
Solvent	Ethanol
Texture	Smooth
Colour of crude extract	Orange-brown
Consistency	Thick
Percentage yield	3.43%

Table 2: Antifungal Activity of Ethanol Extract of *Curcuma longa* Against Clinical Isolates of *Candida albicans* as Measured by Zone of Inhibition (mm)

Clinical Isolates	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Ketoconazole (25 mg/ml)
CA1 (Wound swab)	ND	ND	ND	ND	ND	17
CA2 (High vaginal swab)	ND	ND	ND	ND	11	19
CA3 (Ear swab)	ND	ND	ND	ND	11	20
CA4 (Catheter tip)	ND	ND	ND	ND	ND	15
CA5 (Urine)	ND	ND	ND	11	13	14

Key: *CA = Candida albicans, ND = No detectable inhibition zone*

Table 3: Susceptibility Profile of Clinical Isolates of *Candida albicans* to Ethanol Extract of *Curcuma longa*

Concentration (mg/ml)	Number of Susceptible Isolates (n = 5)	Percentage Susceptibility (%)
25	0	0
50	0	0
100	0	0
200	1	20
400	3	60

Table 4: Antifungal Activity of Ethanol Extract of *Curcuma longa* Combined with *Lactobacillus bulgaricus* Metabolites Against Clinical Isolates of *Candida albicans* as Measured by Zone of Inhibition (mm)

Clinical Isolates	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Ketoconazole (25 mg/ml)
CA1 (Wound swab)	ND	ND	ND	11	15	17
CA2 (High vaginal swab)	ND	ND	11	13	14	19
CA3 (Ear swab)	ND	ND	ND	ND	12	20
CA4 (Catheter tip)	ND	ND	ND	11	13	15
CA5 (Urine)	ND	ND	11	13	15	14

Key: *CA = Candida albicans, ND = No detectable inhibition zone*

Table 5: Susceptibility Profile of Clinical Isolates of *Candida albicans* to Ethanol Extract of *Curcuma longa* Combined with *Lactobacillus bulgaricus* Metabolites According to Specimen Source

Concentration (mg/ml)	Number of Susceptible Isolates (n = 5)	Percentage Susceptibility (%)
25	0	0
50	0	0
100	2	40
200	4	80
400	5	100

Table 6: Comparative Susceptibility of Clinical Isolates of *Candida albicans* to Ethanol Extract of *Curcuma longa* Alone and in Combination with *Lactobacillus bulgaricus* Metabolites

Concentration (mg/ml)	Extract Alone (%)	Extract + *Lactobacillus* (%)
25	0	0
50	0	0
100	0	40
200	20	80
400	60	100

Table 7: Minimum Inhibitory Concentration (MIC) of Ethanol Extract *Curcuma longa* Against Clinical Isolates of *Candida albicans*

Clinical Isolates	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	MIC (mg/ml)
CA1 (Wound swab)	G	G	G	G	>100
CA2 (High vaginal swab)	G	NG	NG	NG	25
CA3 (Ear swab)	G	G	NG	NG	50
CA4 (Catheter tip)	G	NG	NG	NG	25
CA5 (Urine)	G	G	G	NG	100

Key: *G = Growth, NG = No Growth, MIC = Minimum Inhibitory Concentration.*

Table 8: Minimum Inhibitory Concentration (MIC) of Ethanol Extract of *Curcuma longa* Combined with *Lactobacillus bulgaricus* Metabolites Against Clinical Isolates of *Candida albicans*

Clinical Isolates	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	MIC (mg/ml)
CA1 (Wound swab)	NG	NG	NG	NG	12.5
CA2 (High vaginal swab)	G	NG	NG	NG	25
CA3 (Ear swab)	G	G	NG	NG	50
CA4 (Catheter tip)	G	G	NG	NG	50
CA5 (Urine)	G	G	NG	NG	50

Key: *G = Growth, NG = No Growth, MIC = Minimum Inhibitory Concentration.*

Table 9: Minimum Fungicidal Concentration (MFC) of Ethanol Extract of *Curcuma longa* Against Clinical Isolates of *Candida albicans*

Clinical Isolates	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	MFC (mg/ml)
CA1 (Wound swab)	G	G	G	G	NG	200
CA2 (High vaginal swab)	G	G	NG	NG	NG	50
CA3 (Ear swab)	G	G	G	NG	NG	100
CA4 (Catheter tip)	G	G	G	G	NG	200
CA5 (Urine)	G	G	G	G	NG	200

Key: *G = Growth, NG = No Growth, MFC = Minimum Fungicidal Concentration*

Table 10: Minimum Fungicidal Concentration (MFC) of Ethanol Extract of *Curcuma longa* Combined with *Lactobacillus bulgaricus* Metabolites Against Clinical Isolates of *Candida albicans*

Clinical Isolates	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	MFC (mg/ml)
CA1 (Wound swab)	G	G	G	NG	NG	100
CA2 (High vaginal swab)	G	G	G	NG	NG	100
CA3 (Ear swab)	G	G	NG	NG	NG	50
CA4 (Catheter tip)	G	G	G	NG	NG	100
CA5 (Urine)	G	G	G	NG	NG	100

Key: *G = Growth, NG = No Growth, MFC = Minimum Fungicidal Concentration*

DISCUSSION

The results of this study demonstrated that ethanol extract of *Curcuma longa* exhibited concentration-dependent antifungal activity against clinical isolates of *Candida albicans*. This finding agrees with previous studies that have reported the inhibitory effects of turmeric extracts against *Candida* species. Salih *et al*. reported significant growth inhibition of *Candida* species by ethanol extract of *C. longa*, highlighting its potential as a plant-derived antifungal agent (5). Similarly, Kasta demonstrated the inhibitory activity of ethanol turmeric extracts against *C. albicans* and other microbial pathogens (11). The concentration-dependent response observed in the present study is also consistent with the findings of Murugesh *et al*., who reported increased antifungal activity at higher extract concentrations (12). These effects may be attributed to curcumin and other bioactive constituents that disrupt fungal cell wall integrity, interfere with membrane function, and inhibit biofilm formation (13). Since biofilm formation contributes significantly to antifungal resistance, these properties may enhance the antifungal potential of turmeric extracts.

Complete fungicidal activity was achieved at higher extract concentrations, suggesting the existence of a threshold concentration required for maximal antifungal efficacy.

Similar findings were reported by Al-Najjar *et al*., who demonstrated potent antifungal activity of alcoholic turmeric extracts against *C. albicans*, including resistant strains (14). These observations support the potential of *C. longa* as a source of bioactive compounds for antifungal development.

The combination of ethanol extract *C. longa* with *Lactobacillus* metabolites exhibited enhanced antifungal activity compared with the extract alone, particularly at lower concentrations. Although the overall differences were not statistically significant in the MIC and MFC assays, the combination produced larger inhibition zones in the agar diffusion assay. These findings suggest an apparent enhancement of antifungal activity rather than confirmed synergism. Previous studies have shown that *Lactobacillus* species inhibit *C. albicans* through mechanisms including organic acid production, pH reduction, bacteriocin secretion, and competitive exclusion (15). Bhola and Bhadekar also reported greater antimicrobial activity for a turmeric–*Lactobacillus* synbiotic preparation than for turmeric alone (16). Furthermore, Ogidi *et al*. observed enhanced antifungal effects when turmeric-derived compounds were combined with other antifungal agents (17). Curcumin biotransformation by *Lactobacillus* species has also been reported to improve biological activity, providing a possible explanation for the enhanced antifungal effects observed in this study (18).

The inhibition zone analysis further supported these findings. While ethanol turmeric extract alone produced relatively modest inhibition zones, the combination treatment generated larger zones at equivalent concentrations, indicating improved antifungal activity. Nevertheless, ketoconazole consistently produced the largest inhibition zones across all concentrations tested, confirming its superior antifungal potency as previously reported by Siddique *et al*. (19) and Trigo-Gutierrez *et al*. (20). Therefore, although the plant–probiotic combination showed promising activity, it cannot presently be considered a substitute for conventional antifungal therapy.

Susceptibility varied according to the clinical source of the isolates. High vaginal swab isolates exhibited the greatest susceptibility, whereas urine isolates showed the lowest susceptibility. This variation suggests that differences in host microenvironment, adaptation mechanisms, and biofilm-forming capacity may influence antifungal responsiveness. Similar isolate-dependent variations have been reported by Salih *et al*. (5) and Murugesh *et al*. (12), who observed greater susceptibility among mucosal isolates than among isolates from non-mucosal sites. Although MFC values differed among specimen sources, these differences were not statistically significant, indicating that fungicidal activity may be influenced more strongly by concentration than by isolate origin.

Limitations of the Study

A limitation of this study is the relatively small number of clinical isolates evaluated, which restricts the generalizability of the findings. In addition, formal synergy testing methods such as checkerboard assays and fractional inhibitory concentration index (FICI) analysis were not performed. Consequently. Future studies involving larger numbers of clinical isolates, phytochemical standardization of turmeric extracts, and formal interaction analyses are recommended.

Overall, the findings indicate that the susceptibility of *C. albicans* to ethanol *C. longa* extract varies among clinical isolates. While the extract alone demonstrated moderate antifungal activity, its combination with *Lactobacillus* metabolites appeared to enhance antifungal efficacy in some assays. These findings support further investigation of plant–probiotic combinations as potential adjunct antifungal strategies.

Conclusion

In conclusion, ethanol extract of *Curcuma longa* demonstrated concentration-dependent antifungal activity against clinical isolates of *Candida albicans*. Increased extract concentrations resulted in greater inhibitory and fungicidal effects, confirming the antifungal potential of turmeric-derived bioactive compounds. The combination of ethanol extract *C. longa* with *Lactobacillus* metabolites appeared to enhance antifungal activity in some assays, particularly with respect to inhibition zone diameters and earlier fungicidal activity at certain concentrations. However, these observations do not constitute definitive evidence of synergism and should be interpreted cautiously.

Despite the observed antifungal effects, ketoconazole remained more potent than both the extract alone and the combination treatment across all assays. In addition, variations in susceptibility among isolates from different clinical sources suggest that isolate origin may influence antifungal responsiveness.

Overall, the findings indicate that ethanol *C. longa* extract possesses antifungal activity against *C. albicans* and that its combination with *Lactobacillus* metabolites warrants further investigation. These findings support further investigation of plant-probiotic combinations as potential adjunct antifungal strategies. Future studies involving larger numbers of clinical isolates, phytochemical standardization of the extract, formal synergy testing, and in vivo evaluations are recommended to better establish the therapeutic potential of this plant–probiotic approach.

DECLARATIONS

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Conflict of Interest: The authors have no conflicts of interest to declare

Data availability: The data that support the findings of this study are available on request.

Ethical Approval: Ethical Approval was obtained from School of Basic Medical Sciences Ethics Committee, University of Benin, Benin City with approval number, CMS/REC/2025/836. Only archived clinical isolates were used, and all patient identifiers were removed prior to laboratory analysis. All laboratory procedures were carried out in compliance with ethical standards.

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Author Contributions: Chukwurah J. N. and Animhiaga E. C. contributed to designing the study, Animhiaga E. C. collected the data, Animhiaga E.C. analysed the data, Chukwurah J. N. and Animhiaga E. C. wrote the final report and manuscript.

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