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Antimicrobial activities of *Alchornea cordifolia* leaf extract on *Candida albican* and some selected Probiotics

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ABSTRACT

The quest for novel and effective antimicrobial agents has become increasingly urgent because of the increasing threat of drug-resistant microbial pathogens. *Alchornea cordifolia*, a plant species widely used in traditional medicine, has been reported to possess antimicrobial properties. However, its antifungal activity and interactions with beneficial probiotic microorganisms remain poorly understood. This study aimed to investigate the antifungal efficacy of *Alchornea cordifolia* extracts and their bioactive components against *Candida albicans* and explore their interactions with probiotic microorganisms, *Lactobacillus acidophilus* and *Sacchromyces bourdii*, *which are* often used for control. By elucidating the antimicrobial potential and probiotic interactions of *Alchornea cordifolia*, this study aims to contribute to the development of innovative and sustainable solutions for the prevention and treatment of *Candida albicans* infections while also promoting a balanced microbiota. The crude extract was subjected to phytochemical screening. The bioactive components were isolated and purified via column chromatography and thin layer chromatography, whereas FTIR was used to analyse the functional groups present in the extract and isolated components. The extract and isolated components were further subjected to antimicrobial, minimum inhibitory concentration (MIC) and minimum fungicidal/bactericidal concentration (MFC/MBC) assays against *C. albicans*, *S. bourdii* and *L. acidophilus* via the agar diffusion and broth macrodilution technique. Phytochemical analysis revealed the presence of various bioactive components, including tannins, flavonoids, phenols, saponins, alkaloids, anthocyanins, cardiac glycosides, cyanogenic glycosides, oxalates, carotene and sterols. Four spots with Rf values between 0.73 and 0.75, 0.83 and 0.73, 0.87 and 0.98 and 0.32 and 0.23 assigned as components A, B, C and D, respectively, were isolated after elution with a gradient of solvents. The FTIR results of the crude extract revealed functional groups consistent with those of secondary metabolites, such as hydroxyl groups (O-H, peaks at 3812–3500 cm-1), amines (N–H stretching peaks at 3431 cm-1), alkenes (C-H stretching CH3 CH2, CH peaks at 3175–2800 cm-1), and C=C peaks at 1623 cm-1. These functional groups were identified during preliminary tests. The crude extract and components A, B, and C exhibited similar activities against *Candida albicans*, with inhibition zones ranging from 21 mm to 32 mm. Component C had the highest activity, with an inhibition zone of 32 mm. Component D showed moderate activity, with an inhibition zone of 26 mm. Antimicrobial activity against *Sacromyces boulardii* and *Lactobacillus acidophilus* varied among the components, with Component C showing the highest activity against *Sacromyces boulardii*. There was some selectivity for the pathogen rated as the crude extract >C >B = A >D. The crude extract and components A, B, and C had MICs of 100 µg/ml against *Candida albicans*. Component C had an MIC of 1000 µg/ml against *Sacromyces boulardii*. Component D had an MIC of 10 µg/ml against *Candida albicans*. The methanol crude extract and its components have shown promising antimicrobial activity against *Candida albicans*, making it a potential natural solution for candidiasis management. Further research will be crucial to unlock its full potential and explore its use in combination with probiotics.

Keywords: *Alchornea cordifolia*, candidiasis, probiotics and antimicrobial

INTRODUCTION

Candida albicans is a common normal flora found in healthy individuals at diverse sites in the body, such as the gastrointestinal tract, vagina, mouth, skin and respiratory tract [1] ,[2] It transforms into a pathogen when the immune system of the host becomes compromised or the microbiota is disturbed. As opportunistic fungal

pathogens, they cause a wide range of mild to severe infections that can be superficial, affect the skin or mucous membrane, are localized and confined to a particular site, are systemic, spread throughout the body, invasive, and enter deep tissues and organs. In addition to the well-known vulvovaginal candidiasis (VVC), some other diseases include oral candidiasis (thrush), systemic candidiasis, chronic mucocutaneous candidiasis characterized by persistent candida infections of the skin, nails and mucous membranes and candida diaper rash [3]; [4]; [5]; [6]. The management of *Candida albicans* infections often requires a multifaceted approach that incorporates both topical and oral treatments. Currently, four major groups of antifungal drugs are used in the clinical management of *Candida albicans* infections (candidiasis). These drugs include pyrimidine analogues, e.g., 5-flucytosine; azoles, e.g., fluconazole, itraconazole, voriconazole, clotrimazole, and miconazole; and echinocandins, e.g., caspofungin, micafungin & anidulafungin; and polyenes, e.g., nystatin and amphotericin B [7] Topical antifungal agents, such as clotrimazole and miconazole, are effective in treating vulvovaginal candidiasis (VVC) and skin infections [8]. However, oral antifungal agents such as fluconazole and echinocandins are necessary for systemic *Candida* infections [4]. While antifungal agents are commonly used to target and kill *Candida* cells, probiotics have emerged as valuable adjunct therapies to restore the balance of the gut microbiome and prevent reinfection [9]. Probiotics, live microorganisms that confer health benefits, have been shown to be effective in preventing and treating *Candida albicans* infections. Probiotics work by restoring the balance of the gut microbiome, which is often disrupted in individuals with *Candida* overgrowth [9]. By promoting the growth of beneficial bacteria, probiotics can crowd out *Candida* and reduce its ability to colonize and cause infection [10]. The oral administration of probiotics can increase the effectiveness of antifungal agents and reduce the risk of resistance [10]. Additionally, probiotics can stimulate the immune system, increasing its ability to fight off *Candida* [11]. Studies have demonstrated the efficacy of probiotics in reducing *Candida* colonization and symptoms. [12], reported that probiotic supplementation reduced *Candida* colonization in patients with irritable bowel syndrome. [13] reported that probiotic yeast strains inhibited *Candida albicans* growth. The reported most effective probiotic strains against *Candida albicans* include *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Saccharomyces boulardii*. These strains have been shown to inhibit *Candida* growth, reduce adhesion to epithelial cells, and modulate the immune response [14]. Despite these available treatment options, healthcare providers face several challenges in managing *Candida* infections, such as increased resistance to commonly used antifungal medications, particularly azoles, which has been reported, making some infections more difficult to treat. Recurrent infections, especially VVC and chronic mucocutaneous candidiasis, lead to frustration for both patients and healthcare providers. Additionally, there are limited treatment options, particularly in resource-limited settings, where access to effective antifungal medications is often limited, leading to suboptimal treatment outcomes [4]. Addressing these challenges requires a multifaceted approach, one of which involves the combination of antifungal agents and probiotics given concurrently. The combination of topical antifungal agents and oral probiotics has been shown to be effective in treating VVC [15]. However, in systemic or invasive candidiasis, this combination option is limited, especially if the administered probiotic is a fungus, since the antifungal agent inevitably exerts its action against both the pathogen and the probiotics without selectivity. To address this challenge, a bacterial probiotic is often given instead, but the benefit of this option also appears challenging since antibiotics are also administered alongside the antifungal agent to eliminate opportunistic bacterial infections and obtain a better treatment outcome [13]. Thus, there is a growing need for alternative, natural, and holistic approaches to manage candidiasis. There is a search for an antifungal agent that not only overcomes resistance but also possesses selective toxicity against the pathogen. One such proposed alternative is the use of medicinal plants. Plants produce a wide range of secondary metabolites that possess antimicrobial properties. These compounds have evolved as a defense mechanism against microbial pathogens. Several studies have demonstrated the antifungal activity of these plants [16]. Plant-based compounds exert their antimicrobial effects through various mechanisms. Some compounds disrupt the cell membrane, leading to leakage of cellular contents and eventual cell death [17]. Others inhibit essential enzymes involved in microbial metabolism, such as DNA gyrase and RNA polymerase [8]. Additionally, certain plant compounds can interfere with quorum sensing, a process crucial for microbial virulence and biofilm formation [18]. *Alchornea cordifolia*, commonly known as 'Christmas bush', a shrub found in tropical zones, has a rich history of traditional use for the treatment of various ailments, including infectious diseases [19]. This plant belongs to the family Euphorbiaceae and is native to Africa, where it is widely distributed across different regions, including Nigeria, Ghana, and Cameroon [19]. Studies have revealed that *Alchornea cordifolia* possesses significant pharmacological potential due to the presence of various bioactive components, such as alkaloids, phenolics, flavonoids, and terpenoids [20]; [21]. These components are known for their antimicrobial activity and have been shown to inhibit the

growth of bacteria, fungi, and other microorganisms. However, despite the wealth of traditional knowledge and some isolated studies on the properties of *Alchornea cordifolia*, there are critical knowledge gaps in our knowledge of its antifungal properties and how it affects probiotic microbes. Its specific effects on *Candida albicans* and the common probiotic strains *Lactobacillus acidophilus* and *Saccharomyces boulardii*, which are used in the management of candidiasis, have not been extensively studied. Hence, this study aimed to investigate the antifungal activity of *Alchornea cordifolia* extract against *Candida albicans* and evaluate its effects on the growth and survival of *Lactobacillus acidophilus* and *Saccharomyces boulardii*. The findings of this study will contribute to the understanding of the potential of Alchornea cordifolia as a natural remedy for candidiasis management and its impact on the microbiota. By elucidating the antimicrobial potential and probiotic interactions of *Alchornea cordifolia*, this study contributes to the development of innovative and sustainable solutions for the prevention and treatment of *Candida* infections.

METHODOLOGY

Plant Material and Preparation of Extracts

Alchornea cordifolia samples were obtained from Afikpo and its environs, taxonomically identified and authenticated at the Department of Plant Science, Faculty of Physical Sciences, Michael Okpara University of Agriculture, Umudike Abia State, and deposited at the hebarium. The leaves from this plant were carefully separated, washed and dried in a dark environment and ground by using a laboratory mill. The plant extract was obtained via the maceration technique. The methanol (MeOH) mixture was stirred at a ratio of 1:4 per weight of ground sample at room temperature for 24 h with a magnetic stirrer. The mixture was then filtered and dried by evaporation in a hot air oven. The extract was stored at 4 °C until experimentation.

Phytochemical determination

The crude extract obtained was screened to assess the presence of phytoconstituents via the methods described by [22].

Column chromatography separation

The methanolic extract (10 ml) was subjected to column chromatography on silica gel (100–200 mesh – Merck) packed and eluted with a mixture of n-hexane, chloroform, ethyl acetate, ethanol, methanol and water of increasing polarity to obtain fractions. A total of 10 ml of the extraction method was chromatographed over a silica gel column (100–200 mesh). The admixture was packed on a silica gel column (Merck, India), eluted starting with 100% hexane and increased with the solvent polarity chloroform, ethyl acetate, ethanol, methanol, and water at ratios of 90:10, 80:20, 70:30, 50:50, 30:70, 20:80, and 10:90, respectively. Each of the selected compounds in ethanol (70:30) presented different colours, such as red, blue, green and yellow. For further purification with acetone and methanol, the isolated compound yield was 200 mg.

Fraction separation via the TLC method

Thin-layer chromatography was used to separate the sample into different fractions (differing color development) with respect to its retention factor (RF). The sample was spotted on a slurry of silica gel. The silica gel G was prepared with 5 ml of distilled water and tipped over glass plates to form a thin layer (merck TLC aluminum sheets, silica gel 60F254 (20 × 20 cm) precoated plates). The prepared plates were air dried and, for activation, kept in an oven at 100–110°C (30 min). The samples were dissolved in the corresponding solvents (24 ml of chloroform and 4 ml of methanol) and spotted over a stimulated plate (1 cm above the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase and allowed to run 3/4th of the height of the prepared plate. The chromatogram was developed in a mixture of suitable solvents. The plates were air dried, the number of spots was noted, and the Rf values were calculated. The spots were visualized with ultraviolet light at 254 nm. The Rf values of the coloured spots were recorded. Each of the colored dried samples was scraped out via a spatula and labelled separately.

Distance moved by the molecule to the spot) Distance moved by the molecule (location by the spot).

$$\text{Rf Value} = \frac{\text{Distance moved by Mobile}}{\text{Phase (Solvent Front)}}$$

Fourier Transform Infrared Spectrometer (FTIR) Analysis

The crude extract and obtained dried fractions (A, B, C, D) were subjected to FTIR analysis (Buck Scientific M530 USA FTIR). This instrument was equipped with a detector of deuterated triglycine sulfate and a beam splitter of potassium bromide. The Gram A1 software was used to obtain the spectra and manipulate them. Approximately 1.0 g of sample and 0.5 ml of nujol were added, mixed properly and placed on a salt pellet. During measurement, FTIR spectra were obtained in the frequency region of 4,000–600 cm-1 and were added at 32 scans and 4 cm-1 resolution. The FTIR spectra are displayed as transmitter values.

Sample Preparation

1M stock solutions were prepared with 10 g of the extract in 100 ml of 0.1 M $DMSO_4$ and 1 g of the compound isolates in 10 ml of 0.1 M $DMSO_4$. Serial tenfold dilutions of the stock solutions were made via sterile water to obtain concentrations of 10,000 µg/ml, 1000 µg/ml, 100 µg/ml, 10 µg/ml and 0.1 µg/ml.

Antimicrobial Assay

Sabouraud dextrose agar (SDA) and Muller–Hinton agar (MHA) media were prepared following the manufacturer's instructions and autoclaved. The mixture was cooled to 45 °C, poured into pre-labelled Petri dishes and left to solidify. Specifically, 100 µl of each test organism cell suspension matched with the0.5 Macfarland standard was placed on solidified agar and spread with a sterile rod spreader to obtain cell lawns of equal growth on the plate's surface area. Seven wells (6 mm) per plate were made on solidified agar via a sterile borer. Specifically, 400 µl of each dilution of extract/compound isolate was added to the respective well. Ciprofloxacin (5 µg/ml) served as a positive control for bacteria, and fluconazole (10 µg/ml) was the positive control for fungi, while 0.1 M $DMSO_4$ was used as a negative control in each plate. Each plate was made in triplicate, and the anaerobes in the anaerobic jar were incubated for 24 h at 37 °C. The zone of growth inhibition was then measured in millimeters (mm) with the help of a meter ruler. The mean of the readings of the triplicate plates served as the obtained reading.

Determination of the Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) of the crude extract and component isolates were determined via the broth macrodilution method, as described previously (Agboke *et al.*, 2020). The stock solutions used for the antimicrobial assay and the subsequent serial tenfold dilutions of the stock solutions at concentrations of 10,000 µg/ml, 1000 µg/ml, 100 µg/ml, 10 µg/ml and 0.1 µg/ml were prepared via SDB and MHB. Each tube was aseptically inoculated with 0.2 ml of the respective microbial suspension, and the tubes were incubated at 37°C for 24 hours, after which they were observed visually for the presence or absence of turbidity as an indication of the presence or absence of growth, respectively. The lowest concentration that inhibited the growth of the microorganisms after 24 hours of incubation was reported as the MIC against the various test organisms.

Determination of the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC).

Before the MIC tubes, the MIC tubes that did not exhibit any growth and three more dilutions before the MIC tube were used for MFC and MBC. A 0.2 ml aliquot from each tube was aseptically streaked on sterile Muller Hilton agar and Sabouraud dextrose agar. The tubes were then labelled with the proper concentrations and incubated at 37°C for 24 hours. This process was performed for each test organism. After 24 hours of incubation, the plates were inspected to determine whether growth was occurring in relation to the indicated concentrations. The lowest concentration that killed the organisms (or allowed less than 0.1% of the original inoculums to survive) after 24 hours of incubation was taken as the MFC.

Data analysis

Microsoft Excel 2016 and GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, CA, USA) were used for all data analyses and graphs. The values are expressed as the means ± SEMs (n = 3).

RESULTS

Table 1: Phytochemicals

S/N	Tannin (mg/100 g)	Flavenoid (mg/100 g)	Phenol (mg/100 g)	Saponin (mg/100 g)	Alkaloid (mg/100 g)	Anthocyanin (mg/100 g)	Cardiac glucoside (mg/100 g)	Cyanogenic glycosides (mg/kg)	Oxalate (mg/100 g)	Total Carotene (ug/g)	Sterol (mg/100 g)
Crude Extract	++	+++	+	+	+++	+	+++	++	+	+++	+

Table 2: Peak table for the PTLC profile of the crude extract

Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Component Present
0.72	12.89	0.75	111.67	16.78	0.45	106.78	4565.9	16.73	A
0.83	16.90	0.73	179.67	18.67	0.48	111.87	315.6	8.61	B
0.87	20.67	0.98	145.76	20.44	0.65	109.65	245.8	3.24	C
0.32	14.56	0.23	134.87	14.67	0.84	105.67	3556.4	8.01	D

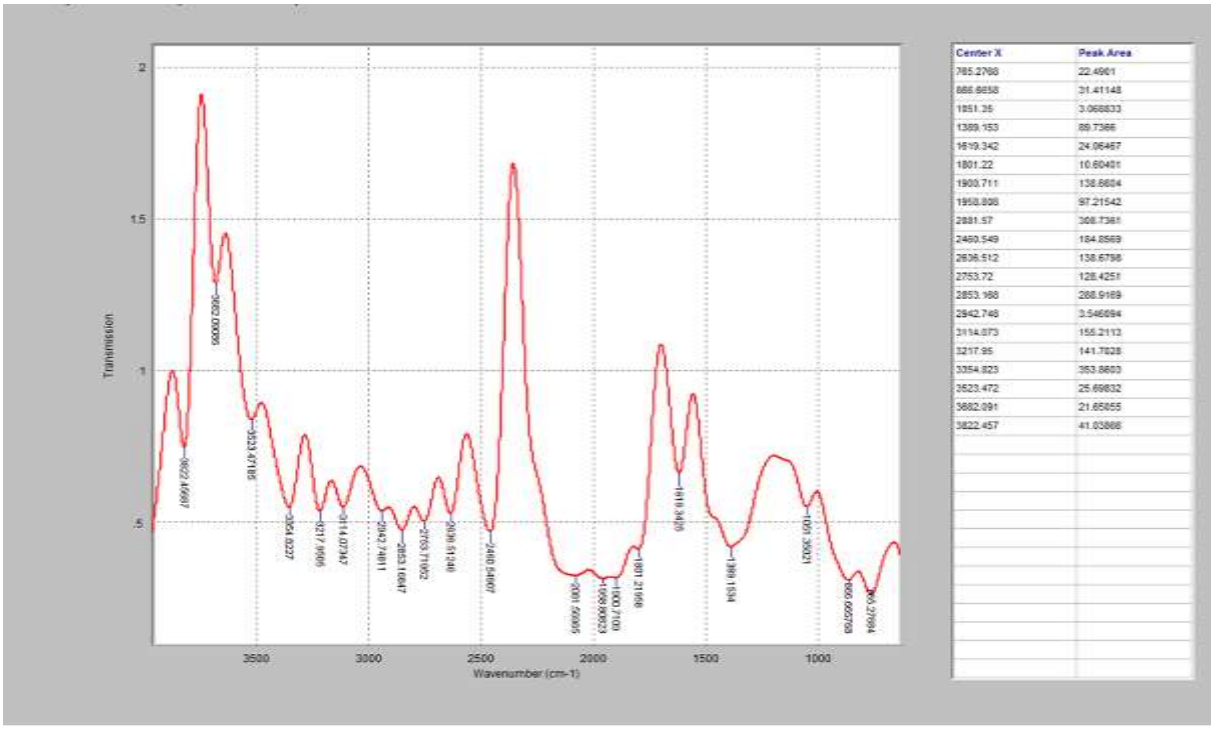
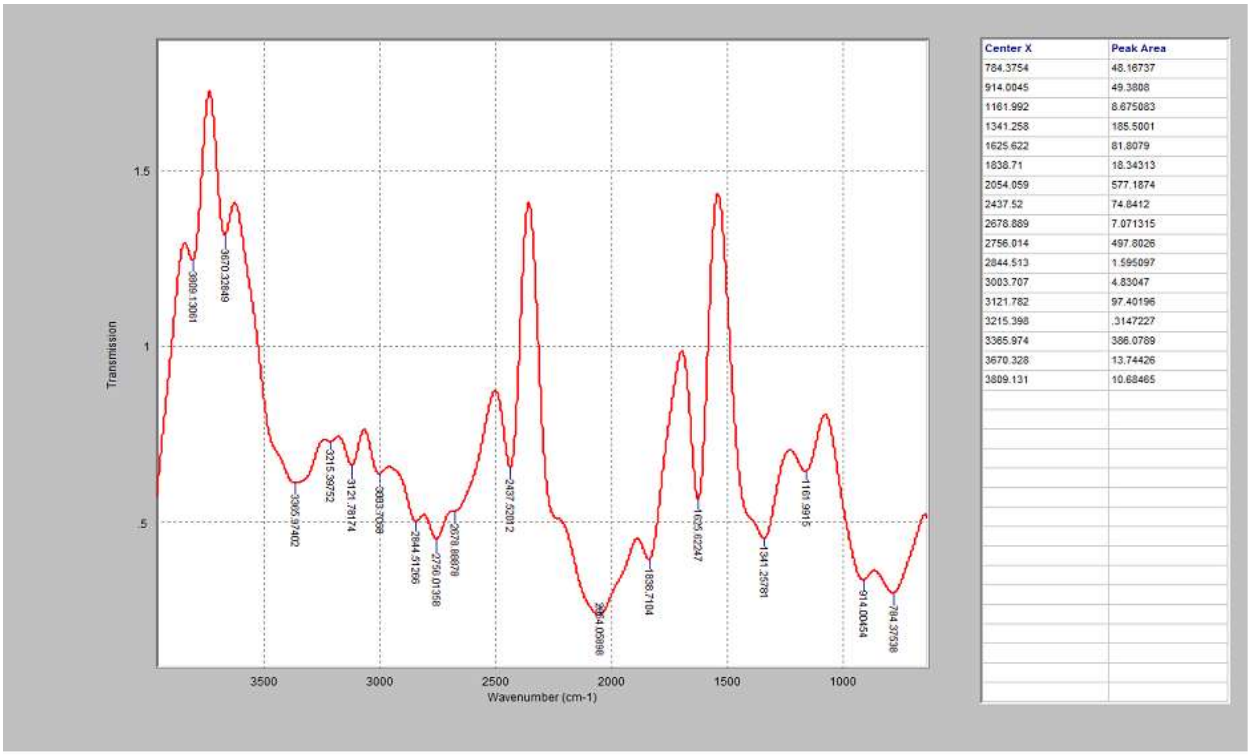
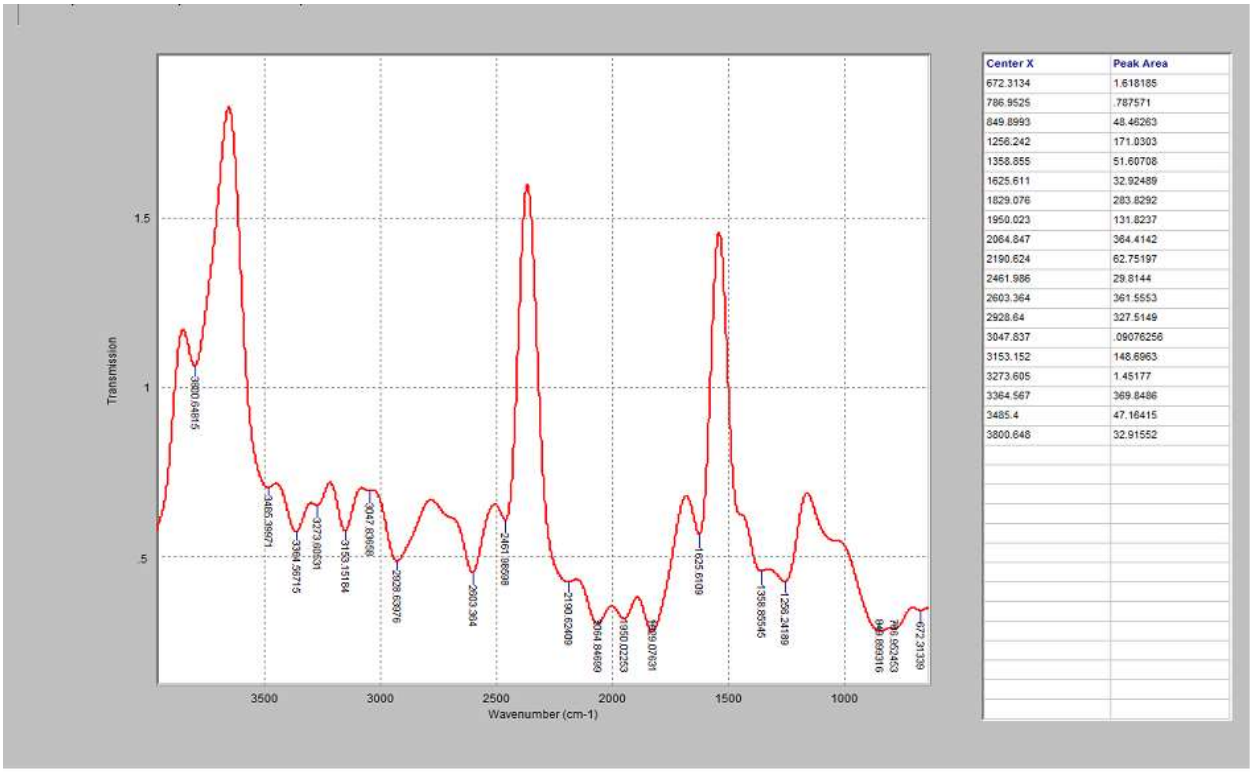


Fig. 1: FTRI graph of component A



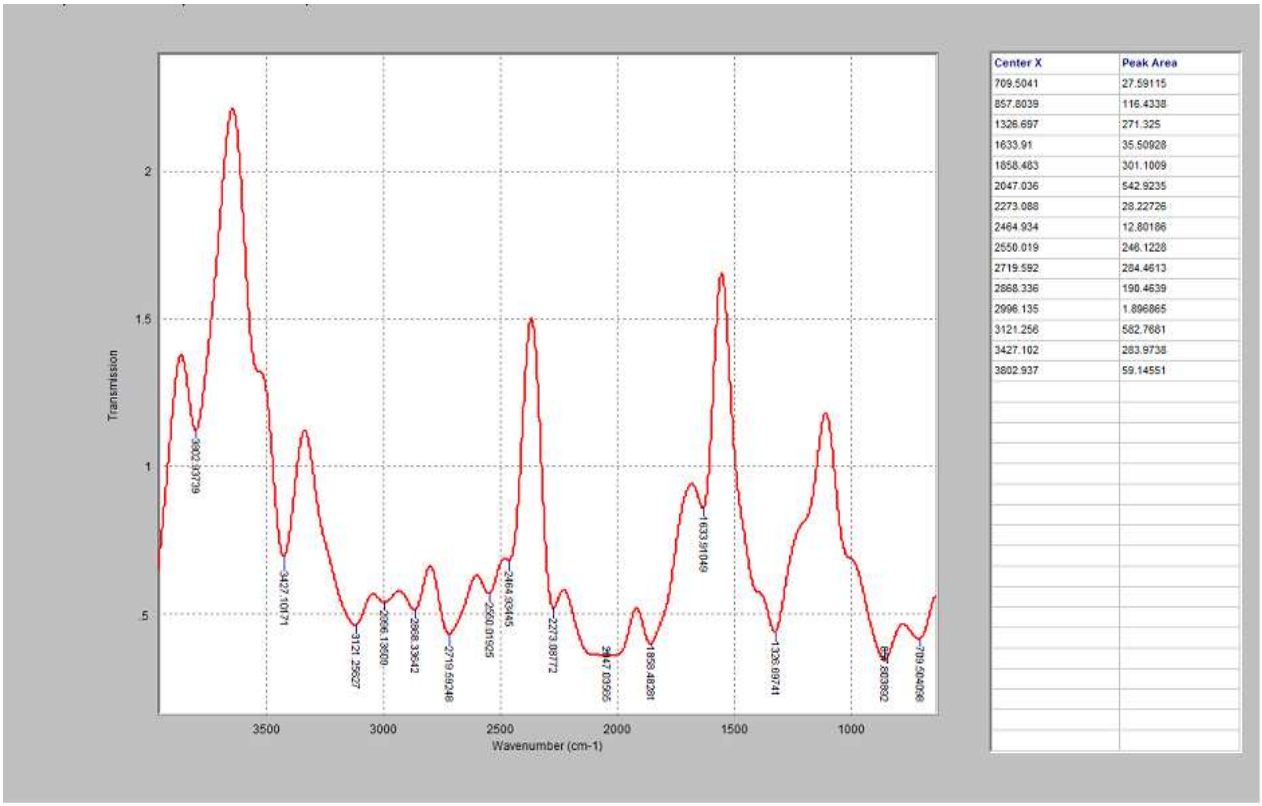
Center X	Peak Area
784.3754	48.16737
914.0045	49.3808
1161.992	8.675083
1341.258	185.5001
1625.622	81.8079
1838.71	18.34313
2054.059	577.1874
2437.52	74.8412
2678.889	7.071315
2756.014	497.8026
2844.513	1.595097
3003.707	4.83047
3121.782	97.40196
3215.398	.3147227
3365.974	386.0789
3670.328	13.74426
3809.131	10.68465

Fig. 2: FTRI graph of component B



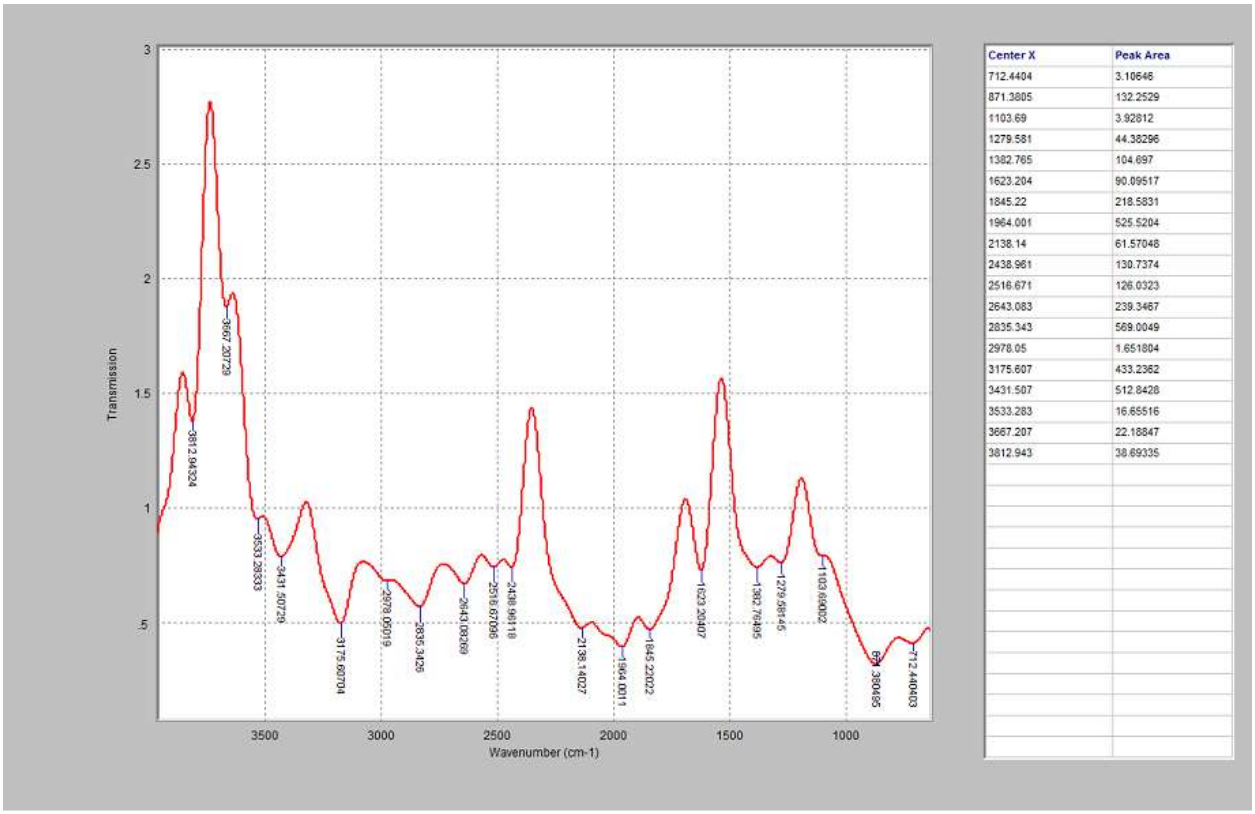
Center X	Peak Area
672.3134	1.618185
786.9525	.787571
849.8993	48.46263
1256.242	171.0303
1358.855	51.60708
1625.611	32.92489
1829.076	283.8292
1950.023	131.8237
2064.847	364.4142
2190.624	62.75197
2461.986	29.8144
2603.364	361.5553
2928.64	327.5149
3047.837	.09076256
3153.152	148.6963
3273.605	1.45177
3364.567	369.8486
3485.4	47.16415
3800.648	32.91552

Fig. 3: FTRI graph of component C



Center X	Peak Area
709.5041	27.59115
857.8039	116.4338
1326.697	271.325
1633.91	35.50928
1858.483	301.1009
2047.036	542.9235
2273.088	28.22726
2464.934	12.80186
2550.019	246.1228
2719.592	284.4613
2868.336	190.4639
2996.135	1.896865
3121.256	582.7681
3427.102	283.9738
3802.937	59.14551

Fig. 4: FTRI graph of Component D



Center X	Peak Area
712.4404	3.10646
871.3805	132.2529
1103.69	3.92812
1279.581	44.38296
1382.765	104.697
1623.204	90.09517
1845.22	218.5831
1964.001	525.5204
2138.14	61.57048
2438.981	130.7374
2516.671	126.0323
2643.083	239.3467
2835.343	569.0049
2978.05	1.651804
3175.607	433.2362
3431.507	512.8428
3533.283	16.65516
3667.207	22.18847
3812.943	38.69335

Fig. 5: FTRI graph of the crude extract

Table 3: Antimicrobial activity of the methanol crude extract against selected microbes

Organism	Dilutions in µg/ml						
	100,000	10,000	1000	100	10	Cipro (5 µg/ml)	Fluconazole (10 µg/ml)
Candida albican	21mm	15 mm	11mm	09mm	00 mm	-	22 mm
Sacromyces boulardii ATCC CNCM 1-745	19 mm	14 mm	00mm	00mm	00 mm	-	22 mm
Lactophillus acidophilus ATCC 4356	16 mm	10 mm	00mm	00mm	00 mm	16mm	-

Table 4: Antimicrobial activity of Component A against selected microbes

Organism	Dilutions in µg/ml						
	100,000	10,000	1000	100	10	Cipro (5 µg/ml)	Fluconazole (10 µg/ml)
Candida albican	21 mm	18 mm	15mm	12mm	00mm	-	22 mm
Sacromyces boulardii ATCC CNCM 1-745	20 mm	15 mm	12mm	00mm	-	22 mm	
Lactophillus acidophilus ATCC 4356	20 mm	12 mm	06mm	00mm	00mm	16 mm	-

Table 5: Antimicrobial activity of Component B against selected microbes

Organism	Dilutions in µg/ml						
	100,000	10,000	1000	100	10	Cipro (5µg/ml)	Fluconazole (10 µg/ml)
Candida albican	26 mm	22 mm	14mm	12mm	00mm	-	22 mm
Sacromyces boulardii ATCC CNCM 1-745	18 mm	15 mm	09mm	00mm	00mm	-	22 mm
L. acidophilus ATCC 4356	28 mm	16 mm	08mm	00mm	00mm	16 mm	-

Table 6: Antimicrobial activity of component C against selected fungi

Organism	Dilutions in µg/ml						
	100,000	10,000	1000	100	10	Cipro (5 µg/ml)	Fluconazole (10 µg/ml)
Candida albican	32 mm	29 mm	22mm	15mm	00mm	-	22 mm
Sacromyces boulardii ATCC CNCM 1-745	30 mm	25 mm	13mm	10mm	00mm	-	22 mm
Lactophillus acidophilus ATCC 4356	16 mm	08 mm	00mm	00mm	00mm	16 mm	-

Table 7. Antimicrobial activity of Component D against selected microbes

Organism	Dilutions in µg/ml						
	100,000	10,000	1000	100	10	Cipro (5 µg/ml)	Fluconazole (10 µg/ml)
Candida albican	26 mm	24 mm	22mm	17mm	10mm	-	22 mm
Sacromyces boulardii ATCC CNCM 1-745	22 mm	21 mm	15mm	13mm	08mm	-	22 mm
Lactophillus acidophilus ATCC 4356	18 mm	12 mm	06mm	00mm	00mm	16 mm	-

Table 8. MICs and MBCs of the Microbes to the various Antimicrobial Agents

Organism	Crude Extract	Component A	Component B	Component C	Component D
Candida albican	MIC: 100 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 100 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 100 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 100 µg/ml, MFC: > 00, 000µg/ml (+)	MIC: 10 µg/ml, MFC > 100, 000 µg/ml (+)
Sacromyces boulardii ATCC CNCM 1-745	MIC: 10, 000 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 1000 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 1000 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 100 µg/ml, MFC: > 100, 000 µg/ml (+)	MIC: 10 µg/ml, MFC > 100,000 µg/ml (-)
Lactobacillus acidophilus ATCC 4356	MIC: 1000 µg/ml, MBC: > 100, 000 µg/ml (+)	MIC: 1000 µg/ml, MBC: > 100, 000 µg/ml (+)	MIC: 1000 µg/ml, MBC: > 100, 000 µg/ml (+)	MIC: 10, 000 µg/ml, MBC: > 100, 000 µg/ml (+)	MIC: 1000 µg/ml, MBC: > 100, 000 µg/ml (+)

DISCUSSION

The phytochemical results revealed various bioactive compounds, including phenolic compounds (tannins, flavonoids, and phenols), saponins, alkaloids, anthocyanins, cardiac glycosides, cyanogenic glycosides, oxalate, total carotene and sterol [23]. These phytochemicals have significant implications for the antimicrobial potential of *Alchornea cordifolia*. These substances support the plant's defense mechanisms and have been reported to exhibit antimicrobial activity against various microorganisms, inhibiting their growth [24]; [25]; [26]. These phytochemicals have been shown to perform multiple functions [27]. Although some compounds, such as cyanogenic glycosides and oxalates, are known not only to be therapeutic but also to be toxic at high concentrations, which calls for caution in their use. This result is comparable to those of similar studies on *Alchornea cordifolia* and other plants and agrees with the work of [28], who reported similar findings. [29], reported that plants with antimicrobial properties often contain a combination of bioactive compounds, including phenolic compounds, saponins, and alkaloids. Four spots with Rf values between 0.73 and 0.75, 0.83 and 0.73, 0.87 and 0.98 and 0.32 and 0.23 assigned as components A, B, C and D respectively, were isolated after elution with a gradient of solvents. The FTIR results of the crude extract revealed functional groups such as hydroxyl groups (O-H, peaks at 3812–3500 cm-1), amines (N–H stretching peaks at 3431 cm-1), alkenes (C-H stretching CH3 CH2, CH peaks at 3175–2800 cm-1), and C=C peaks at 1623 cm-1. These functional groups are consistent with secondary metabolites already identified during preliminary tests [30]. Fourier transmission infrared (FTIR) spectroscopy analysis of the crude extract and its four isolated components (A, B, C, and D) revealed a wealth of information about their functional groups and potential antimicrobial properties. Component A, isolated at an RF of 0.72- 0.75, exhibited a range of functional groups, including hydroxyl, amine, alkane, alkene, and carbonyl groups. These groups suggest that Component A may be a heterocyclic amino acid or diamine carboxylic acid [31]; [32]; [34]; [35]; [36]. Component B, isolated at RF values of 0.83 - 0.73, presented strong absorption

bands indicative of anhydride, carbonyl, and ester groups. These functional groups are characteristic of flavonoids, specifically flavones or flavonols, which are known for their antimicrobial properties [37]; [38]. In contrast, Component C, isolated at RF values of 0.87- 0.98, displayed functional groups consistent with flavonols, including hydroxyl, alkane, alkene, carbonyl, and aromatic ring groups. The similarity of the RF value and functional groups to those of kaempferol, a previously isolated flavonol, suggests that Component C may be related to this compound [38]; [39]. Finally, Component D, isolated at RFs of 0.32 - 0.23, exhibited a broad range of functional groups, including hydroxyl, amine, alcohol, carboxylic acid, lactam, and anhydride groups. These groups may contribute to the antimicrobial activity of Component D through various mechanisms. The presence of these functional groups in the isolated components suggests that they may contribute to the antimicrobial activity of the crude extract. The hydroxyl, alkane, and alkene groups may disrupt cell membranes, whereas the amine and carbonyl groups may interfere with protein function. The anhydride, ester, and flavonoid groups may inhibit enzyme activity, further contributing to the antimicrobial properties of the extract [40]. The [41] stipulates that good antimicrobial agents from natural sources, which are worthy of consideration as good sources of antimicrobials, are those with activity at low concentrations of ≤ 1000 µg/ml. While some researchers have taken benchmark activity at 100 µg/ml, others have adopted bench mark activity at 1000 µg/ml as an indicator of a good source [42]. This study examined the potency of the extract and its components at concentrations of ≤ 1000 µg/ml. The efficacy of the extract was demonstrated through a series of inhibition zone tests, which revealed a dose-dependent response. At concentrations of 100,000 µg/ml, 10,000 µg/ml, 1000 µg/ml, and 100 µg/ml, the extracts resulted in inhibition zones of 21 mm, 15 mm, 11 mm, and 9 mm, respectively. Notably, the activity of the extract was comparable to that of fluconazole, a standard antifungal treatment, at a concentration of 10 µg/ml. The extract's activity against the fungi was pronounced, with *Candida albicans* showing a zone of inhibition of 11 mm at 1000 µg/ml and 9 mm at 100 µg/ml. Its activity was comparable to that of the standard antifungal agent fluconazole (10 µg/ml) 22 mm, which was effective only at 100,000 µg/ml, with a zone of inhibition of 21 mm. *S. boulardii* and *L. acidophilus* became susceptible at high concentrations of 10,000 and 100,000 µg/ml and presented relatively lower zones of inhibition (ZIs), suggesting positive selectivity and microbiome-friendliness at lower concentrations. The activity of component A was moderately significant, with *Candida albican*, resulting in a zone of inhibition of 15 mm, and *Sacromyces boulardii* ATCC CNCM 1 Blueprints for Health: Integrating Construction Engineering Solutions to Enhance Global Health Outcomes and Resilience" 745 resulting in a zone of inhibition of 12 mm. Additionally, the activity was consistent across different concentrations, with zones of inhibition ranging from 12 mm to 21 mm. Activity was also recorded against *Candida albicans* at a dilution of 100 µg/ml, suggesting that this component is a good antifungal agent. It also showed activity against *Lactobacillus acidophilus* ATCC 4356, with a zone of inhibition of 20 mm. Notably, *Lactobacillus acidophilus* ATCC 4356 became susceptible to Component A at concentrations ranging from 10,000 µg/ml, which may be attributed to low activity [42]. Component B showed low to moderate activity against the tested fungi, with *Candida albican*, resulting in a zone of inhibition of 14 mm, and *Sacromyces boulardii* ATCC CNCM 1- 745, 9 mm. *Candida albicans* was the most susceptible zone of inhibition to 12 mm at a low concentration of 100 µg/ml. Notably, *L. acidophilus* ATCC 4356 presented the highest zone of inhibition at the highest concentration but a lower zone of inhibition (8 mm) at 1000 µg/ml. This finding suggests that Component B is a good broad-spectrum antimicrobial agent but is not a likely candidate for combined therapy. Component C had significant activity against *Candida albicans* at 1000 µg/ml, with a zone of inhibition of *22 mm, compared with Saccharomyces boulardii* ATCC CNCM 1-745, (13 mm.) At the highest concentration of 100,000 µg/ml, this difference appeared to have almost disappeared, as *Candida albicans* recorded a zone of inhibition of *32 mm, and Saccharomyces boulardii* ATCC CNCM 1- 745 recorded a zone of inhibition of 30 mm. Additionally, at 100 µg/ml, the fungi exhibited susceptibility, with zones of inhibition of 15 mm and 10 mm. Notably, the fungal strains were the most susceptible at the lowest concentration, as *L. acidophilus* ATCC 4356 was susceptible at only 10,000 and 100,000 µg/ml, with zones of inhibition of 13 mm. Component D had moderate activity at higher dilutions (10,000–100,000 µg/ml) against *L. acidophilus* ATCC 4356 (12 mm and 18 mm). The component showed significant activity against fungal strains at all the tested dilutions, (10–100000 µg/ml), with zones of inhibition in the range of 8–26 mm, making it the most potent component against the selected fungi. Its activity against *Candida albicans* at 1000 µg/ml was comparable to that of the standard drug fluconazole, as both recorded the same zone of inhibition of 22 mm. At the highest dilution of 100,000 µg/ml, its activity (zone of inhibition = 26 mm) was greater than that of the standard drug. There appeared to be no selectivity in its action against the probiotic strain or the clinical strain. Notably, this component recorded a good magnitude of zone of inhibition against *Candida albicans* (10 mm) and *Sacromyces boulardii* ATCC CNCM 1-745 (8 mm) at a low concentration of 10 µg/ml, fulfilling the criteria of a very potent antimicrobial (antifungal) agent (CLSI, 2018). The MICs of the crude extracts against the strains were 100 µg/ml and 10,000 µg/ml. Component

A presented an MIC value of ≤ 1000 µg/mL component B presented an MIC value of ≤ 1000 µg/mL, and component D presented a reduced MIC value of 10 µg/mL in relation to *Candida albicans and Saccharomyces boulardii*. Broad-spectrum antimicrobial action was demonstrated by Component D, which has a reduced minimum inhibitory concentration (MIC) against the fungi and probiotic bacteria [43]. This implies that Component D is the most effective component in preventing infections from these microorganisms. The MBC value for all the antimicrobial agents was above 100,000 µg/ml (MBC ≥100,000). This was indicated by the presence of microbial growth observed in the plates inoculated with aliquots from the tubes of this dilution. The antimicrobial agents may not be bactericidal at the tested concentrations, as indicated by the MBC values, which were primarily higher than 100,000 µg/mL [41]; [44]. This finding may also suggest the agents are inhibitory (static) and not cidal. Additionally, these findings imply that components may find use in therapeutic settings, especially in the fight against antibiotic-resistant fungi [45]; [46]. Because a crude extract's antimicrobial activity is the result of the combined activities of all of its constituents, semipurified extracts made by fractionation should exhibit an improvement in activity (lower MICs), enabling further purification and isolation of the active compound (or compounds) [47]. However, an increase in activity was detected only against a few microbial strains, possibly because the active component(s) in the original crude extract function in synergy with one another or because the active component(s)' structural changes during fractionation [48], [47].

CONCLUSION

Crude methanol extracts, which exhibit significant antimicrobial activity against *Candida albicans*, have shown promising results in the management of candidiasis. This finding is particularly noteworthy, as candidiasis is a common fungal infection that can be challenging to treat, especially in immunocompromised individuals. Furthermore, the selectivity of the extract was highlighted by its lack of activity against *Sacromyces boulardii*, a probiotic yeast, at relatively low concentrations. These findings suggest that the extract may be able to target pathogenic fungi while sparing beneficial microorganisms. However, the extract *inhibited Lactobacillus acidophilus*, a probiotic bacterium, at relatively high concentrations. This finding underscores the potential for interactions between the extract and probiotics, which warrants further investigation. The components, A, B and C are unlikely candidates for combined therapy, as their activity showed no selectivity between the clinical strain and the probiotic strain. These agents may be used only in combined therapy, where the bacterial probiotic strain is administered alongside the fungal agent. While component D, although the most potent component with the lowest MIC of 10 µg/ml, showed no selectivity against the strains, it may not be a good candidate for any form of combined therapy except where it is administered topically for candidiasis management.

The implications of these results are significant, as they suggest that the methanol crude extract may be a valuable adjunct in candidiasis management, particularly when used in conjunction with probiotics. The natural origin and potential selectivity of the extract make it an attractive alternative to traditional antifungal treatments. To fully realize the potential of the extract, further research is necessary to investigate optimal dosing and formulation, and elucidate the mechanisms of action and interactions with probiotics. Clinical trials will also be essential to confirm the efficacy and safety of the extract in humans.

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