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In Vitro Antibacterial and Antifungal Activities of Ethanolic Leaf Extract and Solvent Fractions of *Chionanthus africanus* with Acute and Sub-Acute Toxicological Safety Profile

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Abstract: **Background: *Chionanthus africanus* (Knobl.) Stearn (Oleaceae) is a medicinal tree used in African traditional healthcare for infectious and inflammatory conditions, yet no in vitro antimicrobial or toxicological data exist for this species. This study evaluated the antibacterial and antifungal activities of the crude ethanol extract and solvent fractions of the leaf, and assessed the acute and sub-acute toxicological safety profile of the crude extract in Wistar rats.**

Methods: The crude ethanol extract and n-hexane, ethyl acetate, butanol, and aqueous fractions were screened against ten microbial isolates — comprising Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella* spp., *Shigella* spp.), and fungi (*Candida albicans*, dermatophyte isolates D1 and D2) — using the agar well diffusion method, with minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations determined by the agar dilution method. Acute toxicity was evaluated up to 5000 mg/kg (OECD 425), and sub-acute toxicity was assessed over 28 days at 100, 250, and 500 mg/kg (OECD 407) with haematological and serum biochemical endpoints measured.

Results: The n-hexane fraction was inactive against all organisms. The crude extract, ethyl acetate, butanol, and aqueous fractions exhibited concentration-dependent, selective antimicrobial activity, predominantly against Gram-negative bacteria and fungi. MIC values ranged from 0.63 to >5 mg/mL, with the ethyl acetate fraction showing the lowest MIC (0.63 mg/mL) against dermatophyte D1 and *Candida albicans*. MBC/MFC values mirrored MIC trends, with the ethyl acetate fraction demonstrating fungicidal activity against D1 at 0.63 mg/mL. Acute toxicity testing yielded an LD_{50}>5000 mg/kg. Sub-acute administration produced no significant alterations ($p>0.05$) in haematological parameters (RBC, Hb, PCV, WBC, platelets) or serum biochemical markers (ALT, AST, ALP, total protein, urea, creatinine) relative to controls.

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Conclusion: *C. africanus* leaf extract and fractions exhibit selective weak-to-moderate antimicrobial activity, with the ethyl acetate fraction showing the broadest spectrum and lowest MIC/MBC values, particularly against fungal isolates. The extract is orally safe at doses up to 5000 mg/kg acutely and up to 500 mg/kg in sub-acute exposure, with no hepatotoxic or nephrotoxic effects. These findings provide the first antimicrobial and toxicological evidence base for this medicinally relevant species.

***Keywords: Chionanthus africanus*; antibacterial activity; antifungal activity; MIC; acute toxicity; sub-acute toxicity.**

1. INTRODUCTION

Infectious diseases caused by bacteria and fungi remain a significant burden on global public health, particularly in sub-Saharan Africa where access to conventional antimicrobial agents is often limited and the prevalence of antimicrobial resistance (AMR) is rising (WHO, 2020). The emergence of multidrug-resistant (MDR) strains of clinically important pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* has severely compromised the efficacy of existing therapeutic options, underscoring the urgent need for novel antimicrobial agents from alternative sources (Newman and Cragg, 2020).

Medicinal plants represent a rich reservoir of chemically diverse secondary metabolites with established antimicrobial properties. Plant-derived phenolics, flavonoids, tannins, terpenoids, and alkaloids have been shown to disrupt microbial membrane integrity, inhibit essential enzymes, interfere with quorum sensing, and modulate efflux pump activity — mechanisms that are distinct from those of existing antibiotics and therefore less susceptible to existing resistance mechanisms (Cowan, 1999; Gibbons, 2004). Ethnomedicinal plants in particular have demonstrated higher rates of in vitro antimicrobial activity compared with randomly selected plant specimens, validating the traditional knowledge base as a rational starting point for drug discovery (Fabricant and Farnsworth, 2001).

Chionanthus africanus (Knobl.) Stearn (Oleaceae), a small to medium-sized evergreen tree distributed across sub-Saharan Africa, is used in Nigerian traditional medicine for the management of infectious and inflammatory conditions. Despite this documented ethnopharmacological usage, no peer-reviewed study has reported on the antibacterial or antifungal activity of this species, nor has any toxicological profile been established. Phytochemical profiling of the leaf, conducted in a companion study, revealed a rich phenolic, flavonoid, and tannin content — phytochemical classes with well-established antimicrobial mechanisms of action — providing a rational basis for the present antimicrobial investigation (see companion paper).

Toxicological safety evaluation is an indispensable prerequisite for the scientific validation of any traditionally used medicinal plant, and is particularly important where crude extracts rather than isolated compounds are being investigated. In vitro antimicrobial activity data alone are insufficient to support therapeutic use without complementary evidence of safety in biological systems. The present study therefore aimed to evaluate the in vitro antibacterial and antifungal activities of the crude ethanol extract and n-hexane, ethyl acetate, butanol, and aqueous fractions of *C. africanus* leaf, and to assess the acute and sub-acute oral toxicological safety profile of the crude extract in Wistar rats, in order to generate the first integrated antimicrobial and toxicological evidence base for this species.

2. MATERIALS AND METHODS

2.1 Plant Material Collection, Authentication, and Extraction

Fresh leaves of *Chionanthus africanus* were collected from Nsukka, Enugu State, Nigeria, and authenticated by a plant taxonomist. A voucher specimen was deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka. Leaves were air-dried at room temperature, pulverized, and 1000 g of the powdered material was macerated in ethanol for 72 hours with intermittent agitation. The filtrate was concentrated using a rotary evaporator at 40°C to yield the crude ethanol extract (58 g; 5.8% w/w). The crude extract was reconstituted in distilled water and subjected to liquid-liquid fractionation in order of increasing polarity: n-hexane, ethyl acetate, and butanol, with the remaining aqueous layer retained as the aqueous fraction. Each fraction was concentrated and weighed to determine the respective yield.

2.2 Microbial Isolates and Culture Conditions

Ten microbial isolates were used: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*, *Salmonella* spp., and *Shigella* spp.), and fungi (*Candida albicans* and clinical dermatophyte isolates D1 and D2). Bacterial isolates were maintained on Mueller-Hinton Agar (MHA) and fungal isolates on Sabouraud Dextrose Agar (SDA). Microbial suspensions were standardized to 0.5 McFarland turbidity standard prior to use.

2.3 Agar Well Diffusion Assay

The agar well diffusion assay was performed as described by Okezie *et al.* (2022). Standardized microbial suspensions were inoculated onto MHA plates (bacteria) and SDA plates (fungi). Five wells (8 mm diameter) were bored per plate using a sterile cork borer. Aliquots of 80 µL of each extract or fraction, reconstituted in dimethyl sulfoxide (DMSO) at concentrations of 5, 2.5, 1.25, 0.63, and 0.31 mg/mL, were dispensed into respective wells. Ciprofloxacin (5.6 mg/mL) and fluconazole (4.8 mg/mL) served as positive controls for antibacterial and antifungal assays, respectively. DMSO served as the negative control. Bacterial plates were incubated at 37°C for 24 hours; fungal plates at 25°C for 48 hours. Zones of inhibition were measured in millimetres (excluding well diameter) in triplicate.

2.4 Minimum Inhibitory Concentration (MIC)

MIC was determined by the agar dilution method following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocol with slight modifications (Mounyr *et al.*, 2016). Serial two-fold dilutions of each extract and fraction were incorporated into MHA or SDA to give final concentrations of 0.31 to 5 mg/mL. Inoculated plates were incubated under the same conditions as the diffusion assay. The MIC was defined as the lowest concentration at which no visible microbial growth was observed after incubation.

2.5 Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

Plates from the MIC assay showing no visible growth were sub-cultured onto antibiotic-free MHA and SDA and incubated for a further 24 hours at 37°C (bacteria) or 28°C (fungi). The MBC/MFC was defined as the lowest extract concentration yielding no visible growth on sub-culture, representing a ≥99.9% reduction in the original inoculum. All determinations were performed in triplicate.

2.6 Acute Oral Toxicity Study

Acute oral toxicity was evaluated in adult female Wistar rats (180–200 g) following OECD Guideline 425 (OECD, 2001) in a limit test procedure. Animals were administered a single oral dose of the crude extract at 10, 100, 1000, 2000, and 5000 mg/kg body weight. Animals were observed individually for signs of toxicity at 30 minutes and 1, 2, 4, and 24 hours post-administration, and then daily for 14 days. Mortality, body weight changes, and gross behavioural signs were recorded. The LD_{50} was estimated from the results.

2.7 Sub-Acute Oral Toxicity Study

Sub-acute toxicity was assessed in adult Wistar rats (male and female, 180–220 g) following OECD Guideline 407 (OECD, 2008). Animals were randomized into four groups (n = 5 per sex per group): Group 1 (control, distilled water 10 mL/kg), Groups 2–4 (crude extract at 100, 250, and 500 mg/kg/day, respectively). Doses were administered orally once daily for 28 days. On day 29, blood was collected via ocular puncture under light ether anaesthesia. Haematological parameters — red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC), and platelet count — were determined using standard methods. Serum biochemical markers — alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, urea, and creatinine — were determined using commercial enzyme-linked assay kits following the manufacturers'protocols. All animal procedures were conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals.

2.8 Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Differences among groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test where applicable. A p-value of ≤0.05 was considered statistically significant. Analyses were performed using SPSS version 25.0.

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3. RESULTS

3.1 Extraction Yields

Maceration of 1000 g of powdered leaf in 70% ethanol yielded 58 g of crude extract (5.8% w/w). Liquid-liquid partitioning of the crude extract gave aqueous (37.3%) as the highest-yielding fraction, followed by ethyl acetate (26.6%), butanol (15.3%), and n-hexane (13.3%) fractions. Extraction yields are presented in Table 1.

Table 1: Extraction and fractionation yields of Chionanthus africanus leaf

Extract/Fraction	Yield (g)	% Yield (w/w)	Colour
Crude Ethanol Extract	58.00	5.80	Dark brown
n-Hexane Fraction	7.73	13.30	Pale yellow
Ethyl Acetate Fraction	15.43	26.60	Yellow-brown
Butanol Fraction	8.87	15.30	Dark brown
Aqueous Fraction	21.65	37.30	Dark brown

Percentage yield of fractions calculated relative to crude extract weight.

3.2 Agar Well Diffusion — Crude Ethanol Extract

The crude extract exhibited limited, concentration-dependent antimicrobial activity (Table 2). At the highest concentration (5 mg/mL), mild inhibitory effects were recorded against *Pseudomonas aeruginosa* (6 ± 0 mm), *Salmonella* spp. (5 ± 0 mm), *Shigella* spp. (4 ± 0 mm), dermatophyte D2 (5 ± 0 mm), and *Candida albicans* (6 ± 0 mm). No inhibitory activity was detected against *Staphylococcus aureus*, *Bacillus subtilis*, or *Escherichia coli* at any concentration tested. Activity was absent at concentrations ≤2.5 mg/mL across all organisms.

Table 2: Zone of inhibition (mm) of the crude ethanol extract of Chionanthus africanus leaf against test organisms

Conc. (mg/mL)	S. aureus	B. subtilis	E. coli	P. aeruginosa	Klebsiella spp.	Salmonella spp.	Shigella spp.	D1	D2	C. albicans
5.00	0	0	0	6±0	0	5±0	4±0	0	5±0	6±0
2.50	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0
0.63	0	0	0	0	0	0	0	0	0	0
0.31	0	0	0	0	0	0	0	0	0	0
Cipro (5.6)	—	—	2	9	—	—	—	—	—	—
Fluco (4.8)	—	—	—	—	—	—	—	—	—	24

Values expressed as mean ± SD (n = 3). Zones measured excluding well diameter (8 mm). 0 = no activity. Cipro = ciprofloxacin; Fluco = fluconazole; — = not applicable. D1, D2 = dermatophyte isolates.

3.3 Agar Well Diffusion — Ethyl Acetate Fraction

The ethyl acetate fraction exhibited selective activity concentrated against fungal isolates (Table 3). At 5 mg/mL, inhibition zones were recorded against *Pseudomonas aeruginosa* (6 ± 0 mm), *Salmonella* spp. (7 ± 0 mm), dermatophyte D2 (10 ± 0 mm), and *Candida albicans* (6 ± 0 mm). Antifungal activity against D2 persisted down to 0.63 mg/mL (3 ± 0 mm), indicating concentration-dependent inhibition. No activity was recorded against Gram-positive bacteria at any concentration.

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Table 3: Zone of inhibition (mm) of the ethyl acetate fraction of Chionanthus africanus leaf against test organisms

Conc. (mg/mL)	S. aureus	B. subtilis	E. coli	P. aeruginosa	Klebsiella spp.	Salmonella spp.	Shigella spp.	D1	D2	C. albicans
5.00	0	0	0	6±0	0	7±0	0	0	10±0	6±0
2.50	0	0	0	0	0	0	0	0	7±0	0
1.25	0	0	0	0	0	0	0	0	5±0	0
0.63	0	0	0	0	0	0	0	0	3±0	0
0.31	0	0	0	0	0	0	0	0	0	0
Cipro (5.6)	—	—	2	9	—	—	—	—	—	—
Fluco (4.8)	—	—	—	—	—	—	—	—	—	24

Values expressed as mean ± SD (n = 3). 0 = no activity. Cipro = ciprofloxacin; Fluco = fluconazole.

3.4 Agar Well Diffusion — Butanol Fraction

The butanol fraction showed the most restricted activity of the polar fractions (Table 4). At 5 mg/mL, inhibition was recorded only against *Shigella* spp. (6 ± 0 mm) and *Candida albicans* (4 ± 0 mm). No activity was detected at concentrations ≤2.5 mg/mL against any organism.

Table 4: Zone of inhibition (mm) of the butanol fraction of Chionanthus africanus leaf against test organisms

Conc. (mg/mL)	S. aureus	B. subtilis	E. coli	P. aeruginosa	Klebsiella spp.	Salmonella spp.	Shigella spp.	D1	D2	C. albicans
5.00	0	0	0	0	0	0	6±0	0	0	4±0
2.50	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0
0.63	0	0	0	0	0	0	0	0	0	0
0.31	0	0	0	0	0	0	0	0	0	0
Cipro (5.6)	—	—	2	9	—	—	—	—	—	—
Fluco (4.8)	—	—	—	—	—	—	—	—	—	24

Values expressed as mean ± SD (n = 3). 0 = no activity. Cipro = ciprofloxacin; Fluco = fluconazole.

3.5 Agar Well Diffusion — Aqueous Fraction

The aqueous fraction produced the most limited direct antimicrobial activity (Table 5). Only mild inhibition of *Salmonella* spp. was observed at 5 mg/mL (6 ± 0 mm). All other organisms showed no inhibitory zones across the concentration range tested.

Table 5: Zone of inhibition (mm) of the aqueous fraction of Chionanthus africanus leaf against test organisms

Conc. (mg/mL)	S. aureus	B. subtilis	E. coli	P. aeruginosa	Klebsiella spp.	Salmonella spp.	Shigella spp.	D1	D2	C.albicans
5.00	0	0	0	0	0	6±0	0	0	0	0
2.50	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0
0.63	0	0	0	0	0	0	0	0	0	0
0.31	0	0	0	0	0	0	0	0	0	0
Cipro (5.6)	—	—	2	9	—	—	—	—	—	—
Fluco (4.8)	—	—	—	—	—	—	—	—	—	24

Values expressed as mean ± SD (n = 3). 0 = no activity. Cipro = ciprofloxacin; Fluco = fluconazole.

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3.6 Minimum Inhibitory Concentration (MIC)

MIC values of the crude extract and fractions are presented in Table 6. The n-hexane fraction was inactive against all organisms (MIC >5 mg/mL). Among the active extracts, the ethyl acetate fraction achieved the lowest MIC values: 0.63 mg/mL against dermatophyte D1 and *Candida albicans*. The crude extract inhibited *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* at 1.25 mg/mL and *B. subtilis* at 2.50 mg/mL. The butanol fraction recorded an MIC of 1.25 mg/mL against *Salmonella* spp. Gram-positive bacteria were generally less susceptible, and no extract inhibited *Shigella* spp. or *E. coli* at ≤5 mg/mL across most fractions.

Table 6: Minimum Inhibitory Concentration (MIC) values (mg/mL) of Chionanthus africanus leaf extract and fractions

Extract/Fraction	S. aureus	P. aeruginosa	B. subtilis	E. coli	K. pneumoniae	Salmonella spp.	Shigella spp.	D1	D2	C. albicans
Crude Extract	1.25	1.25	2.50	>5	1.25	5.00	5.00	5.00	5.00	5.00
Ethyl Acetate Fraction	>5	2.50	>5	5.00	5.00	5.00	>5	0.63	2.50	0.63
Butanol Fraction	5.00	5.00	5.00	5.00	2.50	1.25	>5	5.00	5.00	2.50
Aqueous Fraction	>5	5.00	>5	>5	2.50	5.00	>5	0.63	>5	0.63
n-Hexane Fraction	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5

MIC values in mg/mL.>5 = no inhibition at highest concentration tested. D1, D2 = dermatophyte isolates. S. a = S. aureus; P. a = P. aeruginosa; B. s = B. subtilis; E. c = E. coli; K. p = K. pneumoniae; Sal. = Salmonella spp.; Shi. = Shigella spp.; C. a = C. albicans.

3.7 Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

MBC and MFC values are presented in Table 7. The ethyl acetate fraction achieved an MFC of 0.63 mg/mL against dermatophyte D1, the lowest value recorded across all fractions and organisms. MBC/MFC values were generally equal to or twofold higher than corresponding MIC values, indicating a predominantly bactericidal/fungicidal rather than static mode of action for active fractions. The crude extract demonstrated bactericidal activity against *P. aeruginosa* and *K. pneumoniae* at 1.25 mg/mL. The n-hexane fraction showed no cidal activity against any organism.

Table 7: Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values (mg/mL) of Chionanthus africanus leaf extract and fractions

Extract/Fraction	S. aureus	P. aeruginosa	B. subtilis	E. coli	K. pneumoniae	Salmonella spp.	Shigella spp.	D1	D2	C. albicans
Crude Extract	2.50	1.25	2.50	>5	1.25	5.00	5.00	5.00	5.00	5.00
Ethyl Acetate Fraction	>5	5.00	>5	5.00	5.00	>5	>5	0.63	5.00	2.50
Butanol Fraction	5.00	5.00	5.00	5.00	2.50	2.50	>5	2.50	5.00	2.50
Aqueous Fraction	>5	5.00	>5	>5	5.00	5.00	>5	2.50	>5	2.50
n-Hexane Fraction	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5

Values in mg/mL.>5 = no cidal activity at highest tested concentration. Abbreviations as in Table 6.

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3.8 Acute Oral Toxicity

Acute oral administration of the crude extract at doses up to 5000 mg/kg produced no mortality and no observable signs of toxicity in any animal at any dose level across the 14-day observation period. Body weights remained stable throughout. Based on these findings, the LD_{50} of the crude ethanol extract of C. africanus leaf by the oral route exceeds 5000 mg/kg in Wistar rats, classifying it as practically non-toxic per the OECD Globally Harmonized System (GHS) classification (Class 5) and per the Lorke scale. Results are presented in Table 8.

Table 8: Acute oral toxicity profile of the crude ethanol extract of Chionanthus africanus in Wistar rats

S/N	Dose (mg/kg)	Number of Animals	Mortality	Signs of Toxicity
1	10	3	0/3	None observed
2	100	3	0/3	None observed
3	1000	3	0/3	None observed
4	2000	3	0/3	None observed
5	5000	3	0/3	None observed
	LD_{50}		>5000 mg/kg	Practically non-toxic (Class 5, GHS)

OECD 425 limit test. GHS = Globally Harmonized System of Classification.

3.9 Sub-Acute Toxicity — Haematological Parameters

Following 28-day repeated oral administration of the crude extract at 100, 250, and 500 mg/kg/day, no statistically significant differences ($p>0.05$) were observed in any haematological parameter — RBC, Hb, PCV, WBC, or platelet count — compared with the distilled water control (Table 9). All values remained within the normal physiological ranges for adult Wistar rats, indicating that the extract did not adversely affect erythropoiesis, immune function, or haemostatic balance following sub-acute exposure.

Table 9: Effect of 28-day oral administration of crude ethanol extract of Chionanthus africanus on haematological parameters in Wistar rats

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	p-value
RBC ($\times 10^6/\mu L$)	Within normal range	No significant change	No significant change	No significant change	>0.05
Hb (g/dL)	Within normal range	No significant change	No significant change	No significant change	>0.05
PCV (%)	Within normal range	No significant change	No significant change	No significant change	>0.05
WBC ($\times 10^3/\mu L$)	Within normal range	No significant change	No significant change	No significant change	>0.05
Platelets ($\times 10^3/\mu L$)	Within normal range	No significant change	No significant change	No significant change	>0.05

Values expressed as mean ± SD. One-way ANOVA; $p>0.05$ vs. control for all parameters. RBC = red blood cell count; Hb = haemoglobin; PCV = packed cell volume; WBC = white blood cell count.

3.10 Sub-Acute Toxicity — Biochemical Parameters

Sub-acute administration of the crude extract produced no significant alterations ($p>0.05$) in serum ALT, AST, ALP, total protein, urea, or creatinine levels at any dose relative to the control group (Table 10). All biochemical parameters remained within normal physiological limits, indicating preserved hepatic and renal functional integrity following 28 days of treatment.

Table 10: Effect of 28-day oral administration of crude ethanol extract of Chionanthus africanus on serum biochemical parameters in Wistar rats

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	p-value
ALT (U/L)	Within normal range	No significant change	No significant change	No significant change	>0.05
AST (U/L)	Within normal range	No significant change	No significant change	No significant change	>0.05
ALP (U/L)	Within normal range	No significant change	No significant change	No significant change	>0.05
Total Protein (g/dL)	Within normal range	No significant change	No significant change	No significant change	>0.05
Urea (mg/dL)	Within normal range	No significant change	No significant change	No significant change	>0.05
Creatinine (mg/dL)	Within normal range	No significant change	No significant change	No significant change	>0.05

Values expressed as mean ± SD. One-way ANOVA; p>0.05 vs. control for all parameters. ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

4. DISCUSSION

This study provides the first in vitro antibacterial and antifungal activity data for *Chionanthus africanus* leaf and establishes the first toxicological safety profile for this species, thereby addressing a significant gap in the pharmacological characterization of this ethnomedicinally relevant African plant.

The observed selectivity of antimicrobial activity — predominantly against Gram-negative bacteria and fungal isolates — is consistent with patterns widely reported for crude plant extracts and is attributable in part to the structural differences between Gram-positive and Gram-negative cell walls. The outer membrane of Gram-negative bacteria, while classically thought to confer resistance, may paradoxically facilitate the uptake of certain lipophilic phytochemicals via non-specific diffusion through porin channels (Cowan, 1999). The absence of activity against *S. aureus* and *B. subtilis* at the tested concentrations is not uncommon for polar plant extracts and may reflect the thick peptidoglycan barrier or inherent efflux mechanisms of these organisms (Gibbons, 2004).

The ethyl acetate fraction consistently demonstrated the broadest spectrum and lowest MIC/MBC values, particularly against fungal isolates, with MIC and MFC of 0.63 mg/mL against dermatophyte D1 and *C. albicans*. This is consistent with the qualitative phytochemical profile of the ethyl acetate fraction, which is enriched in moderately polar phenolics, flavonoids, and tannins — compound classes with well-documented membrane-disrupting and enzyme-inhibiting antifungal activities (Cushnie and Lamb, 2005; Scalbert, 1991). The pronounced dermatophytic activity is of particular clinical relevance given the rising global incidence of superficial mycoses and the limited armamentarium of affordable antifungal agents in sub-Saharan Africa (Hay *et al.*, 2017).

The MBC/MFC-to-MIC ratios of ≤2 observed for several fraction–organism combinations indicate a predominantly cidal rather than static mode of antimicrobial action, a property that is pharmacologically advantageous as it implies irreversible cell death rather than growth suppression alone. This finding is in line with reports for flavonoid- and tannin-rich plant fractions, which are known to induce membrane lysis and precipitate membrane proteins (Cushnie and Lamb, 2005).

The acute oral LD_{50} of >5000 mg/kg places the crude extract in the practically non-toxic category under both the OECD-GHS classification system and the widely applied Lorke scale (Lorke, 1983; OECD, 2001). This finding is consistent with safety profiles reported for other Oleaceae leaf extracts and provides preliminary support for the traditional oral use of *C. africanus* in Nigerian herbal medicine (Adeneye *et al.*, 2010).

The absence of significant haematological alterations following 28-day repeated dosing at up to 500 mg/kg is reassuring. Haematological indices — particularly RBC, Hb, PCV, and WBC — are sensitive indicators of bone marrow toxicity,

haemolysis, and immune system perturbation; their preservation across all dose groups confirms that the extract does not target haemopoietic or immune compartments at sub-acute doses (Olson *et al.*, 2000; OECD, 2008).

The maintenance of normal serum ALT, AST, and ALP levels demonstrates hepatic integrity, as these enzymes are released into circulation upon hepatocellular damage and are universally used as sensitive biomarkers of drug-induced liver injury (Adeyemi *et al.*, 2010). Normal urea and creatinine levels further confirm that the extract did not impair glomerular filtration or tubular function — the two primary determinants of renal excretory capacity. Together, these data satisfy the minimum toxicological dataset required under OECD Guideline 407 and support the safety of the extract at sub-acute therapeutic-range doses.

The weak-to-moderate direct antimicrobial activity observed in this study is a recognized limitation of many crude plant extract studies and reflects the dilution of active compounds within a complex mixture. Bioassay-guided fractionation and isolation of individual active constituents from the ethyl acetate fraction, combined with mechanistic studies, represent the most logical next step to improve potency and characterize the mode of action. Synergy studies with conventional antibiotics may also reveal adjuvant potential that would not be apparent from standalone MIC data (Hemaiswarya *et al.*, 2008).

5. CONCLUSION

This study provides the first evidence of in vitro antibacterial and antifungal activity for *Chionanthus africanus* leaf extract and solvent fractions, and establishes the first acute and sub-acute toxicological safety profile for this species. The ethyl acetate fraction exhibited the broadest antimicrobial spectrum, with the lowest MIC and MFC values against *Candida albicans* and dermatophyte isolates. Antimicrobial activity was selective, predominantly targeting Gram-negative bacteria and fungi, and was absent in the n-hexane fraction. The crude extract demonstrated a favourable safety profile: an LD_{50} exceeding 5000 mg/kg orally, with no adverse haematological or serum biochemical effects at sub-acute doses up to 500 mg/kg over 28 days. These findings provide a scientific basis for the ethnomedicinal use of *C. africanus* and identify the ethyl acetate fraction as the most promising candidate for further bioassay-guided fractionation and compound isolation.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

All animal experiments were conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals.

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Authors' Contributions

C.E.E., C.R.C. and C.H.N. conceived and designed the study. C.E.E. conducted the laboratory experiments, collected and analysed the data. A.R.O. contributed to methodology and data validation. C.J.I. and U.M.O contributed to the pharmacognostic and phytochemical analyses. C.E.E. drafted the manuscript. C.H.N. and C.R.C. reviewed and edited the manuscript. C.H.N. and C.R.C. supervised the study. All authors read and approved the final manuscript.

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence/Large Language Models

Artificial intelligence-based tools were used for language editing and formatting of the manuscript only. No AI system was used for data generation, analysis or interpretation.

Use of Research Reporting Tool

The ARRIVE guidelines from the EQUATOR Network were consulted during the design and reporting of this animal study.

REFERENCES

[1] Adeneye, A.A., Adeyemi, O.O., Agbaje, E.O. and Banjo, A.A.F. (2010). Evaluation of the toxicity and reversibility profile of the aqueous seed extract of Hunteria umbellata in rodents. African Journal of Traditional, Complementary and Alternative Medicines, 7(4), 350–369.

[2] Adeyemi, O.S., Fambegbe, M., Daniyan, O.R., and Nwajei, I. (2010). Yoyo bitters, a polyherbal formulation, influenced some biochemical parameters in Wistar rats. Journal of Basic and Clinical Physiology and Pharmacology, 23(1), 135–138.

[3] Cowan, M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.

[4] Cushnie, T.P.T. and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26(5), 343–356.

[5] Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives, 109(Suppl 1), 69–75.

[6] Gibbons, S. (2004). Anti-staphylococcal plant natural products. Natural Product Reports, 21(2), 263–277.

[7] Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (3rd ed.). Chapman and Hall.

[8] Hay, R.J., Johns, N.E., Williams, H.C., Bolliger, I.W., Dellavalle, R.P., Margolis, D.J. and Naghavi, M. (2014). The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions. Journal of Investigative Dermatology, 134(6), 1527–1534.

[9] Hemaiswarya, S., Kruthiventi, A.K. and Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. Phytomedicine, 15(8), 639–652.

[10] Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54(4), 275–287.

[11] Mounyr, B., Moulay, S. and Saad, K.I. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79.

[12] Newman, D.J. and Cragg, G.M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803.

[13] OECD (2001). Test No. 425: Acute Oral Toxicity — Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris.

[14] OECD (2008). Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris.

[15] Okezie, U.M., Nwoke, E.A. and Agbafor, K.N. (2022). Antimicrobial activity of plant extracts from south-eastern Nigeria using agar well diffusion assay. Journal of Pharmacognosy and Phytotherapy, 14(1), 1–9.

[16] Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G. and Heller, A. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicology and Pharmacology, 32(1), 56–67.

[17] Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30(12), 3875–3883.

[18] Sofowora, A. (2008). Medicinal Plants and Traditional Medicine in Africa (3rd ed.). Spectrum Books.

[19] WHO (2020). Antimicrobial Resistance: Global Report on Surveillance. World Health Organization, Geneva.