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Research Article

Antibacterial Activity of *Chrysophyllum albidum* Plant Extracts Against Some Bacteria Isolated from Diarrhoeic Patients

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ABSTRACT
This study was carried out to determine the antibacterial activity of *Chrysophyllum albidum* (African star apple) plant extracts of leaf, stem bark and root against some bacterial isolates from diarrhoeal patients. Both clinical and reference isolates were obtained and used to carry out this research work with the reference isolates serving as positive control. Phytochemical screening and antibacterial activity were determined using standard laboratory techniques. The ethanolic root extract was fractionated and the fractions were also tested for antibacterial activities and the most active was screened for phytocompounds. Tannins, saponins, phenols, terpenoids, phytosteroids, steroids and quinones were present while alkaloids, glycosides and phlobatannins were absent in the different plant parts. The ethanolic extract was more effective against both reference and clinical isolates of *S. aureus* with mean zones of inhibition ranging from 16.0±1.0mm to 26.5±4.5mm, followed by *E. coli* with zones between 17.0±3.0mm to 24.0±1.0mm. However, *S. typhimurium* was less susceptible to the extract with mean zones of inhibition ranging from 16.0±1.0mm to 16.0±2.0mm. The ethanolic root extract exhibited the highest activities against both clinical and reference isolates with MICs ranging from 12.5-50mg/ml and MBCs ranging from 50-100mg/ml with MBC/MIC ratio of less than or equal to 4 for four isolates across all six isolates.

Keywords: Abattoir; *Escherichia coli;* Pathogen; Public Health: Wastewater

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INTRODUCTION

Diarrhoeal diseases remain a significant public health problem, particularly in developing countries, where they contribute substantially to morbidity and mortality. Bacterial agents such as *Escherichia coli, Salmonella* Typhimurium, and *Staphylococcus aureus* are commonly implicated in diarrhoeal infections. The increasing resistance of these pathogens to commonly used antibiotics has led to reduced treatment efficacy and increased healthcare challenges (Adonu *et al.,* 2023).

Medicinal plants have historically played a vital role in disease management due to their bioactive phytochemicals. *Chrysophyllum albidum* (African star apple), belonging to the family *Sapotaceae,* is widely used in Nigerian traditional medicine for the treatment of gastrointestinal disorders, infections, and inflammatory conditions. Despite its extensive traditional use, there is limited scientific validation of its antibacterial potential against diarrhoeal pathogens. This study was therefore undertaken to evaluate the antibacterial activity of different parts of *C. albidum* against selected bacterial isolates from

diarrhoeic patients in Kaduna metropolis (Adekanmi *et al.* 2020).

Previous studies on *C. albidum* in western Africa reported the importance of the species for local community livelihood improving and its potentiality for food industries. For instance, the physical, chemical and nutritional characterization of *C. albidum* fruits have shown a high industrial potential. Despite its importance, *C. albidum* is poorly investigated and this species was mentioned in the group of wild fruits tree species which need more detailed scientific information regarding their use pattern, ecology and reproduction biology in order to define a better conservation strategy (Houessou *et al.,* 2012; Odewade *et al.,* 2023).

Some phytochemicals possessed by *C. albidum* possessed properties that can arrest miscarriage (Akaneme, 2008; Odewade *et al.,* 2023). Flavonoids, tannins and alkaloids were phytochemicals which were suspected to possess those properties and were confirmed by the research work (Akaneme, 2008; Odewade *et al.,* 2023).

MATERIALS AND METHODS

Study Area

The study was carried out in Kaduna metropolis, Kaduna State, Nigeria. All laboratory analyses were conducted at the Department of Microbiology, Faculty of Life Sciences, Kaduna State University (KASU).

Plant Collection and Preparation

Fresh leaves, stem bark, and roots of *Chrysophyllum albidum* were collected from their natural habitat in Enugu state and identified in the Department of Biological Sciences, Faculty of Life Sciences, Kaduna State University. The plant material was washed, air-dried, and pulverized into fine powder. The fine powder was then stored in an air tight container until further use.

Extraction Procedure

The powdered plant materials were extracted and fractionated using solvents of varying polarity, including ethanol and water. The extracts were concentrated and stored for phytochemical and antibacterial screening. The plant powder (100g) was dissolved in 500ml of the various solvents and allowed to stand for 48 hours with intermittent shaking and was then sieved using a Whatmann No. 1 filter paper and was then evaporated to dryness using a water bath and stored in the refrigerator at 4^{o}C until further use. Fractionation was done for the most effective extract which was the ethanolic root extract. It was fractionated using partitioning method by using two immiscible liquids such as a polar solvent (water) and non-polar (organic) to semi-polar solvents such as petroleum ether, ethyl acetate and n-butanol in a fractionating column. Extract fractions were also stored for further phytochemical and antibacterial screening.

Bacterial Isolates

Clinical isolates of *E. coli, S.* Typhimurium, and *S. aureus* were obtained from diarrhoeic patients attending selected hospitals in Kaduna metropolis (General Hospital, Kawo and General Hospital, Sabo) and reconfirmed using standard microbiological methods such as gram staining, biochemical testing assays and cultural identification

Identification of Test Bacteria

Gram-Staining and the following biochemical confirmatory tests was carried out: catalase test, coagulase test, motility test, methyl red test, voges proskauer test, oxidase test, indole test, citrate utilization, glucose and sucrose utilization.

Phytochemical screening of extracts

Phytochemical analysis of the plant extract was carried out using standard laboratory techniques for qualitative determination of the following bioactive compounds; alkaloids, glycosides, saponins, steroids, phenols, tannins, flavonoids, diterpines using the method of Ogbeba *et al.* 2017).

Determination of Antibacterial Activity

Agar well diffusion method

Antibacterial activity was evaluated using the agar well diffusion method. Mueller-Hinton Agar and broth were prepared according to manufacturer's instruction and poured into Petri dishes. Standardised inocula of the selected bacteria were inoculated onto the media using a swab stick and then a 5mm cork borer was used to create wells and it was then aseptically filled with the various selected concentrations of the extract and allowed to stand for 24 hours. This was performed in duplicates. After 24 hours the zones of inhibition were measured using a plastic rule and values expressed as mean ± standard deviation and interpreted according to guidelines of Clinical and Laboratory Standards Institute (CLSI) (2025).

Determination of MIC and MBC using Broth dilution method

The MIC and MBC values were determined using standard broth dilution method. One millilitre (1ml) each of the extract concentrations (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) was dispensed into designated test tubes and 0.1ml (100µl) of the standardised bacterial suspension was inoculated into each of the test tubes and incubated for 24 hours. The lowest concentration in which there is no turbidity was expressed as the MIC. The lowest concentration in which there is no turbidity is sub-cultured onto Mueller-Hinton Agar and the lowest concentration in which there is no growth on the media is the MBC value. This was also done after fractionating the most effective crude extracts which was the ethanol root extract. The various extract fractions (petroleum ether, ethyl acetate and n-butanol fractions) were subjected to antibacterial susceptibility testing and standard broth dilution method testing for MIC and MBC determination.

RESULTS

The physical characteristics and percentage yield of ethanol and aqueous extracts of the leaf, stem bark and root are presented in Table 1. The extracts appeared as gummy, dark-green solid and brown with solid texture. The highest percentage yield was found in the ethanol extract of the stem bark which was (26.12%) followed by the ethanol leaf (26.11%), the ethanol root (16.77%), the aqueous root (16.08%), aqueous leaf (14.94%) and aqueous stem (14.44%) respectively.

Phytochemical screening for the bioactive compounds/metabolites presents in the ethanol and aqueous crude extracts of the leaf, stem bark and root of *Chrysophyllum albidum* were determined. The Phytochemical revealed the presence of tannins, saponins, phenols, terpenoids, phytosteroids, steroids and quinones while alkaloids, glycosides and phlobatannins were absent in the different plant parts (Table 2).

Following the collection, *Staphylococcus aureus, Escherichia coli* and *Salmonella* Typhimurium were tested and reconfirmed using commercial identification systems. *S. aureus* was methyl red, voges proskaeur, catalase positive but indole negative. It is non motile and does not produce hydrogen sulphide gas but ferments glucose, lactose and sucrose. *E. coli* are voges proskaeur, catalase and citrate negative but methyl red, indole positive and do not produce hydrogen sulphide gas, it is motile and ferments glucose, sucrose and lactose. *Salmonella* Typhimurium is methyl red, citrate and catalase positive but indole, Voges Proskaeur negative and produces hydrogen sulphide gas, ferments glucose but does not ferment lactose and sucrose. It is motile. (Table 3).

The results of the antibacterial activity of the ethanol and aqueous extracts of the clinical and reference isolates are presented in Table 4. the isolates were intermediate to susceptible to the extracts at 100mg/ml with mean zone of inhibition ranging from 15±1mm – 26.5±0.5mm. the ethanolic root extract was more effective against both reference and clinical isolates of *S. aureus* with mean zones of inhibition ranging from 16±1mm to 26.5±4.5mm, followed by *E. coli* 17±3mm to 24±1mm However, *S. typhimurium* was less susceptible to the extract with mean zones of inhibition ranging from 16±1mm to 16±2mm.

The Minimum inhibitory concentration (MIC) for the leaf, stem bark and root extracts on the clinical and reference isolates at different concentrations of 100, 50, 25 and 12.5mg/ml for crude ethanol and aqueous extract indicated that the MIC ranged from 25-100mg/ml. likewise, the minimum bactericidal concentration ranged from 50- >100mg/ml depending on the bioactivity of the extract on the test organism at the highest concentration of the extracts (Table 5).

Table 1: Physical Characteristics and percentage yield of Aqueous and Ethanol crude extract of leaf, stem bark and root of *Chrysophyllum albidum*

Plant Part	Solvent	Physical Characteristics	Solubility	%Yield
Leaf	Aqueous	Dark green solid	DMSO	14.94
	Ethanol	Dark green solid	DMSO	26.11
Stem	Aqueous	Brownish-grey solid	DMSO	14.44
	Ethanol	Brownish-grey solid	DMSO	26.12
Root	Aqueous	Brownish solid	DMSO	16.08
	Ethanol	Brownish solid	DMSO	16.77

Table 2: Phytochemical Constituents of Aqueous and Ethanol Crude Extracts of *Chrysophyllum albidum*

Phytochemical Group	Aqueous extract			Ethanol Extract		
	Leaf	Stem Bark	Root	Leaf	Stem Bark	Root
Tannin	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	-
Alkaloids	+	-	-	+	-	-
Quinones	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Steroids	-	-	-	-	-	+
Glycosides	-	-	-	-	-	-

Keys: + = Positive (Detected)**,** - = Negative (Not Detected)

Table 3: Biochemical Characteristics of the isolates

Test	*S. aureus*	*E. coli*	*S. typhimurium*
Indole	-	+	-
MR	+	+	+
VP	+	-	-
Catalase	+	-	+
Gas	-	-	+
H_2S	-	+	+
Glucose	+	+	+
Sucrose	+	+	-
Lactose	+	+	-
Citrate	-	-	+
Coagulase	+	-	-
Motility	N.M	M	M

Key: **N.M** = Non-Motile, **M** = Motile, + = Positive (Detected), - = Negative (Not Detect)

Table 4: Antibacterial Activity of leaf, stem bark and root crude extracts of *Chrysophyllum albidum* against bacterial isolates

			Mean and Standard Deviation of zones of inhibition (mm)					
Plant Part	**Solvent**	**Conc (mg/ml)**	***E. coli****	***E. coli***	***S. typhumurium****	***S. typhimurium***	***S. aureus****	***S. aureus***
Leaf	Aqueous	100	16.0±1.0^{a}	17.5±0.5^{a}	16.0±1.0^{a}	16.0±1.0^{a}	17.5±0.5^{a}	19.5±0.5^{a}
		50	13.0±1.0^{a}	12.5±0.5^{b}	11.0±1.0^{a}	14.5±0.5^{a}	13.0±1.0^{a}	16.5±1.5^{a}
		25	8.0±1.0^{b}	12.0±1.0^{b}	8.0±1.0^{b}	11.0±1.0^{b}	9.5±0.5^{b}	13.5±0.5^{b}
		12.5	5.5±0.5^{d}	5.5±0.5^{d}	6.5±0.5^{d}	7.0±1.0^{d}	6.5±0.5^{d}	7.0±1.0^{d}
	Ethanol	100	16.0±1.0^{a}	23.5±0.5^{a}	19.0±1.0^{a}	19.5±0.5^{a}	15.0±0.5^{a}	20.5±0.5^{a}
		50	13.0±1.0^{b}	18.0±2.0^{b}	14.5±0.5^{b}	13.5±0.5^{b}	11.0±1.0^{b}	15.5±0.5^{b}
		25	11.5±0.5^{d}	11.5±0.5^{c}	9.5±0.5^{b}	11.5±0.5^{d}	9.0±1.0^{b}	12.5±0.5^{b}
		12.5	10.0±1.0d^{c}	6.5±0.5^{d}	7.0±1.0^{d}	10.0±1.0^{c}	7.0±1.0^{d}	10.5±0.5^{c}
Stem Bark	Aqueous	100	16.0±1.0^{a}	22.0±6.0^{a}	24.0±3.0^{a}	16.5±4.5^{a}	20.0±1.0^{a}	18.5±1.5^{a}
		50	12.0±1.0^{b}	10.0±1.0^{b}	14.0±2.0^{b}	8.0±2.0^{d}	18.0±1.0^{a}	17.5±2.5^{a}
		25	10.0±1.0^{b}	10.0±4.0^{b}	11.0±1.0^{b}	0±0^{d}	13.0±1.0^{b}	11.5±0.5^{b}
		12.5	6.5±0.5^{d}	0±0^{d}	7.0±1.0^{d}	0±0^{d}	6.0±1.0^{d}	3.0±3.0^{d}
	Ethanol	100	20.0±1.0^{a}	16.0±1.0^{a}	22.0±1.0^{a}	19.0±2.0^{a}	23.0±1.0^{a}	21.5±0.5^{a}
		50	18.0±1.0^{a}	13.0±1.0^{b}	20.0±1.0^{a}	17.0±1.0^{a}	22.0±1.0^{a}	19.0±1.0^{a}
		25	11.5±0.5^{b}	10.0±1.0^{b}	14.5±0.5^{b}	8.5±0.5^{c}	16.5±0.5^{a}	17.5±0.5^{a}
		12.5	7.0±1.0^{d}	9.0±1.0^{c}	8.0±1.0^{c}	6.5±0.5^{d}	6.5±0.5^{d}	12.5±1.5^{b}
Root	Aqueous	100	16.0±1.0^{a}	22.0±6.0^{a}	24.0±3.0^{a}	16.5±4.5^{a}	20.0±1.0^{a}	18.5±1.5^{a}
		50	12.0±1.0^{b}	10.0±1.0^{b}	14.0±2.0^{b}	8.0±2.0^{d}	18.0±1.0^{a}	17.5±2.5^{a}
		25	10.0±1.0^{b}	10.0±4.0^{b}	11.0±1.0^{b}	0±0^{d}	13.0±1.0^{b}	11.5±0.5^{b}
		12.5	6.5±0.5^{d}	0±0^{d}	7.0±1.0^{d}	0±0^{d}	6.0±1.0^{d}	3.0±3.0^{d}
	Ethanol	100	24.0±1.0^{a}	17.0±3.0^{a}	16.0±1.0^{a}	16.0±2.0^{a}	25.0±1.0^{a}	26.5±4.5^{a}
		50	21.0±1.0^{a}	9.5±1.5^{b}	12.5±0.5^{b}	6.5±0.5^{d}	20.0±1.0^{a}	20.0±2.0^{a}
		25	10.0±1.0^{b}	7.5±0.5^{c}	7.0±1.0^{c}	5.5±0.5^{d}	16.5±1.5a	8.5±0.5^{c}
		12.5	7.0±1.0^{d}	5.5±0.5^{d}	5.5±0.5^{d}	0±0^{d}	13.0±1.0^{b}	6.5±0.5^{d}
Control	Ciprofloxacin	100mg/ml	21.0±0.0^{a}	23.0±0.0^{a}	27.0±0.0^{a}	25.0±0.0^{a}	29.0±0.0^{a}	30.0±0.0^{a}

Keys: Values are expressed as Means ± Standard Deviation. Values/means with the same letters in a column are not statistically different at $p \leq 0.05$ level of significance. CLSI (2025) IZD interpretation: ≤ 8mm = little or no activity; 9 – 12mm = weak activity; 13-18mm = Moderate activity; ≥ 19mm = Strong activity *E. coli = Escherichia coli*, *E. coli* = Escherichia coli (*reference isolate), CLSI (2025) IZD interpretation:<8mm = little or no activity; 8 -14mm = Weak activity; 15-18mm = Moderate activity; ≥ 19mm = Strong activity

Table 5 Minimum inhibitory Concentration and Minimum Bactericidal Concentration of aqueous and ethanol extracts against Bacterial Isolates

Plant Part	**Solvents**	***E. coli* Clin**		***E. coli* Ref**		***S. typhimurium* Clin**		***S. typhimurium* Ref**		***S. aureus* Clin**		***S. aureus* Ref**	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Leaf	Aqueous	50	>100	50	100	25	50	50	>100	50	>100	50	100
	Ethanol	50	100	25	50	100	>100	50	100	50	>100	50	100
Stem	Aqueous	50	>100	50	100	100	>100	50	>100	50	>100	25	100
	Ethanol	50	100	25	50	100	>100	50	100	50	100	25	50
Root	Aqueous	50	100	25	50	50	>100	50	100	100	>100	50	100
	Ethanol	50	100	25	50	50	>100	50	100	25	50	25	50

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

DISCUSSION

The present study investigated the antibacterial activity of aqueous, ethanolic, and fractionated extracts of *Chrysophyllum albidum* leaf, stem bark, and root against *Escherichia coli, Staphylococcus aureus*, and *Salmonella* Typhimurium isolated from diarrhoeic patients. The findings demonstrate that *C. albidum* possesses measurable antibacterial properties, with ethanol extracts particularly the ethanolic root extract and its n-butanol and ethyl acetate fractions exhibiting the strongest inhibition across test bacteria. These results align with previous studies that have attributed the antimicrobial efficacy of *C. albidum* to its rich phytochemical constituents, including tannins, phenols, flavonoids, terpenoids, and saponins (Adebayo *et al.,* 2021; Kareem *et al.,* 2022).

The higher yield observed in ethanolic extracts compared to aqueous extracts supports the widely reported efficiency of ethanol in extracting polar and semi-polar phytochemicals (Kareem *et al.,* 2022). The presence of tannins, phenols, saponins, and terpenoids across extracts in this study agrees with findings by Adebayo *et al.* (2021), who similarly reported these phytochemicals to be abundant in *C. albidum* fruit and leaf extracts. The variations in phytochemical distribution across plant parts such as the absence of alkaloids in some extracts also correspond with earlier observations that phytochemical content in *C. albidum* varies with plant part, season, and extraction solvent (Okoh *et al.,* 2023). The high activity demonstrated by the ethanolic extract compared to the aqueous extract agrees with previous findings where ethanol extracts of *C. albidum* showed superior inhibitory activity against Gram-positive and Gram-negative bacteria compared to water extracts (Ogunlana and Ogunlana, 2021). Ethanol, being a better solvent for extracting antimicrobial compounds such as flavonoids and phenolics, explains this increased potency. The higher susceptibility of *S. aureus* in this study corroborates the results of Umeokoli *et al.* (2022), who also found *C. albidum* extracts to be more active against Gram-positive bacteria. This is likely due to the less complex cell wall structure of Gram-positive bacteria compared to Gram-negative organisms, which possess an outer membrane that restricts phytochemical penetration. However, the relatively lower susceptibility of *Salmonella* Typhimurium contrasts with findings by Adebayo *et al.* (2021), who reported moderate inhibitory activity against *Salmonella* spp. This discrepancy may be due to strain variation, extraction differences, or resistance development among clinical isolates. The low MICs exhibited by ethanolic root and n-butanol fraction are consistent with those obtained by Kareem *et al*. (2022), who also reported MIC values between 6.25–50 mg/mL for *C. albidum* fractions. The high MBC range exhibited by the stem and leaf extracts suggest that the plant extract is primarily bacteriostatic, rather than bactericidal, activity, which also supported the finding reported by Ogunlana and Ogunlana (2021).

CONCLUSION

The findings revealed that all plant parts contained important bioactive phytochemicals which are known contributors to antimicrobial potency. The ethanolic extracts (particularly from the root), demonstrated the highest antibacterial activity against *E. coli*, but lower activity against *S. aureus*. This demonstrates the selective antibacterial potential of *C. albidum* and supports its traditional use in managing gastrointestinal and diarrhoeal infections caused by Gram-positive and Gram-negative bacteria.

In-vivo evaluation and toxicity assessment to validate therapeutic safety, animal studies should be conducted to evaluate the in vivo efficacy, dosage tolerance, pharmacokinetics, and potential toxicity of *C. albidum* extracts and purified fractions. Synergistic studies with antibiotics should also be done since the extracts demonstrated primarily bacteriostatic activity, combining *C. albidum* fractions with standard antibiotics should be explored to determine possible synergistic effects, especially against resistant diarrhoeal pathogens. Development of standardized herbal formulations should also be carried out: Given the promising antibacterial properties, standardized herbal preparations (capsules, syrups, or suspensions) should be developed using optimized solvent systems. These formulations should be subjected to stability testing, quality control, and shelf-life evaluation. Wider spectrum screening: Testing the extracts against more diarrhoeal pathogens such as *Shigella, Campylobacter, Vibrio cholerae*, and other *Salmonella* species. Antidiarrhoeal

mechanism studies: Evaluate effects on gut flora, toxins, biofilm disruption, and quorum sensing.

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Conflict of Interest
The authors declare no conflict of interest.

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