

Antibacterial Activities of Tiger Nuts Extracts against Bacteria Isolated from Diarrheagenic Stools Obtained from Selected Hospitals in Akure, Nigeria

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Abstract: Diarrheal diseases remain a global health burden, especially in developing countries where antibiotic resistance complicates treatment. This study investigated the antibacterial activities of n-hexane and ethanolic extracts of tiger nut (*Cyperus esculentus*) against bacterial isolates obtained from diarrheagenic stool samples collected from selected hospitals in Akure, Nigeria. Stool samples were collected, and bacteria in the samples were isolated and identified using standard microbiological methods. Antibiotic sensitivity patterns of the isolates were determined using disc diffusion method. Ethanolic and n-hexane extracts of tiger nut were prepared and tested for antibacterial activity. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined to quantify the efficacy of the extracts. The results revealed the presence of bacterial isolates including *Lactobacillus*, *Staphylococcus*, and *Citrobacter*. Furthermore, fluoroquinolones demonstrated high inhibitory activity against *Lactobacillus*, *Enterococcus*, and *Staphylococcus*. Gram negative bacterial isolates were resistant to amoxicillin and augmentin. The n-hexane and ethanolic extracts exhibited significant antibacterial activity against the isolated bacteria. Notably, the ethanolic extract showed higher antibacterial activity compared to the n-hexane extract of tiger nut. The MIC values ranged from 60 - 80 mg/ml and the MBC values ranged from 70 - 90 mg/ml. Bioactive phytochemicals such as flavonoids, saponins and alkaloids were detected in the extracts. The findings of this study suggests that ethanolic extracts of tiger nut possess notable antibacterial properties and may serve as a promising complementary therapy to combat multiple antibiotic resistant diarrheagenic bacteria.

Key word: Antibiotic resistance, Diarrheagenic bacteria, Minimum Inhibitory Concentration/Minimum Bactericidal Concentration, Tiger nut extracts

INTRODUCTION

Diarrhoea remains a major global health concern, accounting for approximately 1.3 million deaths annually (Manetu *et al.*, 2021), with infants and young children being the most affected (Black *et al.*, 2019). Diarrhoea is defined as the passage of at least three loose, liquid, or watery stools in a day. It often persists for a few days and may result in dehydration due to fluid loss (Manetu *et al.*, 2021). Signs of dehydration include reduced skin elasticity and irritability (WHO, 2017). The most common cause of diarrhoea is intestinal infection by viruses, bacteria, or parasites, a condition generally referred to as gastroenteritis (Manetu *et al.*, 2021). Transmission often occurs through ingestion of food or water contaminated by faecal matter or by direct person-to-person contact (McNeil *et al.*, 2022). Prevention strategies include improved sanitation, provision of safe drinking water, and regular hand washing with soap (Alum *et al.*, 2024). Additional preventive measures include exclusive breastfeeding for at least six

months and rotavirus vaccination (Alum *et al.*, 2024). Management approaches include oral rehydration solution (ORS), which consists of water, salts, and sugar, as well as zinc supplementation (Alum *et al.*, 2024). However, despite these preventive and therapeutic strategies, diarrhoeal diseases remain a pressing public health issue, particularly in low-resource settings where access to clean water, sanitation, and effective antibiotics is limited (WHO, 2017). This has driven increasing interest in alternative solutions derived from natural products.

Tiger nuts (*Cyperus esculentus*) are small tubers, approximately the size of peanuts (Adenowo and Kazeem, 2020), and can be consumed raw or after soaking in water for several hours. They possess a rich phytochemical profile comprising flavonoids, organic acids, alkaloids, tannins, phytates, and oils (Oluwakemi *et al.*, 2021). Tiger nuts have been associated with several health benefits, including promoting weight loss, alleviating flatulence and diarrhoea, and providing protection against heart attack

(Ankomah, 2022), and anti-nutrients present in tiger nuts may be reduced by boiling or soaking/fermenting the nuts in water (Ankomah, 2022). Tiger nut extract is a concentrated liquid derived from tiger nuts, retaining most of their nutrients. These include dietary fibre, vitamin E, and essential minerals such as magnesium and potassium (Edo and Samuel, 2024). The extract also contains antioxidants that help mitigate oxidative stress and inflammation (Abu, 2024). The high content of resistant starch present in the extract also functions as a prebiotic, promoting the growth of beneficial gut bacteria and supporting digestive health (Abu, 2024).

The potential of plant-based alternatives, particularly those derived from underutilized crops such as tiger nuts remains underexplored. The aim of this study was to evaluate the antibacterial activities of tiger nut extracts against bacteria isolated from diarrheagenic stools obtained from health care facilities in Akure, Ondo State, Nigeria. The objectives were to isolate and identify bacteria in diarrheagenic stool samples; determine the qualitative and quantitative phytochemical constituents in extract of tiger nuts; examine the antibacterial sensitivity pattern of the bacterial isolates; assess the antibacterial activity of tiger nut extract on the bacterial isolates and determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tiger nut extracts against the bacterial isolates.

MATERIALS AND METHODS

Collection of Samples: Diarrheagenic stool samples were obtained from consenting patients clinically diagnosed with diarrhoea at J & E Fatula Hospital and Abitoye Hospital. Ethical approval was secured prior to collection. Samples were collected aseptically into sterile, leak-proof containers of 5 ml capacity and transported immediately to the laboratory under cold-chain conditions to minimize microbial alteration.

Isolation and Identification of Bacterial Isolates from Stool Samples: About one gram (1 g) of each stool sample was aseptically transferred into 9 ml of sterile distilled water to obtain an initial suspension. Serial dilutions were prepared up to 10^{-4} . The diluted samples were inoculated onto selective media, including MacConkey agar, Salmonella–Shigella agar (SSA), and nutrient agar, and incubated at 37°C for 24 hours. Distinct colonies were sub-cultured to obtain pure cultures, which were then subjected to Gram staining as well as cultural, morphological, and biochemical characterization. Biochemical tests performed included methyl-red (MR), Voges–Proskauer (VP), citrate utilization, and carbohydrate fermentation tests using glucose, lactose, sucrose, mannose, maltose, galactose, and glycerol. Identification of isolates was based on standard microbiological reference manuals (Al-Hilali and Al-Mozan, 2023).

Preparation of Tiger Nuts Extracts:

Clean and dried tiger nuts were obtained from Oba market, Akure, Nigeria and sorted to remove debris, washed thoroughly in clean water, and oven dried at 50°C for 72 hours and ground into a fine powder (Cecilia *et al.*, 2021). About 200g of Tiger nut powder was soaked separately in 1 l of 95% ethanol, n-hexane (for 72 hours with intermittent shaking. Extracts were filtered, concentrated, and stored in airtight containers at 4°C until use.

Antibacterial Susceptibility Testing (Disc Diffusion) (Tiger nut extract): The isolated bacteria were subjected to a panel of standard commercial antibiotics discs using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA), zone of inhibition diameters were measured and interpreted according to CLSI guidelines (Zainab *et al.*, 2025). The antibacterial activities of the tiger nut extracts were also tested against the isolates using the agar well diffusion method according to Jorgensen and Turnidge (2007). Zones of inhibition were measured in millimetres (mm).

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Tiger Nut Extract:

Broth micro-dilution protocols according to the Clinical Laboratory Standard Institute (CLSI) as described by Torres-Rodríguez *et al.* (2008) was used to determine MIC. Overnight cultures of the test bacterial isolates was prepared by sub-culturing the isolates in sterile Mueller-Hinton broth (MHB) and incubated at 37°C for 18 h. Thereafter, the cultures were standardized to 0.5 MacFarland standard. The turbidity was adjusted to $0.5-1 \times 10^6$ cfu/ml. Each of the test tubes containing the serially diluted extracts were inoculated with the standardized inocula. The set up was incubated at 37°C for 18 h. Thereafter, the tubes were observed for turbidity and the last tube without any turbidity or visible growth was recorded as the MIC. The sample from each Eppendorf tubes used in minimum inhibitory concentration that showed no visible growth after incubation was streaked over a Muller-Hinton agar surface and incubated at 37°C for 18 h. The lowest concentration of the extracts that showed no growth on the MHA plate after 18 h incubation was taken as minimum bactericidal concentration (MBC).

Statistical analysis of data obtained: All experiments were conducted in triplicates. Data are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using SPSS (version 20.0) at a significance level of $p < 0.05$. Comparative analyses were conducted between zones of inhibition, MIC, and MBC value of tiger nut extracts and the standard antibiotics.

RESULTS

Table 1 shows the bacterial isolates detected in the stool samples. *Escherichia coli* was most common of the Gram-negative isolates. Other Enterobacteriaceae such as *Klebsiella* spp., *Enterobacter* spp. and *Proteus vulgaris* and *Citrobacter* spp. were also isolated including *Salmonella* spp. and *Shigella* spp. In addition, *Lactobacillus* spp., *Bacillus*

subtilis, *Enterococcus faecalis* and *Staphylococcus* spp. were isolated.

Gram-negative isolates from stool samples showed a wide range of susceptibility values for tested antibiotics. The isolates had high susceptibility to ciprofloxacin (CPX), levofloxacin (LEV) and ofloxacin (OFL), but resistant to amoxicillin (AMX) and augmentin (AUG). Gentamicin (GEN) and ceftriaxone (CEF) showed intermediate activity, while chloramphenicol (CHL) was variably inhibitory, only to a few isolates. Ampicillin (APX) was weakly to moderately active against *Klebsiella* spp., *Citrobacter* spp., *Enterococcus faecalis*, *Proteus vulgaris*, and some *Escherichia coli* isolates, while several other isolates exhibited resistance. Streptomycin (STR) was also variably active (Table 2).

Overall, the isolates were found to be resistant to multiple antibiotics, with the activities of fluoroquinolones (Ciprofloxacin, levofloxacin and ofloxacin) being the most active, and β -lactams (Amoxicillin and augmentin), being mostly ineffective. The antibiotic susceptibility patterns showed a wide range of variation between Gram-positive stool isolates. Fluoroquinolones (Levofloxacin - LEV, ciprofloxacin - CPX, ofloxacin - OFL, pefloxacin - PEF) exhibited high inhibitory activity on *Lactobacillus* spp., *Enterococcus faecalis*, *Staphylococcus* spp.) showing zones of inhibition greater than 25 mm. Also, nitrofurantoin (NZY) also exhibited relatively high inhibitory activity against some isolates (*Bacillus subtilis* and *Lactobacillus* spp.), while tetracycline (TEX), and erythromycin (ERY) exhibited moderate to low activity. In contrast, most isolates were resistant to β -lactam antibiotics like amoxicillin (AMX) and ceftriaxone (CEF) (Table 3).

The ethanolic extract of tiger nut showed concentration-dependent antibacterial activity against the antibiotic-resistant stool isolates. At lower concentrations (20-40%) inhibition was weak or absent for most of the isolates apart from *Escherichia coli* that was inhibited with 20% (6.00 ± 1.41 mm).

Activity increased with concentration (60-100%) with *Escherichia coli* showing high susceptibility with 21.00 ± 1.41 mm at 100% extract, but lower than the positive control (ciprofloxacin, 31.00 ± 1.41 mm). A small group of isolates (*Lactobacillus* spp. and *Enterococcus* spp.) had high resistance to the extracts, with only a little inhibition at 100% extract (6 mm). *Lactobacillus* spp., *Citrobacter* spp. and *Enterococcus* spp. were actively inhibited from 40% concentration, whilst *Lactobacillus* spp. was intermediate with significant inhibition at 60-80%, and resistant at 100% (Table 4).

The n-hexane extract of tiger nut showed no antibacterial effect against all the isolates tested as recorded in Table 5.

The presence of key secondary metabolites were detected in both n-hexane and ethanolic extracts, at varying concentrations. The ethanolic extract generally showed higher yields of bioactive compounds compared to the n-hexane extract. Flavonoids, alkaloids and saponins were present in higher concentrations in ethanol than in n-hexane. Tannins were absent in the n-hexane extract, but significantly expressed in the ethanolic fraction (Table 6). Similarly, cardiac glycosides and steroids/terpenoids were detected only in ethanol, while phenols

were found in both solvents at comparable levels (Figure 1).

The MIC revealed that the n-hexane extract of tiger nut showed no inhibitory effect (0 mg/ml) against all isolates (Table 7). In contrast, the ethanolic extract exhibited measurable inhibitory effects against several isolates, with the MIC values ranging from 60 mg/ml to 80 mg/ml. *Escherichia coli* and *Lactobacillus* spp. were inhibited at 60 mg/ml, while *Salmonella* spp., *Shigella* spp., *Lactobacillus* spp., *Citrobacter* spp. and *Enterococcus* spp. required a higher concentration of 80 mg/ml for inhibition. However, *Lactobacillus* spp. and *Citrobacter* spp. were not susceptible to any of the tested concentration.

The MBC of the tiger nut extracted with water had no bactericidal effect (0 mg/ml) against all the isolates, which is similar to the previous observation in MIC. The ethanolic extract demonstrated bactericidal activity at slightly higher concentrations than the MIC values. *Escherichia coli* and *Lactobacillus* spp. were susceptible at 70 mg/ml, while *Salmonella* spp., *Shigella* spp., *Lactobacillus* spp., *Citrobacter* spp., and *Enterobacter* spp. required 90 mg/ml to achieve complete bactericidal activity. *Lactobacillus* spp. and *Citrobacter* spp. exhibited no bactericidal response (Table 7).

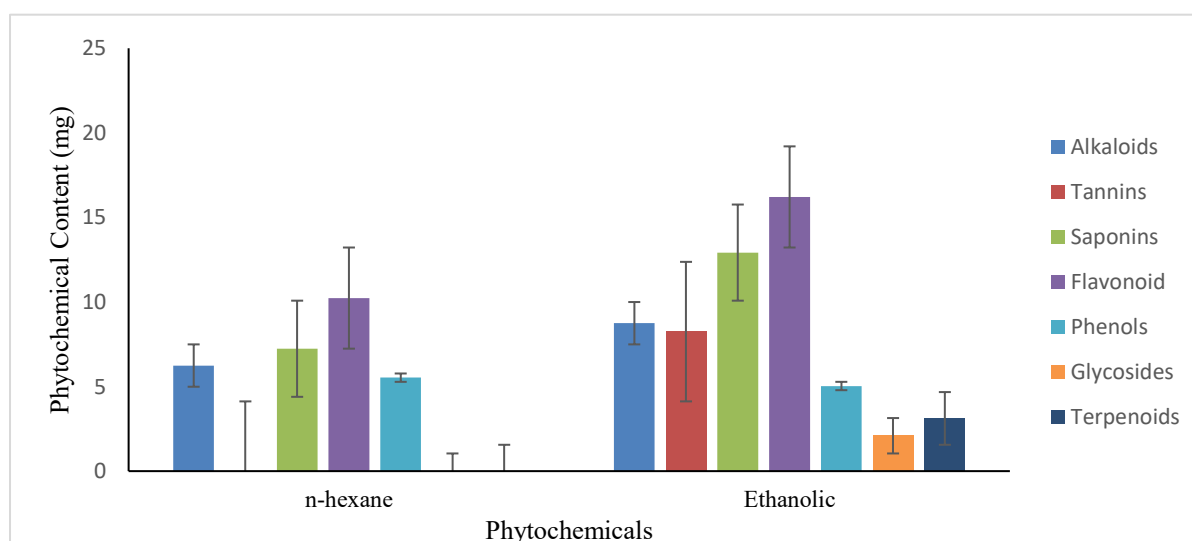


Figure 1: Quantitative Phytochemical Constituents of N-Hexane and Ethanolic Extracts of Tiger Nut (*Cyperus esculentus*)

Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates from Stool Samples

Sample	Gram Staining	Shape	Methyl red	V.P	Manose	Sucrose	Maltose	Glycerol	Citrate	Glucose	Lactose	Galactose	Probable organism
1	-	Short rod	-	-	+	+	-	+	+	+	+	+	<i>Klebsiella/Enterobacter</i> spp.
2	-	Short rod	+	-	+	+	-	+	+	+	+	+	<i>Citrobacter</i> spp.
3	-	Short rod	-	+	+	+	-	+	+	+	+	+	<i>Enterobacter</i> spp.
4	-	Short rod	-	-	+	+	-	+	+	+	+	+	<i>Klebsiella</i> spp.
5	-	Short rod	+	-	+	+	+	+	-	+	+	+	<i>Citrobacter</i> spp.
6	-	Short rod	+	-	+	+	+	+	+	+	+	+	<i>Enterobacter</i> spp.
7	+	Long rod	+	-	+	+	+	+	-	+	+	+	<i>Bacillus subtilis</i>
8	+	Medium rod	+	-	+	+	+	+	+	+	+	+	<i>Lactobacillus</i> spp.
9	+	Medium rod	-	-	+	+	+	+	+	+	+	+	<i>Lactobacillus</i> spp.
10	-	Short rod	+	-	+	+	+	+	+	+	-	+	<i>Escherichia coli</i>
11	+	Medium rod	+	-	+	+	+	+	-	+	+	+	<i>Lactobacillus</i> spp.
12	-	Short rod	+	-	+	+	+	+	-	+	+	+	<i>Citrobacter</i> spp.
13	+	Cocci	+	-	-	+	-	+	+	+	+	+	<i>Enterococcus faecalis</i>
14	+	Cocci	-	-	-	+	+	+	+	+	+	+	<i>Staphylococcus</i> spp.
15	+	Medium rod	+	-	+	-	+	+	+	+	+	-	<i>Lactobacillus</i> spp.
16	-	Medium rod	-	-	+	-	+	+	+	+	+	-	<i>Salmonella/Shigella</i> spp.
17	-	Short rod	+	-	+	+	+	+	+	+	+	+	<i>Escherichia coli</i>
18	+	Medium rod	-	-	+	+	+	+	+	+	+	+	<i>Lactobacillus</i> spp.
19	-	Long rod	-	+	+	+	+	+	+	+	+	+	<i>Proteus vulgaricus</i>
20	-	Short rod	-	-	+	-	+	+	+	+	+	+	<i>Escherichia coli</i>
21	+	Medium rod	-	-	+	+	+	+	-	+	+	+	<i>Lactobacillus</i> spp.
22	+	Cocci	-	-	+	-	+	+	+	+	+	+	<i>Staphylococcus</i> spp.
23	-	Short rod	+	-	+	+	+	+	-	+	+	+	<i>Escherichia coli</i>
24	-	Short rod	+	-	+	+	+	+	-	+	+	+	<i>Escherichia coli</i>
25	-	Short rod	+	-	+	+	+	+	-	+	+	+	<i>Escherichia coli</i>
26	+	Cocci	+	-	-	+	+	+	-	+	+	+	<i>Staphylococcus</i> spp.
27	+	Cocci	+	-	-	+	+	-	+	-	+	+	<i>Enterococcus faecalis</i>
28	+	Cocci	-	-	+	+	+	+	+	+	+	+	<i>Staphylococcus</i> spp.
29	-	Short rod	+	-	+	+	+	+	+	+	-	+	<i>Escherichia coli</i>
30	-	Short rod	+	+	+	+	+	+	+	+	-	+	<i>Escherichia coli</i>

Keys: + positive, - negative

Table 2: Antibiotic Sensitivity Pattern of Gram-Negative Bacterial Isolates from Stool Sample

Isolates	CPX	CHL	AMX	AUG	GEN	OFL	APX	STR	LEV	CEF
<i>Klebsiella</i> spp.	29.00±1.41 ^c	21.50±2.12 ^b	0.00±0.00 ^c	0.00±0.00	21.00±1.41 ^g	22.00±2.83 ^b	26.50±2.12 ^b	10.50±0.71	34.00±2.83 ^b	27.00±1.41 ^d
<i>Citrobacter</i> spp.	20.00± 0.00 ^j	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	26.00±1.41 ^c	25.00±0.00 ^f	14.00±2.83 ^c	14.00± 1.41 ^j	24.50±2.12 ^h	18.50±2.12 ^k
<i>Enterococcus</i> spp.	21.00± 1.41 ^h	20.00±2.83 ^d	0.00±0.00 ^c	0.00±0.00	10.50±0.71 ^k	23.00±1.41 ^g	0.00±0.00 ^j	0.00±0.00 ⁱ	32.00±2.83 ^b	22.50±3.54 ⁱ
<i>Klebsiella</i> spp.	21.00±1.41 ^h	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	20.00±0.00 ^h	22.00±2.83 ^b	0.00±0.00 ^j	6.00±1.41 ^l	22.50±3.54 ⁱ	23.50±0.71 ^h
<i>Citrobacter</i> spp.	20.00± 0.00 ^j	5.00±0.00 ^h	0.00±0.00 ^c	0.00±0.00	15.00±2.83 ^j	27.00±2.83 ^d	0.00±0.00 ^j	6.00±1.41 ^l	37.00±2.83 ^a	26.50±2.12 ^c
<i>Escherichia coli</i>	28.50± 0.71 ^d	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	27.00±1.41 ^a	22.00±2.83 ^h	0.00±0.00 ^j	15.00± 2.83 ⁱ	27.50±2.12 ^g	21.00±1.41 ^j
<i>Enterococcus</i> spp.	0.00±0.00 ⁿ	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	0.00±0.00 ⁿ	0.00±0.00 ⁿ	0.00±0.00 ^j	0.00±0.00 ^m	0.00±0.00 ^k	0.00±0.00 ⁿ
<i>Citrobacter</i> spp.	22.00± 2.83 ^g	21.00±1.41 ^c	0.00±0.00 ^c	0.00±0.00	10.00±0.00 ⁱ	10.00±0.00 ⁿ	12.50±3.54 ^f	21.00±1.41 ^c	16.00±2.83 ^j	0.00±0.00 ⁿ
<i>Enterococcus</i> spp.	24.50± 0.71 ^f	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	21.50±2.12 ^f	26.00±1.41 ^e	21.00±1.41 ^d	26.00± 1.41 ^a	30.00±0.00 ^f	32.00±2.83 ^a
<i>Enterococcus faecalis</i>	6.00±1.41 ^m	11.00±1.41 ^f	0.00±0.00 ^c	0.00±0.00	0.00±0.00 ⁿ	0.00±0.00 ⁿ	11.50±2.12 ^g	17.00± 0.00 ^h	31.00±1.41 ^d	30.00±0.00 ^c
<i>Salmonella/Shigella</i> spp.	32.00± 2.83 ^a	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	23.00±1.41 ^d	18.00±1.41 ^k	21.00±1.41 ^d	25.00± 0.00 ^c	30.50±0.71 ^e	31.00±1.41 ^b
<i>Lactobacillus</i> spp.	31.00± 1.41 ^b	11.00±0.00 ^f	10.00±0.00 ^b	0.00±0.00	26.00±1.41 ^c	21.00±1.41 ^j	10.00±0.00 ⁱ	6.00±1.41 ^l	31.00±1.41 ^d	26.00±1.41 ^f
<i>Proteus vulgaricus</i>	27.50± 2.12 ^c	16.00±1.41 ^c	10.50±0.71 ^a	0.00±0.00	22.50±0.71 ^c	32.00±2.83 ^b	10.50±0.71 ^h	20.00± 0.00 ^g	31.00±1.41 ^d	26.00±1.41 ^f
<i>Escherichia coli</i>	31.00± 1.41 ^b	23.00±0.00 ^a	0.00±0.00 ^c	0.00±0.00	16.00±1.41 ⁱ	30.00±0.00 ^c	21.50±2.12 ^c	21.00± 1.41 ^c	30.00±0.00 ^f	15.50±0.71 ^l
<i>Escherichia coli</i>	20.50± 0.71 ⁱ	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	20.00±0.00 ^h	33.50±4.95 ^a	32.00±2.83 ^a	22.00± 2.83 ^d	30.00±0.00 ^f	25.00±0.00 ^g
<i>Escherichia coli</i>	21.00± 1.41 ^h	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	5.00±0.00 ^m	15.00±0.00 ^m	10.50±0.71 ^h	11.00± 1.41 ^k	0.00±0.00 ^k	0.00±0.00 ⁿ
<i>Escherichia coli</i>	17.50± 0.71 ^k	10.50±0.71 ^g	0.00±0.00 ^c	0.00±0.00	26.50±2.12 ^b	17.00±2.83 ^j	0.00±0.00 ^j	25.50± 0.71 ^b	30.50±0.71 ^e	15.00±0.00 ^m
<i>Escherichia coli</i>	15.50± 0.71 ^l	0.00±0.00 ⁱ	0.00±0.00 ^c	0.00±0.00	25.00±0.00	21.50±2.12 ^j	10.50± 0.71	20.50± 0.71 ^f	33.00±4.24 ^c	16.00 ^k ±1.41

Keys: CPX= Ciprofloxacin, CHL= Chloramphenicol, LEV = Levofloxacin, OFL= Ofloxacin, AMX= Amoxicillin, AUG= Augmentin, GEN= Gentamicin, CEF= Ceftriaxone, APX= Ampicillin, STR= Streptomycin. Values are expressed as mean ± SD. The values with different superscript are statistically significant from each other across the column.

Table 3: Antibiotic Sensitivity Pattern of Gram-Positive Bacterial Isolates from Stool Samples

Isolates	TEX	LEV	PEF	NZY	OFL	CPX	ERY	AMX	CEF	COT
<i>Lactobacillus</i> spp.	11.00±1.41 _f	20.50±0.71 _f	22.00±0.00 _d	20.00±0.00 _d	21.50±2.12 _b	26.50±0.71 _b	16.00±2.83 _c	11.50±0.71 _c	14.00±0.00 _b	7.50±0.71 _c
<i>Bacillus subtilis</i>	13.00±0.00 _e	13.00±0.00 _i	24.00±1.41 _b	25.00±0.00 _a	13.00±1.41 _i	22.00±2.83 _d	25.00±1.41 _a	15.00±1.41 _a	12.00±1.41 _c	14.00±0.00 _a
<i>Lactobacillus</i> spp.	10.50±0.71 _g	23.00±1.41 _d	0.00±0.00 ^h	0.00±0.00 ⁱ	13.50±0.71 _h	16.00±1.41 _g	0.00±0.00 ^g	0.00±0.00 ^e	0.00±0.00 ^f	0.00±0.00 ^e
<i>Staphylococcus</i> spp.	10.50±0.71 _g	15.00±0.00 _h	22.00±2.83 _d	18.00±1.41 _e	25.00±1.41 _c	24.00±0.00 _c	11.00±1.41 _f	0.00±0.00 ^e	0.00±0.00 ^f	0.00±0.00 ^e
<i>Lactobacillus</i> spp.	0.00±0.00 ⁱ	30.00±0.00 _b	30.00±0.00 _a	22.00±2.83 _b	30.00±0.00 _a	11.00±1.41 _i	0.00±0.00 ^g	11.00±1.41 _d	0.00±0.00 ^f	0.00±0.00 ^e
<i>Lactobacillus coli</i>	21.00±1.41 _a	31.50±2.12 _a	23.00±0.00 _c	21.00±1.41 _c	24.00±0.00 _d	21.00±1.41 _e	11.00±1.41 _f	5.50±0.71 ^d	5.00±0.00 ^e	5.00±0.00 ^d
<i>Lactobacillus</i> spp.	15.00±1.41 _d	21.00±1.41 _e	20.00±0.00 _f	14.00±1.41 _i	21.00±1.41 _f	21.00±1.41 _e	11.00±1.41 _f	0.00±0.00 ^e	0.00±0.00 ^f	0.00±0.00 ^e
<i>Staphylococcus</i> spp.	10.00±0.00 _h	20.00±0.00 _g	21.00±1.41 _e	15.50±0.71 _g	21.00±1.41 _f	20.00±0.00 _f	13.00±1.41 _d	0.00±0.00 ^e	0.00±0.00 ^f	0.00±0.00 ^e
<i>Staphylococcus</i> spp.	16.00±1.41 _b	26.00±1.41 _c	20.00±0.00 _f	16.00±1.41 _f	20.00±0.00 _g	31.00±1.41 _a	11.50±0.71 _e	0.00±0.00 ^e	0.00±0.00 ^f	10.50±0.71 _b
<i>Enterococcus faecalis</i>	15.50±0.71 _c	30.00±0.00 _b	16.00±1.41 _g	15.00±0.00 _h	22.00±2.83 _e	13.50±2.12 _h	16.00±1.41 _c	13.00±1.41 _b	6.00±1.41 ^d	0.00±0.00 ^e
<i>Staphylococcus</i> spp.	21.00±1.41 _a	30.00±0.00 _b	22.00±2.83 _d	20.00±0.00 _d	26.00±1.41 _b	20.00±0.00 _f	21.00±1.41 _b	5.50 ± 0.71 ^d	16.00±1.41 _a	0.00±0.00 ^e

Key: TEX= Tetracycline, LEV= Levofloxacin, PEF= Pefloxacin, NZY, Nitrofurantoin, OFL= Ofloxacin, CPX= Ciprofloxacin, ERY= Erythromycin, AMX= Amoxicillin, CEF= Ceftriaxone, COT= Cotrimoxazole. Values are expressed as mean ± SD. The values with different superscript are statistically significant from each other across the column.

Table 4: Antibacterial Activity of Ethanolic Tiger Nut (*Cyperus esculentus*) Extract against Antibiotic-Resistant Gram-Negative Bacterial Isolates from Stool Samples

Isolate	20%	40%	60%	80%	100%	Positive control (Ciprofloxacin)	Negative control (Water)
<i>Escherichia coli</i>	6.00±1.41 ^a	10.00±0.00 ^a	13.50±0.71 ^a	17.00±1.41 ^a	21.00±1.41 ^a	31.00±1.41 ^a	0.00±0.00
<i>Klebsiella</i> spp.	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^f	0.00±0.00 ^c	5.50±0.71 ^d	28.50±0.71 ^c	0.00±0.00
<i>Salmonella/Shigella</i>	0.00±0.00 ^b	6.00±1.41 ^b	8.00±1.41 ^c	10.50±0.71 ^d	17.00±1.41 ^b	20.00±0.00 ^f	0.00±0.00
<i>Enterococcus</i> spp.	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^f	0.00±0.00 ^c	6.00±1.41 ^c	21.00±1.41 ^c	0.00±0.00
<i>Lactobacillus</i> spp.	0.00±0.00 ^b	5.50±0.71 ^c	10.00±0.00 ^b	15.00±1.41 ^b	0.00±0.00 ^f	27.00±1.41 ^d	0.00±0.00
<i>Lactobacillus</i> spp.	0.00±0.00 ^b	0.00±0.00 ^c	6.00±1.41 ^c	10.50±0.71 ^d	0.00±0.00 ^f	30.00±0.00 ^b	0.00±0.00
<i>Citrobacter</i> spp.	0.00±0.00 ^b	0.00±0.00 ^c	7.50±0.71 ^d	12.00±0.00 ^e	0.00±0.00 ^f	30.00±0.00 ^b	0.00±0.00
<i>Enterococcus</i> spp.	0.00±0.00 ^b	3.50±0.71 ^d	6.00±1.41 ^c	10.50±0.71 ^d	4.00±0.00 ^e	30.00±0.00 ^b	0.00±0.00

Key: Values are expressed as mean ± SD. The values with different superscript are statistically significant from each other across the column

Table 5: Antibacterial Activity of N-Hexane Extract of Tiger Nut (*Cyperus esculentus*) against Antibiotic-Resistant Gram-Negative Bacterial Isolates from Stool Samples

Isolate	20%	40%	60%	80%	100%	Positive control (Ciprofloxacin)	Negative control (Water)
<i>Escherichia coli</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	31.00±1.41 ^a	0.00±0.00 ^a
<i>Klebsiella</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	28.50±0.71 ^c	0.00±0.00 ^a
<i>Salmonella/Shigella</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	20.00±0.00 ^f	0.00±0.00 ^a
<i>Enterococcus</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	21.00±1.41 ^c	0.00±0.00 ^a
<i>Lactobacillus</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	27.00±1.41 ^d	0.00±0.00 ^a
<i>Lactobacillus</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	30.00±0.00 ^b	0.00±0.00 ^a
<i>Citrobacter</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	30.00±0.00 ^b	0.00±0.00 ^a
<i>Enterococcus</i> spp.	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	30.00±0.00 ^b	0.00±0.00 ^a

Key: Values are expressed as mean ± SD. The values with different superscript are statistically significant from each other across the column. 00 = Not detected

Table 6: Qualitative and Quantitative Phytochemical Constituents of N-Hexane and Ethanolic Extracts of Tiger Nut (*Cyperus esculentus*)

Phytochemicals	Qualitative phytochemical constituents		Quantitative phytochemical constituents	
	N-hexane extract	Ethanolic extract	N-hexane extract	Ethanolic extract
Alkaloids	+	+	6.2 ± 1.2 ^c	8.8 ± 1.5 ^b
Tannins	-	+	0.0 ± 0.0 ^d	8.0 ± 3.5 ^b
Saponins	+	++	7.2 ± 3.0 ^b	12.8 ± 3.0 ^a
Flavonoids	+	++	10.0 ± 3.0 ^a	16.2 ± 3.0 ^a
Phenols	+	+	5.5 ± 0.3 ^c	5.0 ± 0.3 ^c
Glycosides	-	+	0.0 ± 0.0 ^d	2.2 ± 1.0 ^c
Terpenoids	-	+	0.0 ± 0.0 ^d	3.0 ± 1.5 ^c

Keys: + Present, - Absent

Table 7: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of N-Hexane and Ethanolic Extracts of Tiger Nut against Bacterial Isolates from Stool Samples

Isolate	MIC		MBC	
	n-hexane extract (mg)	Ethanolic extract (mg)	n-hexane extract (mg)	Ethanolic extract (mg)
<i>Escherichia coli</i>	0	60	0	70
<i>Lactobacillus</i> spp.	0	0	0	0
<i>Salmonella/Shigella</i> spp.	0	80	0	90
<i>Enterococcus</i> spp.	0	0	0	0
<i>Lactobacillus</i> spp.	0	60	0	70
<i>Lactobacillus</i> spp.	0	80	0	90
<i>Citrobacter</i> spp.	0	80	0	90
<i>Enterococcus</i> spp.	0	80	0	90

DISCUSSION

The detection of *Escherichia coli* in the stool samples reemphasized the organism as one of the primary gut residents which aligns with Doranga and Conway (2023) who reported the adaptability of *Escherichia coli* and habitual act of colonization in the gut. The observation of *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Salmonella* and *Shigella*- are also associated with a high rate of recovery of Enterobacteriaceae in stools. This finding is similar to the findings of Naidoo and Zishri (2025), where the authors attributed the organism's presence in stool to both commensalism and enteric infections. The presence of beneficial isolates like *Lactobacillus* spp. and/or *Bacillus subtilis* points to a possible use as probiotics, which aligns with the findings of Luise *et al.* (2022), who also emphasized the health-promoting qualities of the isolates. The detection of *Enterococcus faecalis* and *Staphylococcus* spp. indicated the dual character of the gut commensals as opportunists which is consistent with Archambaud *et al.* (2024) and Gardoul *et al.* (2025). Generally, the presence of commensal, useful as well as potentially pathogenic species portrays the dynamic nature of the gut microbiota (Dey and Chaudhuri, 2023).

The high resistance of the isolates to amoxicillin and augmentin in this study is consistent with the report of Bassetti and Garu (2021) where the authors observed wide spread resistance of enteric Gram-negative bacteria to beta-lactam antibiotics.

The high sensitivity of the isolates to ciprofloxacin, levofloxacin, and ofloxacin agrees with the findings of Kibwana *et al.* (2023) who reported fluoroquinolones as one of the best agents against enteric bacteria. In addition, the wide-ranging response to gentamicin and ceftriaxone is consistent with Mirzaei *et al.* (2021), who observed intermediate patterns of susceptibility in Enterobacteriaceae from clinical isolates. The multiple antibiotic resistance profile of the isolates obtained in the present study corroborates the report of Portal *et al.* (2024) on the progressive emergence of multiple antibiotic -resistant Gram-negative enteric bacteria in Nigeria. The concentration dependent activity of the ethanolic tiger nut extract against multiple antibiotic-resistant Gram-negative bacteria in this study corroborates that of Zhang *et al.* (2022), who reported that *Cyperus esculentus* extracts showed a consistent increase in antibacterial potency with increasing concentration due to the presence of bioactive phytochemicals (flavonoids, tannins and saponins). The antagonistic activity recorded in isolate 25 (*Escherichia coli*) is in agreement with Imran *et al.* (2021) who reported that ethanolic plant extracts were able to elicit zones of inhibition similar to ciprofloxacin in susceptible strains of *Escherichia coli*. On the other hand, the resistance of *Lactobacillus* spp. and *Enterococcus* spp. to the ethanolic extract of tiger nut is consistent with the findings of Nam and Yoo (2024), who reported strain-specific resistance among

Enterobacteriaceae presumably caused by efflux pumps or modification in membrane permeability. The delayed susceptibility observed in *Lactobacillus* spp., *Enterococcus* spp. and *Enterococcus* spp. is in agreement with the report of Melander *et al.* (2023) where the authors observed that some Gram-negative bacteria require higher concentrations of the plant extracts for overcoming the intrinsic resistance barriers. The statistically significant zones of inhibition ($p < 0.05$) over the concentration ranges indicate the therapeutic potential of tiger nut extract and further establish its role as a promising adjunctive therapy against multiple-antibiotic resistant enteric pathogens.

The higher concentration and diversity of phytochemicals in the ethanolic extract of tiger nut agree with previous reports that ethanol generally extracts more secondary metabolites than n-hexane. For example, Azeez *et al.* (2022) reported that ethanolic extracts of *Cyperus esculentus* yielded significantly higher flavonoid and saponin content compared to n-hexane extracts, which aligns with the findings in this study. Similarly, Nwosu *et al.* (2022) observed that tannins were absent in aqueous extract of tiger nut, but strongly expressed in ethanol extract, corroborating the findings of this study. The presence of cardiac glycosides and terpenoids only in ethanol extract also agrees with the findings of Lee *et al.* (2024), who emphasized ethanol's polarity as a factor enabling the dissolution of less water-soluble compounds. These findings suggest that ethanol is more effective solvent than n-hexane for extracting a broader spectrum of phytochemicals especially flavonoids, tannins, and glycosides, which are often associated with antimicrobial and antioxidant activities.

Moreover, flavonoids, saponins, and tannins are widely reported to contribute to antimicrobial potential by disrupting microbial membranes and inhibiting enzymes. The observed antibacterial activity of ethanolic extracts of tiger nut against multiple antibiotic-resistant bacteria isolated

from the stool samples, suggests that the higher bioactivity of the ethanol extracts may be as a result of its higher phytochemical constituents. Comparable reports by Wigraboon *et al.* (2024) demonstrated that ethanol-extracted flavonoids and phenols in tiger nut significantly enhanced antimicrobial potency relative to n-hexane extracts. Mehmood *et al.* (2022) also demonstrated that ethanol is more efficient in extracting phenolic compounds, flavonoids, and saponins, known contributors to antimicrobial activity. The observed inactivity of the n-hexane extract aligns with reports that non-polar solvents often extract fewer bioactive secondary metabolites due to their low solubility in polar solvents (Barthwal and Mahar, 2024). The MIC values (60–80 mg/ml in this study) are comparable to those reported by Imran *et al.* (2021), who found similar inhibitory concentrations of ethanolic plant extracts against enteric bacteria. The resistance observed by *Lactobacillus* spp. and *Citrobacter* spp. is consistent with the findings of Davin-Regli *et al.* (2021), who observed variability in bacterial susceptibility due to differences in cell wall composition, efflux pumps, and adaptive resistance mechanisms among Gram-negative stool isolates. The MBC values being higher than MIC values are consistent with standard antimicrobial patterns, where bactericidal concentrations typically exceed inhibitory concentrations (Clinical and Laboratory Standards Institute, 2021). This confirms that ethanolic extract of tiger nut exhibited bacteriostatic activity at lower concentrations and bactericidal activity at higher doses.

CONCLUSION

The findings of this study demonstrated the antibacterial efficacy of tiger nut extracts against common bacterial pathogens isolated from diarrheagenic stool samples. The findings revealed that both the ethanol and n-hexane extracts possess significant inhibitory properties against a range of Gram-negative and Gram-positive bacteria,

including *Escherichia coli* and *Shigella* spp. The ethanolic extract of tiger nut showed significant antibacterial activity, with concentration-dependent inhibitory and bactericidal effects against multiple antibiotic-resistant bacteria from the stool samples. Its superior performance compared to n-hexane extracts showed the importance of solvent type in extracting bioactive compounds. These findings suggest that tiger nut is a valuable source of antimicrobial agents, capable of contributing to the global fight against antibiotic resistance. Further research should focus on isolating, purifying, and identifying the

specific phytochemical compounds within the ethanolic extract that are responsible for the observed antibacterial activity. *In vivo* studies should also be conducted to confirm the suitability of tiger nut extracts for human use.

Acknowledgement

The authors are grateful to the Department of Microbiology, School of Life Sciences, The Federal University of Technology, Akure, Nigeria for providing appropriate support in terms of equipment and laboratory used for the study.

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