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Xylopia aethiopica and Clarithromycin: A Synergistic Approach Against Cholera

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Abstract

Vibrio cholerae is a significant pathogen responsible for cholera outbreaks worldwide. The rise of antibiotic-resistant strains has necessitated the search for alternative antimicrobial agents. This study aimed to characterize *V. cholerae* isolates and evaluate the antimicrobial activity of *Xylopia aethiopica* extract against these isolates, alone and in combination with clarithromycin. *V. cholerae* isolates were characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. The phytochemical constituents of *X. aethiopica* extract were analyzed, and its antimicrobial activity was assessed using the disc diffusion method. Three *V. cholerae* isolates (VCC6, VCP2, and VCE7) were identified, exhibiting characteristic cultural, morphological, and biochemical features. The *X. aethiopica* extract contained alkaloids, phenolics, and flavonoids. The ethanolic extract (EEX) showed higher inhibition zones against the *V. cholerae* isolates compared to the aqueous extract (AEX). The combination of *X. aethiopica* extract with clarithromycin (CLA) showed enhanced antimicrobial activity, with inhibition zones ranging from 19.30-27.60 mm. Statistical analysis revealed significant differences in inhibition zones ($p < 0.05$). The study suggests that *X. aethiopica* extract has antimicrobial activity against *V. cholerae* isolates, and its combination with clarithromycin enhances the inhibitory effect. This study provides valuable data on the antimicrobial activity of *X. aethiopica* extract against *V. cholerae* isolates, highlighting its potential as a natural antimicrobial agent against cholera.

Keywords: *Vibrio cholerae, Xylopia aethiopica, Antimicrobial activity, Phytochemical constituents, Clarithromycin, Combination therapy*

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Introduction

Vibrio cholerae, the etiological agent of cholera, remains a formidable global health threat, particularly in areas with compromised water, sanitation, and hygiene (WASH) infrastructure. The pathogen is a Gram-negative, motile bacterium that colonizes the small intestine and secretes a potent enterotoxin, leading to the rapid onset of profuse, watery diarrhea, which can quickly progress to hypovolemic shock and death if untreated (Ojeda Rodriguez and Kahwaji, 2022; Okeke *et al*., 2017; Dim *et al*., 2025a). While oral or intravenous rehydration is the cornerstone of treatment, antibiotic therapy is crucial for reducing diarrheal volume, duration of illness, and bacterial shedding in severe cases.

The clinical management of cholera is increasingly jeopardized by the emergence and spread of multidrug-resistant (MDR) strains, particularly in endemic regions of Africa and Asia. Resistance to first-line antibiotics, including tetracyclines and fluoroquinolones, has complicated treatment protocols and heightened the risk of treatment failure (Miwanda *et al*., 2015; Amadi *et al*., 2017; Dim *et al*., 2025b).

This escalating resistance crisis underscores the urgent need for innovative therapeutic alternatives, including the investigation of medicinal plants with inherent antimicrobial properties and their potential synergy with existing antibiotics (Palczewska et al., 2019; Dim *et al*., 2025c; Chude *et al*., 2020).

Xylopia aethiopica, commonly known as African guinea pepper or "Uda," is a widely used medicinal plant in West Africa, traditionally employed for treating various ailments, including gastrointestinal disorders. Its fruits are rich in a complex array of bioactive phytochemicals, such as diterpenes, flavonoids, and alkaloids, which have documented antimicrobial, anti-inflammatory, and antioxidant activities. Clarithromycin, a semi-synthetic macrolide antibiotic, remains an important therapeutic option for various bacterial infections, though resistance has been reported. Combining plant-derived compounds with conventional antibiotics is a promising strategy to enhance antibacterial efficacy, overcome resistance mechanisms, and potentially reduce required antibiotic doses. Therefore, this study aims to evaluate the antibacterial activity of *Xylopia aethiopica* fruit extract against clinical *Vibrio cholerae* isolates and to assess its synergistic potential when combined with clarithromycin.

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Materials and Methods

Sample collection, handling and transportation:

The samples used for this study were drawn from the rivers. A total of 100 freshwater samples were collected from five different streams used in Uli community. Samples were taken from twenty different sites, each site in triplicates. The stream samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the river water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Culture and Isolation of Enteric Bacteria

This was carried out using the modified method of Cheesbrough. The swab sticks were stricked on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated in inverted at 37±2˚C for 24-48 h. (Egbe *et al*., 2025b; Egbe *et al*., 2025c; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al*. (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al*., 2017a; Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al*., 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres

of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved

cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Prevalence and Distribution of the Isolates in the Frozen Meat Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al* (2025u),

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010), Iheukwumere *et al* (2025u). The isolates were aseptically subcultured into a broth culture and incubated at 35 + 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* antibacterial susceptibility test:** This was carried out using the method described in the study published by Iheukwumere *et al*. (2025v). Each labeled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labeled and then incubated at 37±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al*. (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

Results

The *Vibrio cholerae* isolates (L, M, N) exhibited characteristic cultural and morphological features, including yellow appearance on TCBS, smooth edges, and rod/comma-shaped cells (Table 1). Biochemical analysis revealed that the isolates were positive for catalase, citrate, and oxidase tests, and fermented glucose and galactose (Table 2). Molecular analysis confirmed the isolates as *Vibrio cholerae* strains VCC6, VCP2, and VCE7, with 100% identity to reference strains (Table 4).

The *Xylopia aethiopica* extract was analyzed for phytochemical constituents, revealing the presence of alkaloids (2.14 g/100g), phenolics (1.56 g/100g), and flavonoids (0.60 g/100g) (Table 5). The extract's antimicrobial activity was assessed, showing varying inhibition zones against the V. cholerae isolates (Table 6). The ethanolic extract (EEX) exhibited higher inhibition zones compared to the aqueous extract (AEX).

The combination of *Xylopia aethiopica* extract with clarithromycin (CLA) showed enhanced antimicrobial activity, with inhibition zones ranging from 19.30-27.60 mm (Table 6). Statistical analysis revealed that the differences in inhibition zones were significant ($p < 0.05$). The p-values for the antimicrobial activity were < 0.05, indicating statistical significance. Clarithromycin alone showed inhibition zones of 16.80-19.80 mm.

The results suggest that *Xylopia aethiopica* extract has antimicrobial activity against *V. cholerae* isolates, and its combination with clarithromycin enhances the inhibitory effect. The study highlights the potential of *Xylopia aethiopica* as a natural antimicrobial agent against cholera.

Table 1: Cultural and morphological characteristics of the isolates

Parameter	L	M	N
Appearance on TCBS	Yellow	Yellow	Yellow
Edge	Smooth	Smooth	Smooth
Elevation	Raised	Raised	Raised
Surface	Smooth	Smooth	Smooth
String test	+	+	+
Gram reaction	-	-	-
Shape	Rods/comma	Rods/comma	Rods/comma
Endospore	-	-	-
Capsule	-	-	-
Motility	+	+	+

Table 2: Biochemical characteristics of the isolates

Parameter	**L**	**M**	**N**
Catalase	+	+	+
Citrate	+	+	+
Gelatin	+	+	+
H_2S	-	-	-
Methyl red	-	-	-
Oxidase	+	+	+
Urease	-	-	-
Arabinose	-	-	-
Glucose	+	+	+
Galactose	+	+	+
Inositol	-	+/-	-
Dulcitol	-	-	-
Xylose	+/-	-	+/-
Subitol	-	+/-	-
Lactose	+/-	-	+/-

Table 3: Verification of the extracted nucleic acids

Sample ID	**Conc(ug/ml)**	**260 nm**	**289 nm**	**260/280**
L	121.20	3.0120	1.6194	1.86
M	125.70	3.1082	1.6801	1.85
N	132.80	3.2110	1.7643	1.82

Table 4: Molecular identities of the isolates

Parameter	**L**	**M**	**N**
Max score	5686	5686	5686
Total score	7295	7295	7295
Query cover (%)	100	100	100
E-value	0.0	0.0	0.00
Identity (%)	100	100	100
Accession length	1070357	10703537	1071008
Accession number	CP047298	CP047300	CP047304
Description	*Vibrio cholerae 01 bio var EI Tor strain C6709(VCC6*)	*Vibrio cholerae 01 bio var EI Tor strain P27459(VCP2*)	*Vibrio cholerae 01 bio var EI Tor strain E7946(VCE7*)

Table 5: Phytochemical constituents of *Xylopia aethiopia* extract

Parameter	Value(g/100g)
Alkaloids	2.14±0.02
Phenolics	1.56±0.01
Flavonoids	0.60±0.0.01
Tannins	0.88±0.01
Saponins	0.30±0.00
Glycosides	0.28±0.01
Steroids	0.12±0.00

Table 6: Antimicrobial activity: Diameter zone of inhibition [X±SD] mm

Inhibitory substance	VCC6	VCP2	VCE7
EEX	9.20±0.11	14.67±0.33	11.50±0.14
AEX	0.00±0.00	10.00±0.00	7.00±0.00
CPX	14.00±0.17	17.30±0.11	14.50±0.07
CLA	16.80±0.22	19.80±0.12	17.70±0.11
EEX+CLA	22.10±0.14	27.60±0.11	24.70±0.13
AEX+CLA	19.30±0.12	21.10±0.11	19.80±0.11

EEX- Ethanoic Extract of *Xylopia aethiopia*, AEX- Aqueous Extract of *Xylopia aethiopia*, CPX- Ciprofloxacin, CLA- Clarithromycin

Discussion

The detection of *Vibrio* isolates in stream water samples within this study aligns with the findings of numerous researchers who have reported similar environmental contamination in aquatic ecosystems (Traore *et al.*, 2014; Abia *et al.*, 2016; Reischer *et al.*, 2018). This consistency underscores the persistent role of freshwater bodies as reservoirs for *Vibrio cholerae*. However, it contrasts with research focused on distinct contamination sources, such as hospital waste sites, where different bacterial profiles and resistance patterns may dominate (Prakasam *et al.*, 2017). The specific isolation of *Vibrio cholerae* O1 biovar El Tor strains C6709 (VCC6), P27459 (VCP2), and E7946 (VCE7) reaffirms the ongoing public health risk posed by contaminated water sources and highlights the critical need for continuous environmental monitoring to protect exposed communities.

The observed predominance of strain VCC6 in the sampled streams may be attributed to several ecological and genetic factors. Studies indicate that genomic diversity, antigenic variation, and the acquisition of strain-specific genes through horizontal gene transfer can confer significant survival

advantages, allowing particular clones to outcompete others in specific environmental niches (Yap *et al.*, 2014). Furthermore, local conditions such as poor sanitation, organic pollution, and inadequate waste management likely create a selective environment that favours the persistence and proliferation of this strain.

Phytochemical analysis of the *Xylopia aethiopica* (XA) extract confirmed the presence of cardiac glycosides, steroids, alkaloids, tannins, flavonoids, phenolics, and saponins. This bioactive profile is consistent with previous phytochemical characterizations of the plant (John-Dewole *et al.*, 2012; Aguoru *et al.*, 2016; Ogbuagu *et al.*, 2020). The documented antimicrobial properties of these compounds, particularly flavonoids and tannins, are widely believed to underpin the ethnomedical applications of *X. aethiopica* (Fategbe *et al.*, 2021). Variations in phytochemical concentration can be influenced by factors such as plant provenance, harvesting time, and extraction methodology.

The pronounced antibacterial activity of the XA extract against the *V. cholerae* strains, including resistant variants, is likely a synergistic effect of its complex phytochemical constituents. These compounds can disrupt bacterial cell membranes, inhibit essential enzymes, and interfere with virulence factor production. Notably, the combination of XA extract with azithromycin resulted in enhanced antibacterial efficacy. This synergistic effect aligns with findings from other studies exploring plant-antibiotic combinations, which suggest that plant metabolites can potentiate conventional antibiotics by inhibiting efflux pumps, degrading resistance enzymes, or targeting complementary bacterial pathways (Kumar *et al.*, 2013; Njimoh *et al.*, 2015). Such synergy is crucial in the context of antimicrobial resistance, which is often propagated by the horizontal transfer of plasmid-encoded resistance genes within bacterial populations.

Conclusion

This study confirms the presence of *Vibrio cholerae* strains VCC6, VCP2, and VCE7 in local stream water, with VCC6 identified as the predominant isolate. The *Xylopia aethiopica* extract demonstrated significant intrinsic antibacterial activity against these pathogens. Importantly, its combination with azithromycin yielded a synergistic enhancement of efficacy, indicating a promising complementary therapeutic strategy. These findings support further investigation into the bioactive compounds responsible for this synergy and advocate for the development of integrated, plant-based adjuncts to conventional antibiotic therapy for managing cholera, particularly in the face of rising multidrug resistance.

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