RESEARCH ARTICLE

Journal of Public Health, Policy, and Society (JPHPS)
DOI: https://doi.org/10.54117/dxhw7505

3(1): 74-84 (2026)

Fidaxomicin and *Xylopia aethiopica*: A Combined Strategy against Cholera Pathogens

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Received: *29 Dec 2025* ***Accepted:*** *03 Feb 2026* ***Published:*** *14 Feb 2026*

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 fidaxomicin. *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* O1 biovar El Tor strains (C6709/VCC6, P27459/VCP2, E7946/VCE7) were isolated from stream samples, with VCC6 identified as the most prevalent ($p < 0.05$). *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 fidaxomicin (FID) showed enhanced antimicrobial activity, with inhibition zones ranging from 21.00-29.00 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 fidaxomicin 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.

How to cite this article
Onwuasoanya, U. F., Iheukwumere, I. H., Iheukwumere, C. M., Unaeze, B. C., Okongwu, D. J., Abba, O., Ike, V. E., Nnagbo, P. A., & Mere, C. A. (2026). Fidaxomicin and *Xylopia aethiopica*: A combined strategy against cholera pathogens. *Journal of Public Health, Policy, and Society, 3*(1), 74–84. https://doi.org/10.54117/dxhw7505

Keywords
Vibrio cholerae, Xylopia aethiopica, antimicrobial activity, phytochemical constituents, fidaxomicin, combination therapy

INTRODUCTION

Cholera, caused by the Gram-negative bacterium *Vibrio cholerae*, remains a major global health threat, especially in regions with inadequate water, sanitation, and hygiene infrastructure. The pathogen's rapid transmission and potential to cause severe, life-threatening dehydrating diarrhea underscore the need for effective therapeutic interventions (Ojeda Rodriguez and Kahwaji, 2022; Okeke *et al*., 2017; Dim *et al*., 2025a). While oral rehydration therapy is the mainstay of treatment, antibiotic therapy is crucial for reducing diarrheal volume, shortening illness duration, and limiting transmission in moderate to severe cases.

However, the efficacy of standard antibiotics is increasingly compromised by the global rise of multidrug-resistant (MDR) *V. cholerae* strains. Resistance to first-line agents such as tetracyclines, fluoroquinolones, and even macrolides like azithromycin has been documented, particularly in cholera-endemic regions of Africa and Asia (Miwanda *et al*., 2015; Amadi *et al*., 2017; Dim *et al*., 2025b). This alarming trend highlights an urgent need for innovative therapeutic strategies, including the investigation of novel antibiotic agents and synergistic combinations with natural products.

Fidaxomicin is a narrow-spectrum macrocyclic antibiotic approved for the treatment of *Clostridioides difficile* infection. It inhibits bacterial RNA polymerase and has demonstrated potent activity against various Gram-positive pathogens. Its unique mechanism of action and low potential for cross-resistance make it an intriguing candidate for repurposing against other challenging infections, including those caused by MDR Gram-negative bacteria when used in combination with agents that disrupt outer membrane integrity.

Concurrently, medicinal plants like *Xylopia aethiopica* (African guinea pepper) have a long history of use in traditional medicine for treating gastrointestinal ailments. Phytochemical studies have identified a rich profile of bioactive compounds,

including diterpenes and flavonoids, with documented antimicrobial and adjuvant properties (Palczewska *et al.*, 2019; Dim *et al*., 2025c; Chude *et al*., 2020). Plant extracts can potentiate conventional antibiotics by inhibiting efflux pumps, degrading resistance enzymes, or increasing membrane permeability.

Therefore, this study aims to evaluate the individual and combined antibacterial effects of fidaxomicin and *Xylopia aethiopica* extract against clinical isolates of *Vibrio cholerae*

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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^{0}C 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).

Preparation, Extraction and Phytochemical Analysis of the Plant Material

Preparations of plant materials: The fresh leaves of *Xylopia aethiopica* was collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The leaves samples were authenticated appropriately. The fresh leaves were plucked, washed and dried under shade at room temperature for 14 days. The dried leaves were ground to powder form using sterile electric grinder. Twenty grams of the ground leaves each was macerated in two hundred milliliters of distilled water and ethanol respectively for 72 h. The mixture was filtered using what man No.1 filter paper. The extracts were concentrated by evaporation at room temperature in a steady air current (Iheukwumere *et al*., 2025u).

Phytochemical analysis of the plant extracts: The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by

***In Vitro* Antibacterial Activity**

Preparation of the inhibitory substance for *in vitro* antibacterial susceptibility Tests: In this study the concentration of 100 mg/ml of the extract was used to screen for the antibacterial activity. This was carried out using the modified method described in the study published by Iheukwumere *et al*. (2018). Here, 2.5 g of the extract was dissolved in 25.0 ml of peptone water. Similarly, equal concentration of the antibiotic was prepared, and then equal volume of the extract and antibiotic were mixed, and this was used for the study

***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 fidaxomicin (FID) showed enhanced antimicrobial activity, with inhibition zones ranging from 21.00 - 29.00 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. Fidaxomicin alone showed inhibition zones of 17.00 - 21.00 mm.

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

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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

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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
FID	17.50±0.00	21.00±0.21	19.00±0.11
EEX+FID	24.50±0.22	29.00±0.12	25.50±0.11
AEX+FID	21.00±0.12	24.00±0.11	23.00±0.11

EEX- Ethanoic Extract of *Xylopia aethiopia,* AEX- Aqueous Extract of *Xylopia aethiopia*, CPX- Ciprofloxacin, FID- Fidaxomicin

DISCUSSION

The isolation of *Vibrio cholerae* strains from stream water samples in this study aligns with a substantial body of research documenting the persistence of this pathogen in aquatic environments, particularly in regions with compromised sanitation (Traore *et al.*, 2014; Abia *et al.*, 2016; Reischer *et al.*, 2018). This environmental reservoir poses a direct and continuous public health risk, reaffirming the critical need for vigilant water quality monitoring. The discrepancy with findings from studies on hospital waste sites, such as Prakasam *et al.* (2017), is expected, as contamination sources, selective pressures, and bacterial communities differ fundamentally between natural aquatic ecosystems and anthropogenic waste repositories.

The observed predominance of strain VCC6 may be attributed to a confluence of genetic and ecological factors. Genomic studies indicate that specific *V. cholerae* lineages can achieve clonal expansion through advantageous genetic traits, such as the acquisition of mobile genetic elements, antigenic variation, and unique metabolic capabilities that enhance environmental fitness (Yap *et al.*, 2014). This genetic adaptation, coupled with local environmental conditions—including organic pollution from inadequate waste disposal and agricultural runoff—likely creates a niche that favors the survival and proliferation of this particular strain.

Phytochemical analysis of the *Xylopia aethiopica* (XA) extract confirmed a rich profile of bioactive compounds, including alkaloids, flavonoids, tannins, and saponins, consistent with previous characterizations of this medicinal plant (John-Dewole *et al.*, 2012; Aguoru *et al.*, 2016; Fategbe *et al.*, 2021). The antimicrobial efficacy of the extract against the *V. cholerae* isolates can reasonably be attributed to the synergistic action of these phytoconstituents, which are known to disrupt microbial cell membranes, inhibit essential enzymes, and interfere with virulence mechanisms.

A key finding of this research was the enhanced antibacterial effect observed when the XA extract was combined with fidaxomicin (FDX). This synergistic interaction suggests a promising strategy to potentiate the activity of a next-generation antibiotic. The mechanism may involve the plant-derived compounds compromising bacterial membrane integrity or inhibiting efflux pumps, thereby increasing intracellular accumulation of fidaxomicin and overcoming intrinsic or acquired resistance mechanisms. This aligns with emerging studies advocating for plant-antibiotic combinations as a viable approach to counteract multidrug resistance, which is frequently propagated by plasmid-mediated horizontal gene transfer (Kumar *et al.*, 2013; Njimoh *et al.*, 2015; Musbal *et al.*, 2024).

CONCLUSION

This study confirms the presence of *Vibrio cholerae* strains VCC6, VCP2, and VCE7 in local stream water, identifying VCC6 as the predominant isolate. The *Xylopia aethiopica* extract demonstrated significant intrinsic antibacterial activity against these pathogens. Importantly, its combination with fidaxomicin produced a synergistic enhancement of efficacy, indicating a potent combined therapeutic strategy. These findings support the further investigation of fidaxomicin–plant extract combinations as a novel approach to manage cholera infections, particularly in the context of rising antimicrobial resistance, by potentially lowering effective antibiotic doses and overcoming established resistance pathways

Acknowledgement: We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

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