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Full Length Research Article

ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF *Enantia chlorantha* STEM BARK EXTRACTS AGAINST SELECTED PATHOGENIC BACTERIA

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ABSTRACT
Antimicrobial resistance has become a major global health concern, creating an urgent need for alternative antimicrobial agents. *Enantia chlorantha* (Annonaceae) is a medicinal plant widely used in African traditional medicine for the treatment of infectious diseases. This study investigated the antibacterial activity, phytochemical composition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of methanol stem bark extract and solvent fractions of *E. chlorantha* against selected pathogenic bacteria. Stem bark of *E. chlorantha* was extracted with methanol and partitioned into n-hexane, ethyl acetate, and aqueous fractions. Clinical isolates were identified using morphological, biochemical, and molecular techniques. Antibacterial activity was evaluated using the agar well diffusion method, while MIC and MBC were determined using broth dilution and sub-culturing methods. Quantitative phytochemical analysis was performed using standard spectrophotometric procedures. Molecular characterization confirmed the isolates as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori*. The methanol extract and aqueous fraction exhibited notable antibacterial activity against the test organisms, with inhibition zones ranging from 4.50 ± 0.50 to 15.90 ± 0.10 mm and 5.00 ± 1.00 to 19.50 ± 0.50 mm, respectively. MIC values ranged from 9.38 to 150 mg/mL. Phytochemical analysis revealed high concentrations of phenols (416.07 ± 0.07 mg/100 g), followed by saponins, tannins, and flavonoids. The findings demonstrate that *E. chlorantha* stem bark possesses significant antibacterial properties, likely due to its rich phytochemical constituents, and may serve as a promising source of novel antimicrobial agents.

Keywords: *Enantia chlorantha*; Antibacterial activity; Phytochemical analysis; Antimicrobial resistance; Gram-negative bacteria.

INTRODUCTION

Antimicrobial resistance (AMR) has become a major global public health concern due to the rapid emergence and spread of multidrug-resistant bacterial pathogens. The increasing resistance of microorganisms to commonly used antibiotics has significantly reduced the effectiveness of conventional antimicrobial therapies, thereby increasing morbidity, mortality, and healthcare costs worldwide (World Health Organization [WHO], 2023). Recent reports indicate that antimicrobial resistance threatens the successful prevention and treatment of infectious diseases caused by bacteria, fungi, parasites, and viruses (WHO, 2023). This global challenge has stimulated renewed interest in medicinal plants as alternative sources of novel antimicrobial agents with fewer side effects and improved therapeutic potential (Guefack *et al*., 2022). Medicinal plants have historically played important roles in the treatment and management of infectious diseases, especially in developing countries where access to modern healthcare may be limited. Approximately 80% of the population in many African and Asian countries depend partly on traditional herbal medicine for primary healthcare needs (Abubakar & Ibrahim, 2021). Plant-derived bioactive compounds such as alkaloids, flavonoids, tannins, phenolics, terpenoids, and saponins have been reported to exhibit significant antibacterial, antifungal, antioxidant, and anti-inflammatory activities (Ogunjinmi & Adegbola, 2024). These phytochemicals are increasingly being investigated for their ability to combat resistant microbial strains and serve as templates for the development of new antimicrobial drugs (Imieje *et al*., 2024).
Enantia chlorantha is a tropical medicinal plant belonging to the family Annonaceae and is widely distributed across West and Central Africa. The stem bark of *E. chlorantha* is extensively used in traditional medicine for the treatment of malaria, typhoid fever, jaundice, urinary tract infections, wounds, and gastrointestinal disorders (Etame *et al*., 2019). Previous phytochemical studies have shown that the plant contains important bioactive constituents including protoberberine alkaloids, phenols, flavonoids, tannins, and saponins, which contribute to its therapeutic properties (Imieje *et al*., 2024).

Despite the growing scientific interest in *E. chlorantha*, available studies have largely focused on crude extracts with limited attention given to solvent-partitioned fractions and their comparative antibacterial efficacy against clinically important Gram-negative bacteria. In addition, there is limited information regarding the molecular characterization of bacterial isolates used in evaluating the antibacterial activity of the plant extracts.
The novelty of the present study lies in the integrated assessment of antibacterial activity, molecular identification of bacterial isolates, and phytochemical composition obtained from *E. chlorantha* stem bark extract and fractions against selected pathogenic bacteria. Unlike previous studies that mainly evaluated crude extracts, this study comparatively investigated the antibacterial activities of methanolic crude extract and solvent-partitioned fractions against characterized isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori*. The study also provides additional insight into the phytochemical constituents responsible for the observed antibacterial effects.

Therefore, this study was designed to investigate the antibacterial activities, minimum inhibitory concentration (MIC), minimum

bactericidal concentration (MBC), and phytochemical composition of *E. chlorantha* stem bark extract and fractions against selected pathogenic bacteria.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh stem bark of *Enantia chlorantha* was purchased from fish markets, behind a mobile filling station, along Keteren Gwari Road, Minna, Niger State, authenticated by a taxonomist at the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, University of Abuja, with Voucher number UA/FPS/003. The plant material collected was washed under running tap water, air-dried at room temperature in the laboratory, pulverized and homogenized to a fine powder, and stored in airtight glass containers for further use.

Extraction and fractionation of the methanol stem bark of *Enantia chlorantha.*

The powdered stem bark (100 g) *of E. chlorantha* was extracted using the cold maceration technique with 1 L of methanol in a glass jar for 3 days (72 hours) at room temperature. The extract was filtered; the filtrate was further concentrated using a rotary evaporator and finally evaporated to dryness using a water bath set at 50 °C. Twenty-five grams (25 g) of crude methanol extract was suspended in 100 ml of water and filtered. The filtrate was partitioned thrice with 100 ml of hexane, ethyl acetate and distilled water in increasing order of polarity (n-hexane < ethyl acetate < distilled water) using a separate funnel.

The crude methanol extract and fractions were evaporated to dryness at a reduced temperature of 40°C over a water bath, labelled appropriately and stored for further use (Sarker *et al*., 2006; Nuhu *et al.*, 2022).

Ethical Consideration

Ethical approval was obtained from the ethics and publication committee of General Hospital, Minna, Niger State, to permit the collection of bacterial isolates.

Isolation and Identification of the Bacterial Isolates

Clinical bacterial isolates were obtained from the Microbiology Laboratory of General Hospital, Minna, Niger State, Nigeria. The isolates were initially enriched in nutrient broth and incubated aerobically at 37°C for 24 h. Thereafter, a loopful of each culture was streaked onto Eosin Methylene Blue (EMB) agar, Salmonella–Shigella Agar (SSA), and Nutrient Agar (NA) and incubated at 37°C for 24 h. Distinct colonies were repeatedly sub-cultured on freshly prepared nutrient agar plates to obtain pure bacterial isolates. Preliminary identification of the isolates was carried out based on colonial morphology, Gram staining characteristics, and standard biochemical tests, including indole, methyl red, Voges–Proskauer, citrate utilization, catalase, oxidase, urease, and motility tests, following the methods described by Ravi *et al.* (2015). The presumptively identified bacterial isolates were subsequently confirmed by molecular characterization using 16S *rRNA* gene sequencing.

Molecular Characterization of the Bacterial Isolates

Genomic DNA was extracted from overnight cultures of the purified bacterial isolates using a bacterial genomic DNA extraction kit according to the manufacturer's instructions. The quality and integrity of the extracted DNA were assessed by electrophoresis on a 1.5% agarose gel. Amplification of the bacterial 16S *rRNA* gene was performed by polymerase chain reaction (PCR) using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGATCCAGCC-3') as described by Lane (1991). The PCR reaction consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 min. The amplified PCR products were separated on a 1.5% agarose gel, producing a single DNA fragment of approximately 1,500 bp.

The PCR amplicons were purified and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The resulting nucleotide sequences were edited using BioEdit and compared with sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) for species identification. Multiple sequence alignment and phylogenetic analyses were performed using MEGA version 6.0 to determine the evolutionary relationships among the isolates (Hall, 1999; Tamura et al., 2013). These procedures were carried out according to the methods described by Fifa *et al.* (2019).

Determination of the Antibacterial Activity of Extracts and Fractions

Standardization of the Bacterial Culture

According to the method described by Iyabo *et al*. (2018), two milliliters (0.2 mL) of overnight cultures of the test organism were transferred into 20 mL of sterile nutrient broth, and the culture was incubated for 2-3 hours at 37°C to standardize the culture to 106 CFU/mL McFarland. A loopful of the standardized inoculum was used for the antibacterial assay.

Antibacterial activities

The agar well diffusion method was carried out to determine the antimicrobial activity of the extract and fractions as described by Gupta et al. (2013). Mueller-Hinton agar plates were prepared for *Pseudomonas aeruginosa, Salmonella bongori,* and *Escherichia coli.*

Forty grams (40 g) of the Nutrient agar was dissolved in 1 L of distilled water in a conical flask. The mixture was sterilized in the autoclave at 121°C for 15 minutes, and the medium was allowed to cool to 45 °C. Sterile molten Mueller-Hinton agar (20 mL) at 45 °C was dispensed into sterile Petri dishes and allowed to set. A sterile cork borer of diameter 6 mm was used to bore equidistant wells onto the agar plates. One drop of the molten agar was used to seal the bottom of the bored wells to prevent the extract from seeping beneath the agar. Sterile cotton swab sticks were used to streak on the surface of the agar plates with the standardized test organisms and 100 µL of the methanol extract and fractions (15, 25, 50, 100, 150, 200, 250 and 300 mg/mL) stem bark of *E. chlorantha* were added separately to the bored wells and 5 mg/mL of the standard drug (Ciprofloxacin and Amoxicillin) were used as positive control. At the same time, dimethyl sulfoxide (DMSO) served as the negative control. A 30-minute (30 mins.) pre-diffusion time was allowed, after which the plates were incubated at 37°C for 24 hours. The zones of inhibition were then measured in millimeters. The above method was carried out in duplicate, and

the mean of the results was taken. A test with ten millimeters (10 mm) zones of inhibition was considered sensitive to the plant extract.

Determination of Minimum Inhibitory Concentration (MIC)
The method of Iyabo *et al*. (2018) was employed with slight modification using a spectrophotometer to determine the MIC of the extract against the organisms. A series of two-fold dilutions of extract and fractions ranging from 300 mg/mL to 38.85 mg/mL was made in nutrient broth. Zero point one milliliter (0.1 mL) of each of the standardized test organisms (0.5 McFarl and turbidity standards) was added to each dilution. Two controls were maintained for each batch. These included tubes containing extract/fractions and growth medium without inoculum and a tube containing the growth medium and inoculum (organism control). The tubes were incubated at 37 ^{0}C for 24 hours. At the end of the incubation period, the optical density of the cultures in the test tubes was read using a spectrophotometer at a wavelength of 600 nm. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive control. The concentration/test tube where a significant reduction in absorbance was observed was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)
The minimum bactericidal concentration (MBC) was determined using the broth dilution subculture method as described by Andrews (2001) with slight modifications. Following the determination of the minimum inhibitory concentration (MIC), aliquots (100 µL) were aseptically withdrawn from the test tubes showing no visible bacterial growth, beginning with the MIC tube and all higher extract concentrations, and sub-cultured onto freshly prepared nutrient agar plates. The inoculated plates were incubated aerobically at 37°C for 24 h. After incubation, the lowest concentration of the extract that produced no visible bacterial growth on the agar plates was recorded as the MBC.

Quantitative phytochemical evaluation
The methanol extract of *E. chlorantha* stem bark was analysed quantitatively for the presence of phenols, flavonoids, tannins, and saponins using a spectrophotometric method.

Determination of total phenolic content (TPC)
Total phenol content in methanol extract *of E. chlorantha* stem bark was measured spectrophotometrically by the Folin–Ciocalteu colorimetric method, using gallic acid as the standard and expressing results as Gallic Acid Equivalent (GAE) per gram of sample. Different concentrations (0.01- 0.1 mg/mL) of gallic acid were prepared in methanol. Aliquots of 0.5 mL of the test sample and each sample of the standard solution were taken, mixed with 2 mL of Folin–Ciocalteu reagent (1:10 in deionised water) and 4 mL of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foil and incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analysed in triplicate. The total phenol was determined using a standard curve prepared from a pure phenolic standard (gallic acid) (Alhakmani *et al*., 2013).

Determination of total flavonoid content (TFC)
The TFC of methanol extract of *E. chlorantha* stem bark was determined by aluminium chloride colorimetric assay (Zhishen *et al*., 1999). Briefly, 0.5 mL aliquots of the methanol extract and standard solution (0.01 – 0.1 mg/mL) of Quercetin were mixed with 2 mL of distilled water and subsequently with 0.15 mL of sodium nitrite (5% $NaNO_2$, w/v in methanol). After 6 minutes, 0.15 mL of (10% w/v $AlCl_3$) solution was added. The solution was allowed to stand for 6 min, and after that, 2 mL of sodium hydroxide (4% NaOH, w/v in methanol) solution was added to the mixture. The final volume was adjusted to 5 mL with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm. TFC was determined as mg Quercetin equivalent per gram of sample with the help of a calibration curve of Quercetin. All determinations were performed in triplicate.

Determination of tannin content
The tannins were determined by the Folin-Ciocalteu method. 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu-phenol reagent, 1 mL of 35% Na2CO3 solution, and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (0.01 – 0.1 mg/mL) was prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract (Rajeev *et al*., 2012).

Determination of saponin content
Total saponins were determined according to the method described by Makkar et al. (2007). One gram (1 g) of freeze-dried extract was dissolved in 50% aqueous methanol, and an aliquot (5 mg/mL) was taken. Vanillin reagent (0.25 mL; 8%) was added, followed by sulphuric acid (2.5 mL; 72% v/v). The reaction mixtures were mixed well and incubated at 60°C on a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV-visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from aliquots of diosgenin (0.01 – 0.1 mg/mL in 50% aqueous methanol). The total saponin concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Data Analysis
Data were expressed as mean ± standard error of the mean (SEM)**.** The antibacterial activity data were analysed using two-way analysis of variance (ANOVA) to determine the effects of extract treatment (methanol extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction) and extract concentration (50, 100, 150, 200, 250, and 300 mg/mL)**,** as well as the interaction between these two factors, on the mean zones of inhibition. Where significant differences were observed **(**$p < 0.05$**),** means were separated using Duncan's Multiple Range Test (DMRT)**.** Statistical analyses were performed using IBM SPSS Statistics version 25.0.

RESULTS

Characterization and Identification of Bacterial Isolates
The bacterial isolated were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori.* Table 1 and 2 indicates the findings of the morphological and biochemical tests conducted.

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Molecular Characterization of Bacteria Isolates

Polymerase chain reaction amplifications of total genomic DNA of the bacteria isolates using primer pair 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'-AAGGAGGTGATCCAGCC-3' primers produced a PCR product of about 1500 base pair (bp) (Plate I). The PCR products were sequenced (Gen Bank Accession Number: MK572634.1, MN623691.1 and CP074120.1for *Pseudomonas aeruginosa, Salmonella bongori, and Escherichia coli* respectively) (Table 3). The phylogenetic tree of the isolates shows the relationship among the organisms and their origin using the NCB I data (Figure 1).

Table 1: Cultural growth characteristics on media, microscopic morphological characteristics and Gram staining Reaction

Organism	GC & M	MCM	Gram staining
Escherichia coli	Greenish metallic sheen colonies on EMB	Rod shaped bacilli	Negative
Pseudomonos aeruginosa	Large opaque flat colonies with green	Rod shaped bacilli	Negative
Salmonella bongori	Colourless colonies with black center on SSA	Rod shaped bacilli	Negative

GC & M = growth characteristics on media, MCM= microscopic morphological characteristics

Table 2: Biochemical characteristics of bacteria isolates

Organism	Indole	Methyl-red	Voges-proskaur	Citrate	Catalase	motility	oxidase	Urease
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginosa	-	-	-	+	+	+	+	-
Salmonella bongori	-	+	+	-	+	+	-	-

+ = Positive and - = Negative

Table 3: Molecular Characterization of Bacteria Isolates

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
IDZU7	*Pseudomonas aeruginosa*	287	2623	99%	0	99.93%	1473	MK572634.1
IDZU8	*Salmonella bongori*	54736	2647	99%	0	99.52%	1526	MN623691.1
IDZU9	*Escherichia coli*	562	2630	99%	0	99.58%	4844032	CP074120.1

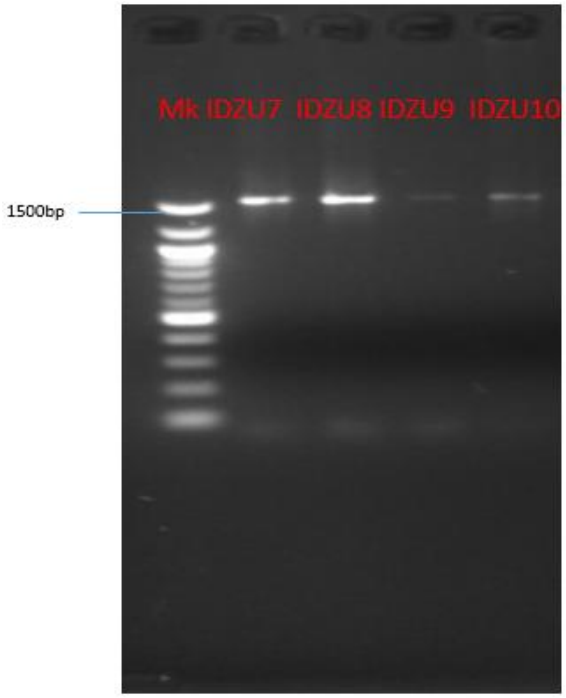

Plate I: PCR amplification of the DNA region of the bacteria isolates using the 27F and 1525R primer

MK = 1500bp DNA ladder, IDZU7, IDZU8, IDZU9, IDZU10 = PCR reaction mixture with DNA of the samples to be identified.

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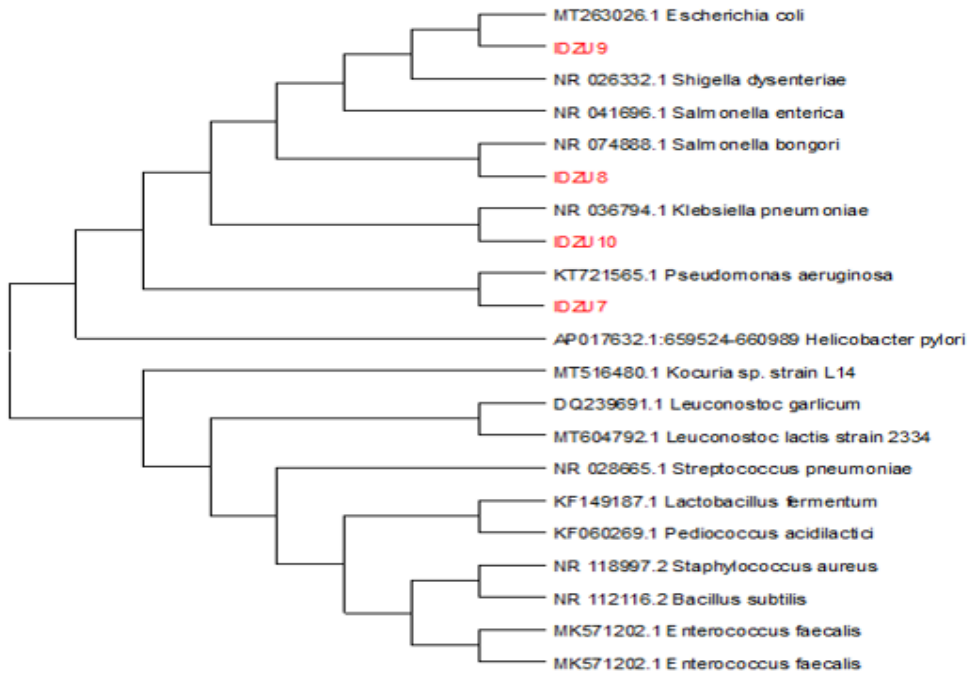

Figure 1: Phylogentic tree showing the relations among the bacteria isolates
IDZU7 = *Pseudomonas aeruginosa,* **IDZU8** = *Salmonella bongori* and **IDZU9** = *Eschrichia coli*

Antibacterial Evaluation of the Crude Methanol Extract and Solvent Fractions

The crude methanol extract of *Enantia chlorantha* stem bark exhibited antibacterial activity against all the test organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori*) at concentrations ranging from 150 to 300 mg/mL. The highest antibacterial activity was observed against *E. coli*, with inhibition zones increasing from 5.35 ± 0.65 mm at 50 mg/mL to 15.90 ± 0.10 mm at 300 mg/mL. Similarly, *S. bongori* showed inhibition zones ranging from 4.50 ± 0.50 mm to 12.50 ± 0.00 mm, whereas *P. aeruginosa* exhibited inhibition only at concentrations of 200–300 mg/mL, with inhibition zones ranging from 9.80 ± 0.50 mm to 11.90 ± 0.60 mm (Table 6). The antibacterial activities of the extract were compared with the positive controls, ciprofloxacin (5 mg/mL) and amoxicillin (5 mg/mL).

The n-hexane fraction exhibited little or no antibacterial activity against the test organisms, with inhibition observed only at higher concentrations. In contrast, the aqueous fraction demonstrated the greatest antibacterial activity among the solvent fractions, producing inhibition zones ranging from 5.00 ± 1.00 mm to 19.50 ± 0.50 mm against *E. coli*, *P. aeruginosa*, and *S. bongori*. The ethyl acetate fraction also exhibited antibacterial activity, with the highest inhibition recorded against *E. coli*, ranging from 9.00 ± 1.00 mm to 17.50 ± 0.50 mm (Table 7).

Table 6: Mean zones of inhibition of methanol extract of *Enantia chlorantha* stem bark against bacterial isolates

Organisms	50	100	150	200	250	300	Cipro 5 mg/mL	Amox 5 mg/mL
E. coli	5.35±0.65b	10.50±0.50c	11.75±0.25c	14.80±0.20d	15.00±0.10d	15.90±0.10d	18.55±1.12e	3.10±0.23a
P. aeruginosa	-	-	-	9.80±0.50a	10.00±0.10a	11.90±0.60a	24.35±1.50d	12.75±0.22c
S. bongori	-	-	4.50±0.50a	6.00±1.00b	8.55±1.55b	12.50±0.00c	23.01±0.20e	7.30±1.11b

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n=3, - = No activity

Table 7: Mean zones of inhibition of n-hexane, ethyl acetate and aqueous fraction extract of *Enantia chlorantha* stem bark against bacterial isolates

Organisms	H150	H250	H300	A150	A250	A300	E150	E250	E300
E. coli	$3.25±0.75^{a}$	$6.65±1.05^{bc}$	$9.85±0.15^{d}$	$5.55±0.45^{b}$	$7.00±0.00^{c}$	$15.50±45.50^{e}$	$5.40±1.05^{b}$	$6.40±0.40^{bc}$	$10.05±0.05^{d}$
P. aeruginosa		_	_	_	$8.55±1.55^{a}$	$11.75±0.75^{b}$	$9.50±0.50^{a}$	$17.00±1.00^{c}$	$21.90±0.10^{d}$
S. bongori		_	_	$9.10±1.70^{c}$	$10.85±1.85^{d}$	$12.50±1.50^{e}$	$5.50±0.00^{b}$	$6.05±0.00^{b}$	$11.75±0.25^{d}$

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n=3, - = No activity. H: n-Hexane fraction, A: Residual aqueous fraction, E: Ethyl acetate fraction

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Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Methanol extract and fractions of *Enantia chlorantha* stem bark

The result of the antimicrobial activities of the extracts as determined by measuring the minimum inhibitory concentrations (MIC) of methanol extract and fractions of *E. chlorantha* stem bark are presented in Figure 2 to 5 respectively. In present study, MIC values ranged between 9.38 - 150 mg/mL and most of the cases were in correlation with the antibacterial activity determined by inhibition zone formation.

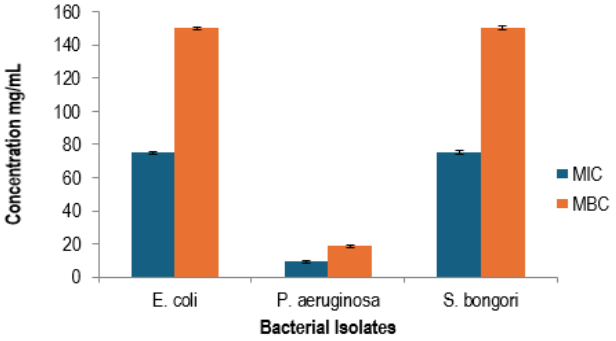

Figure 2: MIC and MBC of methanol crude extract of *Enantia chlorantha* stem bark
Bar of same color with the same alphabet have no significant difference at $p < 0.05$

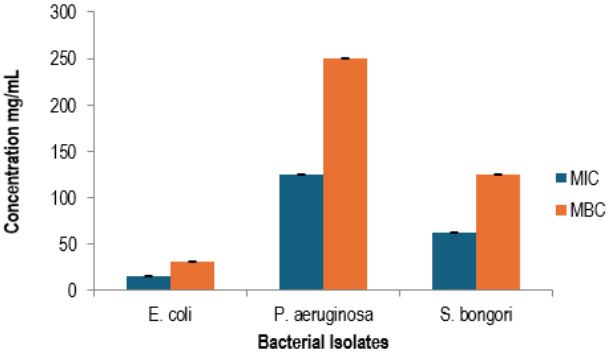

Figure 3: MIC and MBC of n-Hexane fraction of *Enantia chlorantha* stem bark extract
Bars of the same colour with the same alphabet have no significant difference at $p < 0.05$

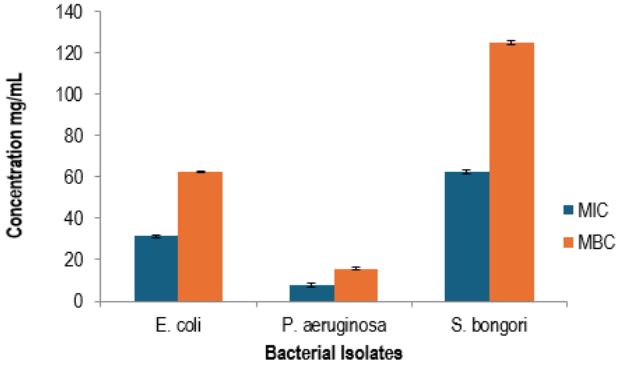

Figure 4: MIC and MBC of ethyl acetate fraction of *Enantia chlorantha* stem bark extract
Bars of the same colour with the same alphabet have no significant difference at $p < 0.05$

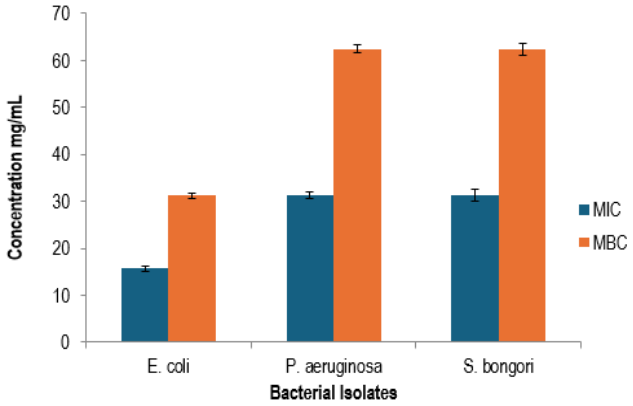

Figure 5: MIC and MBC of aqueous fraction of *Enantia chlorantha* stem bark extract
Bars of the same colour with the same alphabet have no significant difference at $p < 0.05$

Quantitative Phytochemical Analysis

Quantitative analysis of the phytochemical content revealed phenol to be in high concentration in the methanol extract of *E. chlorantha* stem bark. The concentrations of phenol, saponin, tannins and flavonoids were observed to be 416.07 ± 0.07, 117.86 ± 0.22, 26.45 ± 0.17 and 26.09 ± 0.05 (Table 8).

Table 8: Quantitative phytochemical contents of methanol extract of *Enantia chlorantha* stem bark

Metabolites	MEECS (mg/100g)
Total Phenols	416.07±0.07
Total Saponins	117.86±0.22
Total Tannins	26.45±0.17
Total Flavonoids	26.09±0.05

MEBDS: Methanol Extracts of *Enantia chlorantha* Stem Bark

DISCUSSION

The isolates were characterized using morphological characteristics (Table 1 and 2). The bacterial isolate grew slowly on nutrient agar; visible growth was observed after two days. The isolate grew at an optimum temperature of 30ºC. The microbial world is characterized by an incredible metabolic and physiological versatility that permits microorganisms to inhabit hostile ecological niches and to exploit, as carbon and energy sources, compounds unpalatable for higher organisms (Baylis *et al*., 2013). Isolates in this study displayed the typical morphological characteristics for *Escherichia coli, Pseudomonas aeruginosa and Salmonella bongori*. New strains of *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella bongori* arise all the time from the natural biological process of genetic variability; hence, monitoring the levels of this contamination is important (Gulcin *et al*., 2006). The development of highly applicable nucleic acid-based diagnostic analysis has enabled the detection of different bacteria, since culture media isolation and biochemical reactions were used. Among these, PCR is a commonly used molecular method that provides rapid and reliable detection of pathogenic bacterial strains, showing the prevalence and distribution of these pathogens (Gulcin *et al*., 2006).

PCR amplification of the bacterial 16S *rRNA* gene using the universal primers 27F and 1525R produced amplicons of approximately 1,500 bp in all the bacterial isolates (Plate I). The purified PCR products were subsequently sequenced, and the resulting nucleotide sequences were analysed using the Basic Local Alignment Search Tool (BLAST**)** against sequences deposited in the NCBI GenBank database for species identification. The sequences showed high similarity with *Pseudomonas aeruginosa*, *Salmonella bongori*, and *Escherichia coli* and were assigned the GenBank accession numbers MK572634.1**,** MN623691.1, and CP074120.1**,** respectively (Table 3). Phylogenetic analysis based on the 16S *rRNA* gene sequences further confirmed the taxonomic identity of the isolates by clustering them with closely related reference strains retrieved from the NCBI GenBank database (Figure 1).

Although the methanol extract and fractions have potential for antibacterial activity against E. coli, P. aeruginosa, and S. bongori, the methanol extract of the plant and aqueous fraction seem to possess the most significant antibacterial activity compared to the ethyl acetate and n-hexane fractions. The ethyl acetate and n-Hexane fractions showed minimal antimicrobial activity against the isolates at 150, 250 and 300 mg/mL concentrations, while the methanol extract and aqueous fraction gave wider zones of inhibition.

The diameter zones of inhibition of bacterial growth at varying concentrations revealed the antibacterial activity of the methanolic crude extract of E. chlorantha bark (Table 6) and its various fractions (residual aqueous fraction and ethyl acetate fraction) (Table 7). The methanolic extract of E. chlorantha bark revealed significant antibacterial activity against E. coli, P. aeruginosa, and S. bongori with increasing concentration of the extract. The same pattern was observed for each of its fractions, except for *P. aeruginosa* and *S. bongori*, which were resistant to the n-hexane fraction. Even though all three test organisms are Gram-negative and thus possess an outer membrane that serves as an effective barrier in Gram-negative species (Tawyabur *et al.,* 2020), the test organisms were susceptible to the extract. In addition, since the zone of inhibition is equal to or greater than the standard, it shows that the test organisms are sensitive to the plant extracts. *E. coli* was the most susceptible bacterium; in contrast, P. aeruginosa was observed to have minimal activity, which is attributed to the fact that *P. aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and trans envelope multidrug resistance pumps (MDRs) (Thuong *et al*., 2013). The extract has significant antimicrobial activity, meaning that it could be used to fight against bacterial infections effectively. The activity could be due to the different classes of phytochemicals found, their quantities, and the possible interactions with other constituents of the extract.

The studied plants were most active against all the bacteria tested. The significant antibacterial activity of the active plant extracts was comparable to the standard antibiotics Amoxicillin and Ciprofloxacin. The variation in susceptibility of the tested microorganisms could be attributed to intrinsic properties related to the permeability of their cell surface to the extracts. The present results therefore offer a scientific basis for the traditional use of solvent extracts of the three plants as a possible source of new and effective herbal medicines to treat infections caused by multidrug-resistant strains of microorganisms.

In microbiology, large diameters of zones of inhibition correlate with small minimum inhibitory concentrations (MIC) (Tan *et al.,* 2010). The MIC is usually described as the lowest concentration of an antimicrobial agent that results in inhibition of visible growth (that is, colonies on an agar plate or turbidity in broth culture) under standard conditions (Atukpawu & Ozoh, 2014). From the results of this study, E. *chlorantha* stem bark possesses promising antimicrobial activities against the test organisms (Figures 2 to 5).
The basic quantitative measures of the in Vitro activity of antibiotics and plant extracts with antibacterial potential are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (Atukpawu & Ozoh, 2014). It was observed that the Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts (*E. chlorantha* stem bark) varied in most cases (Figures 2 to 5). The MIC values obtained for the entire test organisms ranged from 18.75 mg/mL to 300 mg/mL. The MBC (Figure 2 to 5) in most cases varied from the MIC, indicating a different concentration needed to inhibit the growth of the microorganisms and an entirely different one to kill them.
In the present study, quantitative phytochemical analysis of methanol extract of *E. chlorantha* stem bark showed the presence of flavonoids, tannins, phenols and saponins in all samples tested (Table 9). Moses *et al*. (2019) reported that phenols, tannins, saponins, flavonoids and alkaloids were known to show medicinal activity as well as exhibiting physiological activity. Phenolic compounds are commonly found in plants, including *E. chlorantha* stem and have been reported to show a wide range of biological activities, including antibacterial activity (Moses *et al.,* 2019; Ismaila & Clement, 2017).
Total flavonoids of the methanol extract of *E. chlorantha* stem bark are 26.09 mg/100g. Soad *et al.* (2016) stated that flavonoids are probably the most important natural phenols due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants and as potential therapeutic agents against a wide variety of diseases. They show anti-allergic, anti-microbial and anti-cancer activity, which can be used for different diseases that are generally found in the human body. It has been reported that the presence of phytoconstituents such as flavonoids, tannins and polyphenols prevents several diseases through their free radical scavenging activity (Kokilam & Vasuki, 2014), and these phenolic compounds, which include phenols, tannins and flavonoids, have been found in appreciable amounts in *E. chlorantha* stem bark.
The presence of tannin is also important, as it forms irreversible complexes with proline-rich protein, which results in protein synthesis inhibition (Kindo *et al.,* 2016). Akhlash and Sunil (2019) also reported that tannins react with proteins to provide a tanning effect that helps in the treatment of inflamed ulcerated tissues. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and useful in the treatment of intestinal disorders like diarrhea and dysentery, wounds, sprains, bruises and arresting bleeding (Lyczak *et al*., 2014). Thus, it can be suggested from the above that *E. chloranta* stems are a source of bioactive compounds that could affect the treatment of antimicrobial infections.
Saponins from plants have long been employed for their detergent properties. They are used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins (Moses *et al*., 2019).

Conclusion

This study demonstrated that the methanol extract and solvent fractions of *Enantia chlorantha* stem bark possess notable antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori*. Molecular characterization confirmed the identity of the test organisms, while antibacterial assays revealed that the methanol extract and aqueous fraction exhibited the highest inhibitory effects. The extract also showed promising MIC and MBC values, indicating both growth-inhibitory and bactericidal potentials. Quantitative phytochemical analysis revealed high levels of phenols, along with appreciable amounts of saponins, tannins, and flavonoids, which may account for the observed antibacterial activity. Overall, the findings provide scientific evidence supporting the traditional medicinal use of *E. chlorantha* and highlight its potential as a source of bioactive compounds for the development of alternative antimicrobial agents.

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