Volume 11, Issue 4, April – 2026 International Journal of Innovative Science and Research Technology
ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26apr280

# Molecular Characterization, *In-vitro* and *In-silico* Studies of *Garcinia kola* Ethanol Stem Bark Extract Against Gastrointestinal Pathogens: *E. coli*, *Salmonella typhimurium* and *Shigella dysenteria*

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Publication Date: 2026/04/11

**Abstract: Gastrointestinal infections caused by multidrug-resistant bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Shigella dysenteriae* represent a growing public health challenge, highlighting the need for alternative therapeutic agents. This study aimed to evaluate the phytochemical composition, antibacterial potential, and in-silico binding interactions of *Garcinia kola* ethanol stem bark extract against these pathogens. The objectives were to identify key bioactive compounds, determine antibacterial efficacy, and assess molecular interactions with target bacterial proteins. Phytochemical screening was performed, and thin-layer chromatography was used to profile the chemical constituents. Bacterial isolates were characterized using cultural, biochemical, and 16S rRNA molecular methods. Antibacterial activity was assessed via zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, while molecular docking studies evaluated binding affinities of extract compounds against bacterial protein targets. Results revealed the presence of saponins, anthraquinones, phlobatannins, tannins, cardiac glycosides, and phenols, with multiple compounds detected via TLC. The extract exhibited antibacterial activity with inhibition zones of 19.00 ± 1.00 mm to 35.00 ± 0.00 mm at 500 mg/ml, MIC values of 0.07 ± 0.01 to 0.45 ± 0.05 mg/ml, and MBC of 250–500 mg/ml. Molecular docking indicated strong binding of hesperidin, rutin, and ellagic acid (-5.7 to -9.7 kcal/mol) to bacterial proteins. In conclusion, *Garcinia kola* ethanol stem bark extract exhibits potent antibacterial activity and promising in-silico interactions, supporting its potential as a natural therapeutic agent. It is recommended that further in-vivo studies and formulation development be undertaken to explore clinical applications.**

***Keywords:*** *Garcinia kola, Ethanol Extract, Gastrointestinal Pathogens, Antibacterial Activity, Phytochemicals, Molecular Docking*

**How to Cite:** Gladys O. Anyanwu; Joseph C. Uwaezuoke; Kenneth A. Yongabi; Chinyere N. Ohalete; Pauline O. Nnagbo; Maximus C. Korie (2026) Molecular Characterization, *In-vitro* and *In-silico* Studies of *Garcinia kola* Ethanol Stem Bark Extract Against Gastrointestinal Pathogens: *E. coli*, *Salmonella typhimurium* and *Shigella dysenteria*. *International Journal of Innovative Science and Research Technology*, 11(4), 238-249. https://doi.org/10.38124/ijisrt/26apr280

## I. INTRODUCTION

Gastrointestinal infections caused by pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Shigella dysenteriae* remain a significant global health challenge, particularly in developing regions with limited access to clean water and healthcare facilities. These pathogens are responsible for a wide range of illnesses, including diarrhea, dysentery, and foodborne infections, contributing substantially to morbidity and mortality. The increasing prevalence of antimicrobial resistance among these organisms has further reduced the effectiveness of conventional antibiotics (Dogara *et al.*, 2022). This growing resistance necessitates the exploration of alternative therapeutic agents, especially those derived from natural sources (Abhadionmhen *et al.*, 2023).

Medicinal plants have long served as reservoirs of bioactive compounds with diverse pharmacological properties. *Garcinia kola* (bitter kola), widely used in traditional African medicine, is known for its antimicrobial, anti-inflammatory, and antioxidant activities. Its rich phytochemical composition, including flavonoids, tannins, alkaloids, and phenolic compounds, underpins these biological effects. Several studies have reported the antibacterial potential of *Garcinia kola* extracts against both Gram-positive and Gram-negative bacteria, validating its traditional use in managing infections (Alsalamah, 2022). These findings highlight its relevance as a promising candidate in the search for new antimicrobial agents (Okoronkwo, 2022).

The ethanol extract of *Garcinia kola* stem bark is particularly significant due to its ability to extract a wide range of bioactive constituents with potential therapeutic value. Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical technique used to identify and characterize these compounds, including fatty acids, esters, and phenolic derivatives. The identification of such phytochemicals provides insight into the mechanisms responsible for the antimicrobial activity of the plant. This analytical approach also facilitates the discovery of novel compounds that may serve as leads in drug development (Rufa'i *et al*., 2023). Advances in GC-MS applications have further strengthened its role in phytochemical research and antimicrobial screening (Okoye & Eze, 2023).

The integration of in vitro and in silico approaches has become increasingly important in modern drug discovery. In vitro assays provide direct evidence of antimicrobial activity, while in silico techniques such as molecular docking help predict interactions between bioactive compounds and microbial targets at the molecular level. This combined strategy enhances the reliability and depth of analysis in evaluating plant-based therapeutics. However, limited studies have focused on the stem bark of *Garcinia kola* using this integrated approach against key gastrointestinal pathogens. Therefore, this study aims to characterize the ethanol stem bark extract using GC-MS, evaluate its antibacterial activity, and explore its molecular interactions through computational analysis (Dogara *et al*., 2022; Rufa'i *et al*., 2023).

## II. MATERIALS AND METHODS

➢ *Study Area*

This study was conducted in Imo State, Nigeria. The clinical samples were collected from Federal Teaching Hospital, Owerri, Imo State. Imo State is a state in the South-East geopolitical zone of Nigeria, bordered to the north by Anambra State, Rivers State to the west and south, and Abia State to the East.

➢ *Sample Collection*

- *Collection of Specimen*

Thirty (30) biopsies of mucosa from the gastric antrum of patients who have diagnosed as having peptic ulcer (gastric and duodenal) using endoscopic examination at the endoscopic units of Federal Medical Centre, Owerri by specialized surgeons (Abubakar *et al*, 2020).

- *Collection of Plant Material*

*Garcinia kola* were gotten from *Garcinia kola* plant tree at Umuduru, Umunoha, Mbaitoli Local Government Area of Imo State. The plant parts were identified by a plant Taxonomist Prof. C.M. Duru and were taken to the laboratory for further processing (Mordi 2020).

- *Isolation of Gastrointestinal Tract Organisms from the Clinical Samples*

The method described by Abubakar *et al*, (2020) was adopted in the isolation of gastrointestinal tract organisms from the clinical samples. Biopsy samples were homogenized with a glass rod in the same tube in a laminar flow hood. Three loops of this homogenized sample were inoculated in a ready-to-use selective medium eosin methylene blue agar, MacConkey agar and nutrient agar and the plates were incubated at 37 °C for 24 – 48 hours.

- *Colonial Morphology Identification*

The method described by Bale *et al*. (2021) was adopted in the colonial morphology identification. Presumptive identification of the colonies was done by observing their individual shape, colour, elevation, edge, surface, consistency, and appearance on the media used for isolation. Colonies with characteristic metallic sheen on Eosin methylene blue agar (EMB) agar and lactose fermenters on MacConkey agar was noted.

- *Biochemical Tests with the Bacterial Isolates*

The method described by Bale *et al*. (2021) was adopted in the biochemical characterization of the bacterial isolates. The biochemical tests that were carried out included gram staining, catalase, oxidase, coagulase, citrate utilization, indole production, sugar fermentation and motility test.

- *Molecular Identification of the Bacterial Isolates*

The molecular identification of the bacterial isolates is still ongoing, and the results were carried out.

- *DNA Extraction using ZR Bacterial DNA MINIPREP*

Two milliliters (2 ml) of the bacterial cells were added to the broth in a ZR Bashing$^{TM}$ lysis tube and 750 µl lysis solution was added to the tube. It was fitted in a bead with 2 ml tube holder assembly and processed at maximum speed for >5 minutes. Thereafter, the tube was centrifuged in a microcentrifuge at > 10, 000 x g for 1 minute. Later, 400 µl supernatant to a Zymo-Spin$^{TM}$ IV Spin filter (orange top) in a collection tube and centrifuged at 7, 000 x g for 1 minute. Another 1, 200 µl of bacterial DNA binding buffer was added to the filtrate in the collection tube.

Later, 800 µl of the mixture was transferred to Zymo-Spin$^{TM}$ IIC column in a collection tube and centrifuged at 10, 000 x g for 1 minute. The flow through was discarded from the collection tube and repeated. Thereafter, 200 µl DNA Pre-Wash buffer was added to the Zymo-Spin$^{TM}$ IIC column to a clean 1.5 ml microcentrifuge tube and 100 µl was added (35 µl minimum) DNA elution buffer tube directly to the column matrix. It was centrifuged at 10, 000 x g for 30 seconds to elute the DNA (Abdullah & Hassoni, 2024).

- *Electrophoresis for DNA and PCR*

One gram (1 g) of agarose (for DNA), 2 g of agarose for PCR were weighed and mixed with 100 ml 1 x TAE in a microwable flask. The agarose powder was microwave for 3 minutes until the agarose completely dissolved. The agarose was cooled to 50 $^0$C. After cooling, 10 µl EZ vision DNA stain which binds to the DNA and allows visualization under ultraviolet light. The agarose was poured into a gel tray with well comb in place. The poured gel was placed at 4 $^0$C for 15 minutes. After solidification and loading the buffer, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was filled with 1 x TAE until the gel is covered. A molecular weight ladder was carefully loaded into the first lane of the gel. The samples were carefully loaded into the additional wells of the gel. The gel was run at 80 – 150 V for 1 hour. Thereafter, the power was turned off and the cables were disconnected from the electrodes. The DNA fragments/PCR product under UV transilluminator was visualized (Lam, K.J. & Clelland 2024).

- *Gene Sequencing*

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis (Lam, K.J. & Clelland 2024).

- *Preparation and Extraction of Plant Materials*

The stem barks were dried and ground to powder with a laboratory blender. Fifty grams (50g) of grind plant materials were weighed using a digital weighing balance and transferred into 500ml conical flasks containing 400 mls of ethanol in a ratio of 2:5 (w/v). The conical flask was stirred by vigorous shaking for 60 minutes on a mechanical shaker and allowed to stand for 24hr. The organic solvent extracts were filtered using Ash less No.42 filter paper and the filtrate air-dried to powder (Ajiboye *et al* 2020, Ezeigbo *et al*., 2016).

- *Determination of the Phytochemical Constituents of the Plant Extracts*

The method described by Bukar *et al*. (2019) was adopted in the determination of the qualitative phytochemical screening of the extracts. The phytochemicals that were determined include saponins, tannins, alkaloids, anthraquinones, cardiac glycosides, phenols, and phlobatannins.

- *Thin Layer Chromatography*

Pre-coated silica gel 60 F254 TLC plates were used as the stationary phase. Each plate was cut into dimensions of 5 cm × 10 cm and a faint line was drawn with a pencil 1.5 cm from the bottom margin to serve as the baseline for sample application, following the method described by Oduro *et al.* (2020). Using fine capillary tubes, small concentrated spots of the extract was applied gently on the baseline of the TLC plates. The spots were air-dried to ensure they remained tight and did not spread before development. Each extract was spotted at equidistant points and labeled accordingly.

- *Determination of Retention Factor (Rf) Values*

The distance traveled by each compound spot and the solvent front was measured in centimeters. This allowed for the comparative analysis of phytochemical components across the four extracts (Lawal & Ojo, 2022).

Retention factor (Rf) for each spot was calculated using the formula:

$$Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$

➢ *Antibacterial Screening*

- *Preparation of Test Organisms*

Pure cultures of test organisms for standardization were sub-cultured on nutrient agar and nutrient broth at 37 $^o$C for 24 hours. Organisms were also grown on a slant for preservation (Gotep *et al*., 2009). Test isolates were standardized by McFarland method. McFarland solution consists of Barium Chloride and Sulphuric Acid (Cheesbrough, 2010).

- *Antibacterial Susceptibility Test/Antibiogram using Plant Extracts*

Susceptibility of the test isolates to the extract was done by well-in-agar diffusion assay. Four wells of 6.25 mm deep were made with a sterile cork borer on Mueller-Hinton agar previously seeded with the 24hr old, standardized cultures. The wells were filled with different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml) of the ethanol and aqueous extracts separately. The concentrations were obtained using two-fold dilution. The plates were incubated for 24 hours at 37 $^0$C. After 24 hours, zone of inhibition around the wells were measured and recorded in millimeters (mm). This process was repeated with the other organisms (Zhang *et al*, 2024, Peter *et al*, 2022, Uwimbabazi *et al*, 2015).

- *Minimum Inhibitory Concentration (MIC) Assay*

The Minimum Inhibitory Concentration Assay is a technique used to determine the lowest concentration of a particular antibiotic needed to kill an organism. This assay is typically performed on planktonic (free floating) bacterial cells. To evaluate MIC, the procedure was done according to Atlas *et al.* (1995). Serial dilutions of the extracts (representing different concentrations of 500 mgml$^{-1}$, 250 mgml$^{-1}$, 1.25 mgml$^{-1}$ and 62.5 mgml$^{-1}$) was added to a growth medium (nutrient broth) in separate test tubes. These tubes were then inoculated with the standardized test isolates. The tubes were incubated overnight. Broth tubes that appeared turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. The MIC of the antibiotic/toxicant (plant extract) is the lowest concentration that does NOT show growth. This was confirmed using the spectrophotometer at 420 nm.

- *Minimum Bactericidal Concentration (MBC) Assay*

Minimum bactericidal concentration is the lowest number of bacteria recorded on the plate after 24 h incubation on nutrient agar. A loopful of the different concentrations (after spectrophotometric reading) were streaked on a freshly prepared surface dried nutrient agar and incubated overnight. Concentrations of growth after incubation were used to determine the MBC.

- *Antibiotic Susceptibility/Sensitivity Test using Commercial (Oxoid) Antibiotics*

Commercial antibiotics (oxoid) of known concentrations were placed at equidistant on freshly prepared and surfaced dried Mueller-Hinton agar (MHA) previously seeded with standardized pure cultures of test organisms and incubated at 37 $^0$C for 24-48 h. Zone of inhibition (ZOI) was measured and recorded after incubation. The resistance, sensitivity and intermediate activities of the organisms were compared with the Clinical and Laboratory Standards Institute (CLSI).

➢ *In-silico studies (Molecular Docking)*

- *Ligand and Standard Drug Preparations*

The structural data files (SDF) of the various bioactive compounds identified in the GC-MS analysis and reference drugs (conventional antibiotics) were obtained from PubChem web-platform (https://www.ncbi.nlm.nih.gov/pccompound) in 3D conformation (Klim *et al*., 2023). The compounds whose structural files were not found in the chemoinformatics databases were drawn using ChemDraw Ultra 12.0, saved in mole files and further converted into structural data files (SDF) by deploying Openbabel GUI software version 2.3.2.

- *Protein Targets Selection and Preparation*

The three-dimensional (3D) crystallographic structures of the co-crystallized protein targets; *E. coli* (PDB: 4XO8), *Salmonella typhimurium* (PDB: 5V2W) and *Shigella dysenteriae* (PDB: 2RG7) were retrieved from the Protein Database (PDB) (www.pdb.org/pdb), the protein were prepared for docking through the removal of the co-crystallized ligand and water molecules to produce a nascent receptor, polar hydrogen's were added and the receptor sites were identified using Biovia discovery studio v.24.1.0.23298.

- *Molecular Docking (Pharmacodynamics Properties)*

Virtual screening of the ligands was carried out using PyRx-Python Prescription 0.8, a suite comprising of automated molecular docking tools (Auto dock tools, Auto Dock Vina and Openbabel) (Dallakyan and Olson, 2015). The PDBQT file of the ligands and protein were generated through this software. The specific target sites of the target proteins were set with the help of grid box (X: -18.405, Y: -6.0595, Z: 7.5325 for 4XO8; X: 52.1661, Y: 1.0466, Z: 17.6103 for 5V2W; X: 170208, Y: 9.3790, Z: -22.8236 for 2RG7). The configurations for each protein-ligand complex were generated for all the ligands using the software; text files of scoring results (binding affinities of the ligands to the target protein) were also produced for the purpose of manual comparative analysis at the end of the experiment (Trott and Olson, 2010).

## III. RESULTS

Table 1 Phytochemical Constituents of Ethanol Stem Bark Extracts of *Garcinia kola*.

| **Phytochemical** | **Remark** |
|---|---|
| Saponins | + |
| Anthranoids | - |
| Anthraquinones | + |
| Alkaloids | - |
| Phlobatannins | + |
| Tannins | + |
| Cardiac glycosides | + |
| Phenols | + |

Key:

- = Absence of phytochemicals

+ = Presence of phytochemicals

Table 2 Thin Layer Chromatographic Result of Ethanol Stem Bark Extracts of *Garcinia kola*

| **Sample** | **Spot** | **Observed Color** | **Distance Travelled by Compound (cm)** | **Distance Travelled by Solvent Front (cm)** | **Rf Value** |
|---|---|---|---|---|---|
| Stem bark | A | Light brown | 3.50 | 6.50 | 0.54 |
| | B | Dark brown | 5.50 | 6.50 | 0.85 |

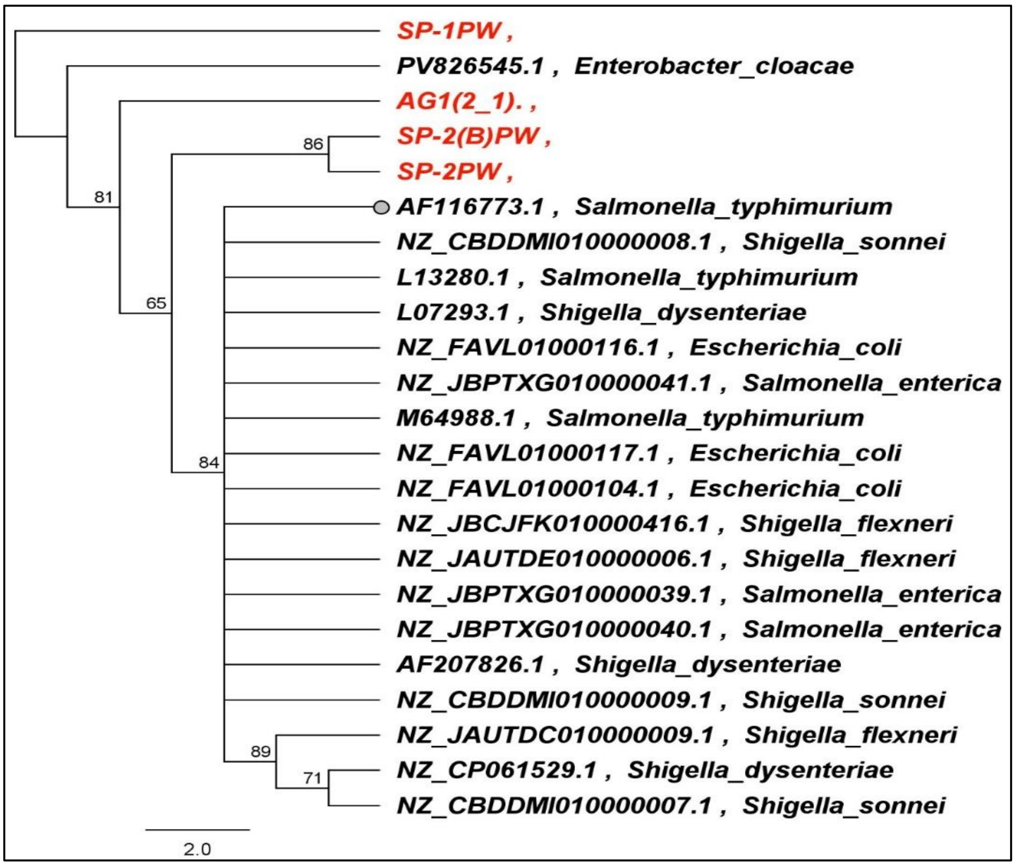


Fig 1 The phylogenetic tree showing the evolutionary relationships among *Salmonella enterica*, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei*, *Escherichia coli* and the four query isolates under study (SP-1PW, SP-2(B)PW, SP-2PW, and AG1).

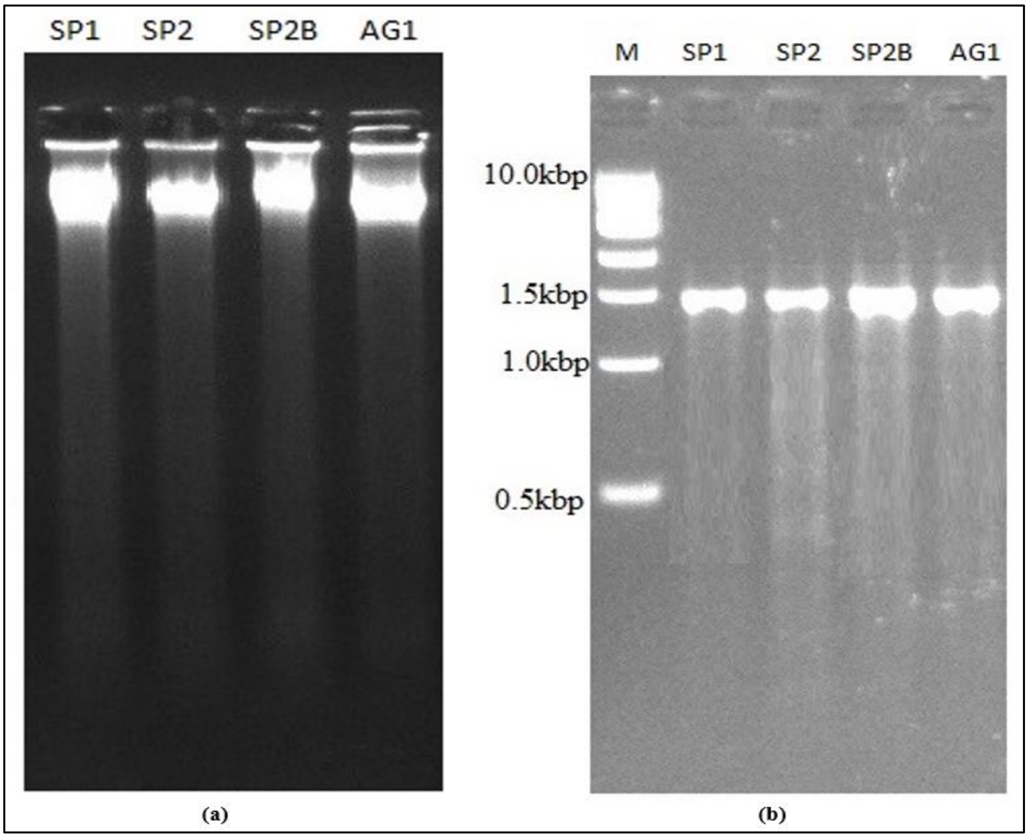
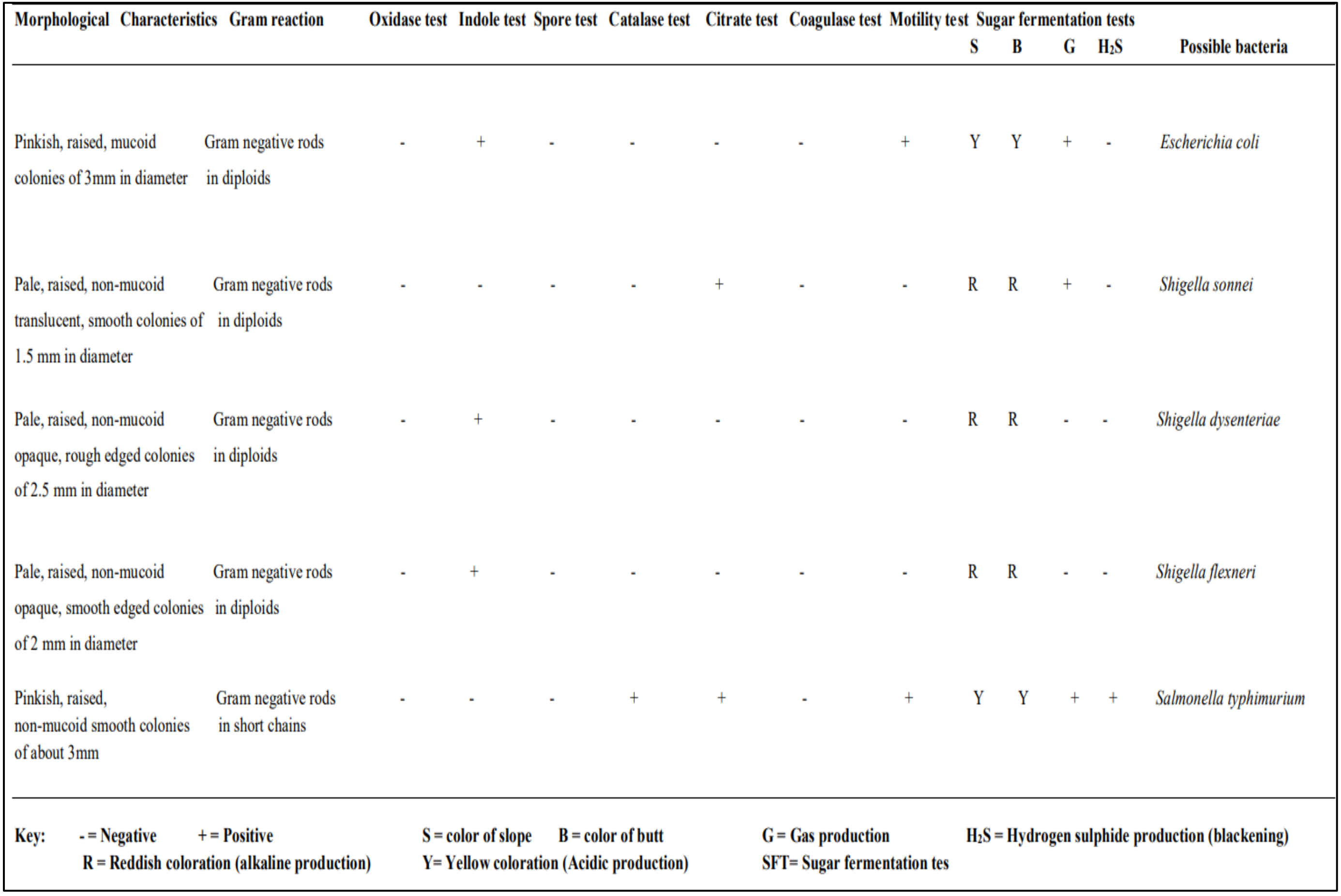


Fig 2 (a) Gel Image showing High Molecular Weight DNA Extracted from the Isolates (b) Gel Image Showing the Amplification of the 16SrRNA Gene at 1500bp. Lane M is 1kbp DNA Ladder from NEB

Table 3 Cultural Morphology and Biochemical Characteristics of the Bacterial Isolates from the Gastrointestinal Tract



| Morphological Characteristics | Gram reaction | Oxidase test | Indole test | Spore test | Catalase test | Citrate test | Coagulase test | Motility test | Sugar fermentation tests | | | | Possible bacteria |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | S | B | G | $H_2S$ | |
| Pinkish, raised, mucoid colonies of 3mm in diameter | Gram negative rods in diploids | - | + | - | - | - | - | + | Y | Y | + | - | *Escherichia coli* |
| Pale, raised, non-mucoid translucent, smooth colonies of 1.5 mm in diameter | Gram negative rods in diploids | - | - | - | - | + | - | - | R | R | + | - | *Shigella sonnei* |
| Pale, raised, non-mucoid opaque, rough edged colonies of 2.5 mm in diameter | Gram negative rods in diploids | - | + | - | - | - | - | - | R | R | - | - | *Shigella dysenteriae* |
| Pale, raised, non-mucoid opaque, smooth edged colonies of 2 mm in diameter | Gram negative rods in diploids | - | + | - | - | - | - | - | R | R | - | - | *Shigella flexneri* |
| Pinkish, raised, non-mucoid smooth colonies of about 3mm | Gram negative rods in short chains | - | - | - | + | + | - | + | Y | Y | + | + | *Salmonella typhimurium* |

**Key:** **- = Negative** **+ = Positive** **S = color of slope** **B = color of butt** **G = Gas production** **$H_2S$ = Hydrogen sulphide production (blackening)**
**R = Reddish coloration (alkaline production)** **Y= Yellow coloration (Acidic production)** **SFT= Sugar fermentation tes**

Table 4 Sensitivity Profile of Commercial Antibiotics Against the Isolates

| **Bacterial Isolate** | **C (30 µg)** | **AK (30µg)** | **RD (5 µg)** | **AMC (30µg)** | **LEV (5µg)** | **TE (10µg)** | **CN (10µg)** | **F (100 µg)** | **NA (30µg)** | **CIP (5 µg)** | **SXT (5µg)** | **TZP (36µg)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SAE | 16.50 ± 2.12 | 31.50 ± 2.12 | 0.00 ± 0.00 | 0.00 ± 0.00 | 31.50 ± 0.71 | 15.00 ± 0.00 | 31.00 ± 1.41 | 0.00 ± 0.00 | 20.00 ± 0.00 | 33.00 ± 1.41 | 0.00 ± 0.00 | 28.00 ± 0.00 |
| SAT | 0.00 ± 0.00 | 21.00 ± 1.41 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 20.00 ± 2.83 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 10.00 ± 0.00 |
| SHF | 12.00 ± 2.83 | 29.00 ± 1.41 | 0.00 ± 0.00 | 0.00 ± 0.00 | 33.50 ± 2.12 | 0.00 ± 0.00 | 30.00 ± 0.00 | 0.00 ± 0.00 | 25.00 ± 0.00 | 43.50 ± 2.12 | 16.50 ± 2.12 | 30.50 ± 0.71 |
| SHD | 23.00 ± 4.24 | 32.00 ± 2.83 | 11.00 ± 1.41 | 0.00 ± 0.00 | 29.00 ± 1.41 | 0.00 ± 0.00 | 29.50 ± 0.71 | 20.00 ± 0.00 | 6.50 ± 2.12 | 10.00 ± 0.00 | 0.00 ± 0.00 | 28.00 ± 0.00 |
| SHS | 0.00 ± 0.00 | 28.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 10.00 ± 0.00 | 0.00 ± 0.00 | 30.00 ± 0.00 | 0.00 ± 0.00 | 21.00 ± 1.41 | 0.00 ± 0.00 | 15.00 ± 0.00 | 28.00 ± 0.00 |
| ESC | 21.00 ± 1.41 | 25.00 ± 0.00 | 10.00 ± 0.00 | 0.00 ± 0.00 | 27.00 ± 1.41 | 13.00 ± 1.41 | 30.50 ± 4.95 | 21.00 ± 1.41 | 26.00 ± 0.00 | 15.00 ± 3.00 | 26.00 ± 2.83 | 23.50 ± 1.41 |

Values expressed as Mean± Standard deviation, n=2**,** SAE = *Salmonella enteric* SAT = *Salmonella typhimurium* SHF = *Shigella Flexner*, SHD = *Shigella dysenteriae,* SHS = *Shigella sonnei,* ESC = *Escherichia coli,* C= Chloroamphenicol, AK= Amikacin, RD= Rifampicin, AMC= Amoxicillin, LEV=Levofloxacin, TE=Tetracycline, CN= Gentamicin, F= Nitrofurantoin, NA= Nalidixic acid, CIP=Ciprofloxacin, SXT= Trimethoprim-sulfamethoxazole, TZP=Piperacilin-tazoba

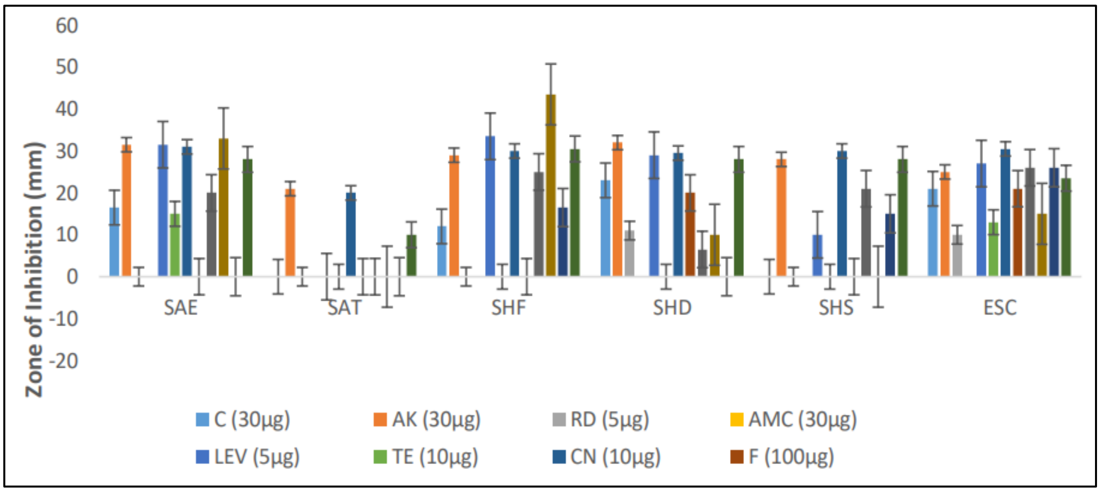


Fig 3 Sensitivity Profile of Commercial Antibiotics Against the Isolates

Table 5 Sensitivity and Zone of Inhibition of Ethanol Stem Bark Extract of *Garcinia kola* on the Isolates

| **Test Isolate** | **CIP (mm)** | **500 mg/ml** | **250 mg/l** | **125 mg/l** | **62.5 mg/l** |
|---|---|---|---|---|---|
| SAE | 22.50 ± 2.50 | 20.00 ± 0.00 | 12.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| SAT | 20.00 ± 0.00 | 23.50 ± 1.50 | 15.50 ± 0.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| SHF | 31.00 ± 1.00 | 35.00 ± 0.00 | 10.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| SHD | 43.50 ± 1.50 | 24.00 ± 2.00 | 18.00 ± 1.00 | 9.00 ± 1.00 | 0.00 ± 0.00 |
| SHS | 40.00 ± 0.00 | 19.00 ± 1.00 | 15.00 ± 0.00 | 11.00 ± 1.00 | 0.00 ± 0.00 |
| ESC | 24.50 ± 0.50 | 23.50 ± 1.50 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Values expressed as Mean± Standard deviation, n=2

CIP, Ciprofloxacin; mg/ml; milligram per milliliter

SAE = *Salmonella enteric* SAT = *Salmonella typhimurium*
SHF = *Shigella flexneri*

SHD = *Shigella dysenteriae* SHS = *Shigella sonnei*
ESC = *Escherichia coli*

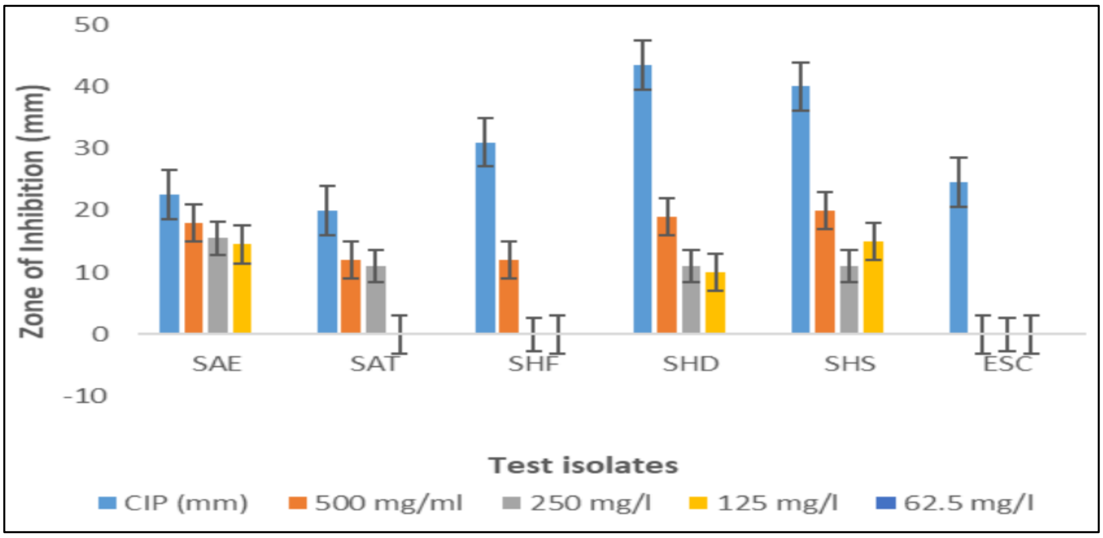


Fig 4 Sensitivity and Zone of Inhibition of Ethanol Stem Bark Extract of *Garcinia kola* on the Isolate

Table 6 Minimum Inhibitory Concentration (MIC) of Ethanol Stem Bark Extract of *Garcinia kola* on the Isolates (λ=420 nm)

| **Bacterial Isolate** | **500 mg/ml** | **250 mg/ml** | **125 mg/ml** | **62.5 mg/ml** |
|---|---|---|---|---|
| SAE | 0.16 ± 0.00 | 0.12 ± 0.01 | 0.24 ± 0.01 | 0.45 ± 0.05 |
| SAT | 0.12 ± 0.00 | 0.14 ± 0.01 | 0.16 ± 0.00 | 0.15 ± 0.01 |
| SHF | 0.13 ± 0.00 | 0.17 ± 0.01 | 0.20 ± 0.00 | 0.22 ± 0.01 |
| SHD | 0.15 ± 0.00 | 0.16 ± 0.01 | 0.24 ± 0.01 | 0.36 ± 0.01 |
| SHS | 0.13 ± 0.01 | 0.07 ± 0.01 | 0.36 ± 0.00 | 0.35 ± 0.00 |
| ESC | 0.13 ± 0.00 | 0.14 ± 0.00 | 0.12 ± 0.00 | 0.38 ± 0.00 |

Values expressed as Mean± Stanardard deviation, n=2 SHD = *Shigella dysenteriae* SHS = *Shigella sonnei* ESC = *Escherichia coli*

SAE = *Salmonella enteric* SAT = *Salmonella typhimurium*
SHF = *Shigella flexneri*

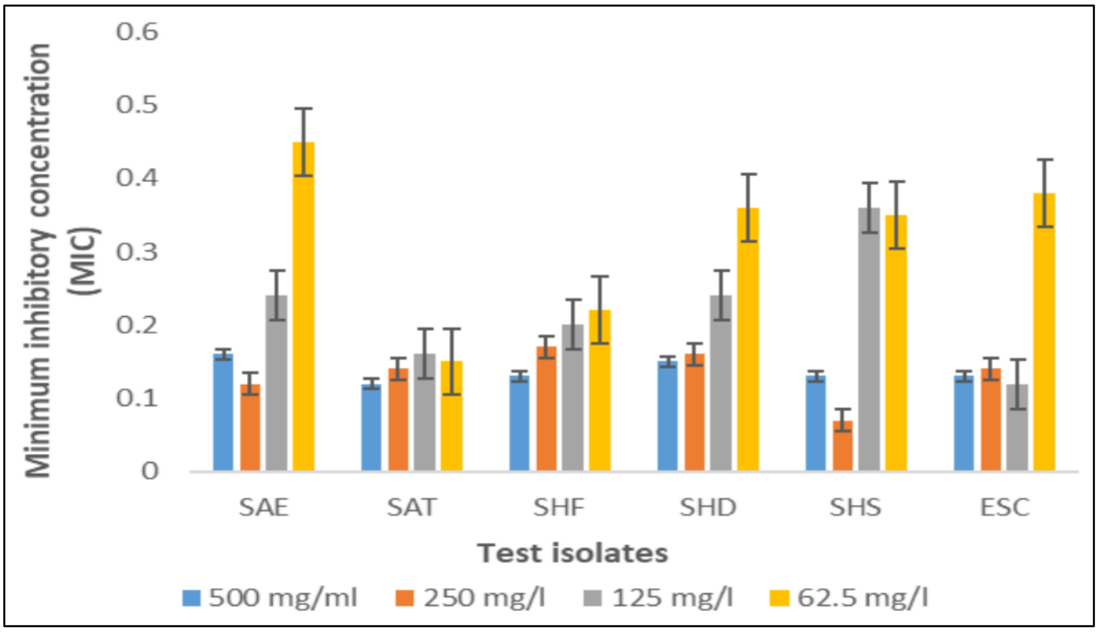


Fig 5 Minimum Inhibitory Concentration (MIC) of Ethanol Stem Bark Extract of *Garcinia kola* on the Isolates (λ=420 nm)

Table 7 Minimum Bactericidal Concentration (MBC) of Ethanol Stem Bark Extract of *Garcinia kola* on the Isolates

| **Bacterial Isolates** | **MBC (mg/ml)** |
|---|---|
| SAE | 500/250 |
| SAT | 500/250 |
| SHF | 500 |
| SHD | 500 |
| SHS | 500/250 |
| ESC | 500/250 |

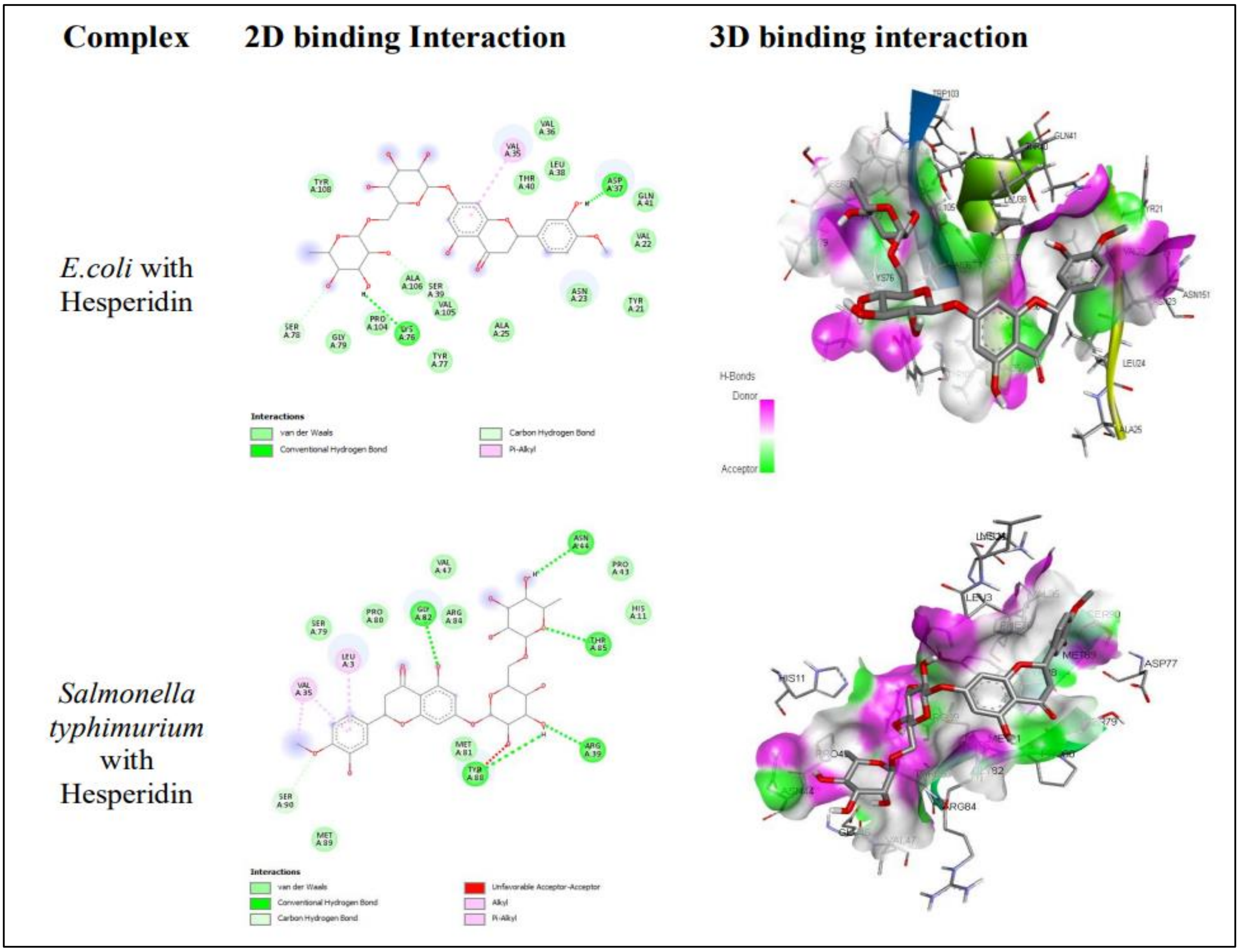
Key: SAE = *Salmonella enteric* SAT = *Salmonella typhimurium* SHF = *Shigella flexneri* SHD = *Shigella dysenteriae* SHS = *Shigella sonnei* ESC = *Escherichia coli*

Table 8 Binding Affinities of the Selected Conventional Antibiotics with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae*

| **Reference Drugs** | **PubChem CID** | **Binding Affinities (Kcal/mol)** | | |
|---|---|---|---|---|
| | | **4XO8** | **5V2W** | **2RG7** |
| Rifampicin | 135398735 | **-11.8** | **-11.8** | **-20.6** |
| Levofloxacin | 149096 | -5.9 | -6.2 | -8.2 |
| Ciprofloxacin | 2764 | -5.9 | -5.8 | -8.7 |
| Amoxicillin | 33613 | -5.8 | -5.8 | -7 |
| Gentamicin | 3467 | -5.4 | -5.9 | -7.3 |
| Trimethoprim-sulfamethoxazole | 358641 | -5.2 | -6.3 | -7.6 |
| Amikacin | 37768 | -4.7 | -5 | -6.9 |
| Piperacillin-tazobactam | 43672 | -6.5 | -6.7 | -8.8 |
| Nalidixic acid | 4421 | -5.2 | -5.2 | -7.6 |
| Tetracycline | 54675776 | -4.2 | -6.7 | -7 |
| Chloroamphenicol | 5959 | -5.2 | -5.5 | -6.9 |
| Nitrofurantoin | 6604200 | -5.5 | -6.1 | -7.6 |

Table 9 Binding Affinities of the Bioactive Compounds in *Garcinia kola* Ethanol Stem Bark Extract with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae*

| **Compounds** | **PubChem CID** | **Binding Affinities (Kcal/mol)** | | |
|---|---|---|---|---|
| | | **4XO8** | **5V2W** | **2RG7** |
| Hesperidin | 10621 | **-7.5** | **-7.8** | **-9.7** |
| 2-(3,4-dihydroxyphenyl) chroman-3,5,7-triol | 1203 | -5.5 | -6.1 | -7.8 |
| Chlorogenic acid | 1794427 | -6.1 | -6.2 | -8.2 |
| Eugenol | 3314 | -4.5 | -4.9 | -5.9 |
| Gallic acid | 370 | -5.1 | -5.3 | -6 |
| Naringenin | 439246 | -5.8 | -6.1 | -8.2 |
| Cinnamic acid | 444539 | -4.4 | -5 | -5.9 |
| Farnesol | 445070 | -4.9 | -5.2 | -6.6 |
| Ferulic acid | 445858 | -5.1 | -5 | -6.8 |
| Quercetin | 5280343 | -6.1 | -6.1 | -8.1 |
| Apigenin | 5280443 | -5.9 | -6.1 | -8 |
| Luteolin | 5280445 | -6 | -6.3 | -8.3 |
| Rutin | 5280805 | -6.3 | -6.4 | -8.9 |
| Kaempferol | 5280863 | -5.9 | -6.1 | -8.2 |
| Erucic acid | 5281116 | -4.1 | -4.4 | -5.3 |
| Fisetin | 5281614 | -5.8 | -5.8 | -8.4 |
| Ellagic acid | 5281855 | -5.7 | -6.2 | -9.3 |
| D-sorbitol | 5780 | -4.4 | -4.2 | -5.7 |
| P-coumaric acid | 637542 | -5 | -5 | -6.3 |
| Caffeic acid | 689043 | -5 | -5.1 | -6.6 |



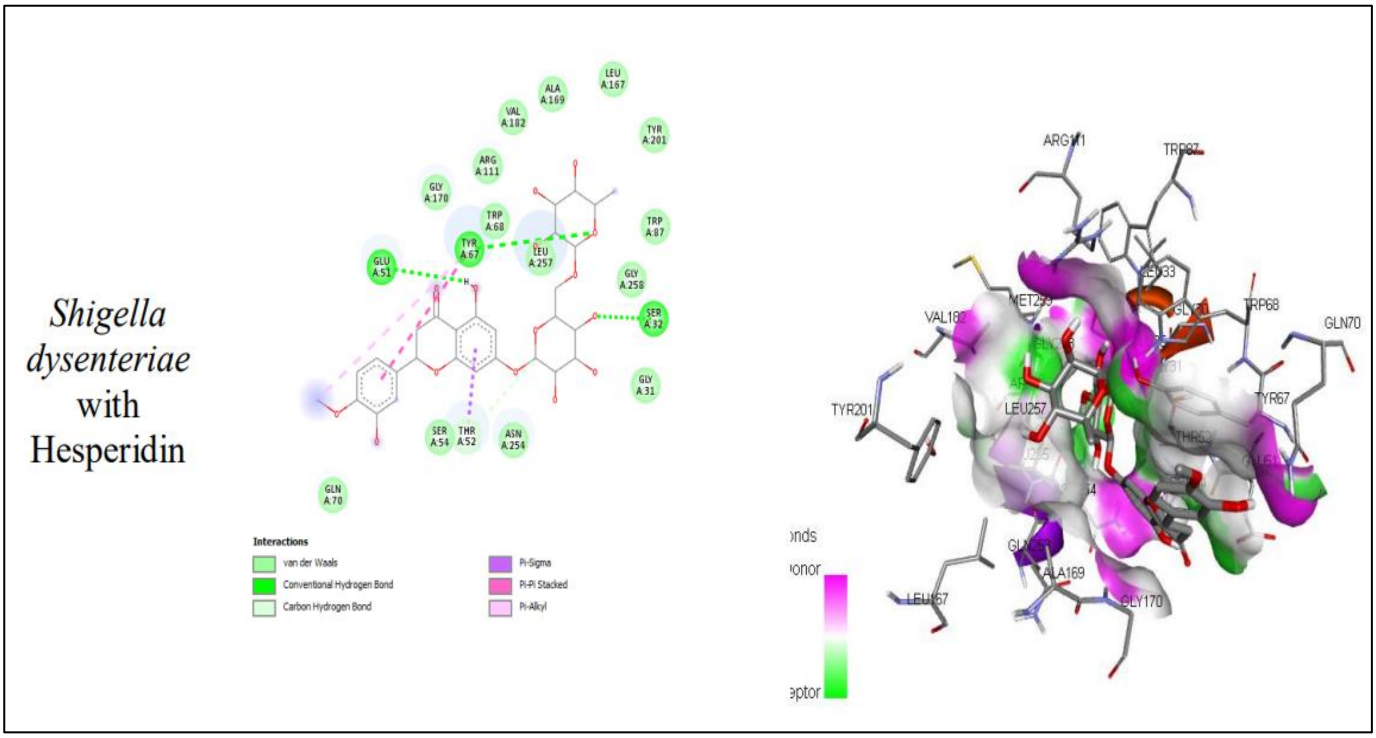


Fig 6 2D and 3D Binding Interactions of the Hit Bioactive Compounds in *Garcinia kola* Ethanol Stem Bark Extract with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteria*

## IV. DISCUSSION

The phytochemical constituents presented in Table 1 revealed the presence of saponins, anthraquinones, phlobatannins, tannins, cardiac glycosides, and phenols, while alkaloids and anthranoids were absent. These bioactive compounds are widely reported to contribute to antimicrobial activity through mechanisms such as protein precipitation, enzyme inhibition, and membrane disruption. The dominance of phenols and tannins in this study is consistent with findings by Ojo *et al*. (2022), who reported that phenolic-rich plant extracts exhibit strong antibacterial effects against enteric pathogens. However, the absence of alkaloids contrasts with the findings of Ibrahim and Abdullahi (2024), who detected alkaloids in *Garcinia kola* root extracts, suggesting that phytochemical composition varies depending on plant part and extraction conditions.

The chromatographic separation shown in Table 2 produced two distinct spots with Rf values of 0.54 and 0.85, indicating the presence of compounds with varying polarity. This confirms the heterogeneity of the ethanol extract and supports the presence of multiple bioactive constituents. Similar Rf ranges have been reported by Adebayo *et al*. (2023), who observed Rf values between 0.50 and 0.88 in TLC analysis of medicinal plant extracts, attributing such variation to differences in compound polarity and solvent systems. The presence of multiple bands further supports the complexity of plant-derived extracts and justifies advanced characterization techniques.

The molecular identification results illustrated in Figure 1 and Figure 2, alongside the biochemical characteristics in Table 3, confirmed the isolates as *Escherichia coli*, *Salmonella typhimurium*, and *Shigella* species. The amplification of the 16S rRNA gene at approximately 1500 bp is consistent with standard bacterial identification protocols. These findings agree with the work of Nwankwo *et al*. (2022), who reported similar molecular and biochemical profiles for gastrointestinal pathogens isolated from clinical samples. The Gram-negative rod morphology and biochemical reactions observed further validate the reliability of the identification process.

The antibiotic susceptibility profile presented in Table 4 and Figure 3 revealed high levels of resistance among the isolates. For instance, *Salmonella typhimurium* showed complete resistance (0.00 mm) to several antibiotics, while demonstrating moderate sensitivity to amikacin (21.00 ± 1.41 mm) and gentamicin (20.00 ± 2.83 mm). *Shigella flexneri* exhibited high susceptibility to ciprofloxacin (43.50 ± 2.12 mm) and levofloxacin (33.50 ± 2.12 mm). These findings are in line with reports by Adewumi *et al*. (2023), who documented increasing resistance of enteric bacteria to commonly used antibiotics, particularly beta-lactams and tetracyclines. Similarly, Eze *et al*. (2024) reported that fluoroquinolones remain among the most effective antibiotics against multidrug-resistant gastrointestinal pathogens.

The antibacterial activity of the ethanol stem bark extract shown in Table 5 and Figure 4 demonstrated significant inhibition at higher concentrations. At 500 mg/ml, zones of inhibition ranged from 19.00 ± 1.00 mm to 35.00 ± 0.00 mm, while at 250 mg/ml, values ranged from 10.00 ± 0.00 mm to 18.00 ± 1.00 mm. The absence of inhibition at 62.5 mg/ml indicates reduced efficacy at lower concentrations. Notably, the extract showed comparable activity to ciprofloxacin against *Salmonella typhimurium*

(23.50 ± 1.50 mm vs 20.00 ± 0.00 mm). These findings agree with Olalekan *et al*. (2022), who reported strong antibacterial activity of *Garcinia kola* extracts at concentrations above 200 mg/ml, attributing this effect to the synergistic action of phytochemicals.

The MIC values presented in Table 6 and Figure 5 further confirmed the antibacterial efficacy of the extract. For example, *Salmonella enterica* showed MIC values ranging from 0.16 ± 0.00 at 500 mg/ml to 0.45 ± 0.05 at 62.5 mg/ml, indicating decreased inhibition at lower concentrations. *Shigella sonnei* exhibited a notably low MIC value of 0.07 ± 0.01 at 250 mg/ml, suggesting high susceptibility. The MBC values in Table 7, which ranged between 250–500 mg/ml, indicate bactericidal activity at higher concentrations. These results are comparable to findings by Chukwu *et al*. (2023), who reported MIC values within similar ranges for plant extracts rich in phenolic compounds, emphasizing their strong antibacterial potential.

The molecular docking results in Table 8 revealed that conventional antibiotics such as rifampicin exhibited the highest binding affinities (-11.8 to -20.6 kcal/mol), followed by ciprofloxacin (-5.8 to -8.7 kcal/mol). The bioactive compounds identified in the extract (Table 9) showed promising binding affinities, with hesperidin exhibiting values of -7.5, -7.8, and -9.7 kcal/mol against the respective targets. Other compounds such as rutin (-6.3 to -8.9 kcal/mol) and ellagic acid (-5.7 to -9.3 kcal/mol) also demonstrated strong interactions, as illustrated in Figure 6. These findings are consistent with the study by Mensah *et al.* (2024), who reported that flavonoids and phenolic acids exhibit strong binding affinities with bacterial enzymes, contributing to their antimicrobial effects.

Comparatively, although the phytochemicals exhibited lower binding affinities than rifampicin, they were comparable to several conventional antibiotics such as tetracycline and amoxicillin. This suggests that the bioactive compounds possess significant inhibitory potential and may serve as alternative therapeutic agents. Similar observations were reported by Bello *et al*. (2022), who demonstrated that plant-derived flavonoids can exhibit binding affinities comparable to synthetic drugs. Overall, the combined findings from Tables 5–9 and Figures 3–6 confirm that *Garcinia kola* ethanol stem bark extract possesses significant antibacterial activity, supported by both experimental and computational evidence, and highlight its potential for development as a natural antimicrobial agent.

## V. CONCLUSION

According to the findings of this study, bitter kola stem bark extracts had antibacterial activities against gastrointestinal tract organisms. From the results, most of the organisms that showed resistant to commercial antibiotics were susceptible to bitter kola stem bark extracts. The extract showed bactericidal effect against the test organisms at lower concentrations. However, the result of the molecular docking of the bioactive compounds in the stem bark of the bitter kola had higher binding affinities compared to the twelve (12) conventional antibiotics except for Rifampicin. The result showed that antibacterial activities of the bark of bitter kola is as result of the abundant phyto-active compounds in the stem bark. The findings has shown that Hesperidin had the highest inhibition potentials against *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae* with higher inhibition on *Shigella dysenteriae.* Hence, Hesperidin could be regarded as potential drug candidate for the treatment of gastrointestinal tract infection especially those caused by *Shigella dysenteriae.*

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