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Phytochemical Profiling and Molecular Docking Analysis of *Garcinia kola* Ethanolic Leaf Extract Against Gastrointestinal Infections

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Abstract: Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs. This study profiled the bioactive components of *Garcinia kola* leaf against clinical isolates from gastrointestinal tract. Preliminary phytochemical screening was adopted in the determination of the phytochemical components of the extracts. Agar well diffusion technique was adopted in the determination of the antibacterial susceptibility pattern of the extracts against *Salmonella enterica, Salmonella typhimurium, Shigella flexneri, Shigella dysenteriae, Shigella sonnei* and *Escherichia coli* were used in this study. Saponins .anthraquinones, phlobatannins, tannins, phenols, anthranoids and cardiac glycosides were detected in the leaf. Antibacterial activity screening revealed zones of inhibition ranging from 10mm to 22mm with leaf ethanol extract while the Minimum bactericidal concentrations ranged from 250mg/ml to 500mg/ml. The result showed the antibacterial activities of the various leaf of bitter kola as result of the abundant phyto-active compounds in the leaves. However, Hesperidin, Proanthocyanidin, Columbin, Cynaroside, Sanguinarine, Silymarin, (22E)-Stigmasta-4,22-dien-3-ol, Siphonaxanthin triacetate, Desmosterol acetate, Stigmasterol methyl ether, Stigmasterol, Desmosterol were reported to have higher binding interactions with the disease targets. 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.* Hence, there should be enlightenment of the general public on the medicinal importance of bitter kola leaf in order to effectively harness the medicinal values.

Keywords: phyto-active, resistant, hesperidin, phytochemical, infections

Introduction

There are reports on ethno-botanical records indicate a consensus on the use of medicinal plants because they contain bioactive compounds from which phyto medicines can be derived to provide cheaper drugs. Meanwhile, there are presently global problems of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradicated diseases. There is therefore, a pressing need to search for new and more potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms (Adegboye *et al.,* 2008). *G. kola* belongs to the family Guittiferae and it is commonly called ‘‘Orogbo’’ in Yoruba language while the English name is bitter kola. The plant has been referred to as a ‘‘wonder plant’’ because every part of it has been found to be of medicinal importance. *Garcinia kola* Heckel (Clusiaceae), a multipurpose tree commonly found in subtropical and tropical moist lowland forests of Nigeria, Cameroon and other countries in sub-Saharan Africa. It is colloquially called bitter kola, false kola or sometimes ‘‘wonder plant’’ because almost every part of this tree has been used in traditional medicine for broad portfolio of ailments since ancient times (Ijomone and Obi, 2013).

Over the last few years, *G. kola* has received quite large research attention, mainly due to content of a very specific biflavonoid complex collectively referred to as kolaviron, whose distribution seems to be limited to *G. kola*. The phytochemical compounds isolated from *G. kola* include oleoresin, tannins, saponins, alkaloids, cardiac glycosides. Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols. Two new chromanols, garcioic and garcinal, together with tocotrienol were reported isolated from *G. kola*. The biflavanones are predominant compounds in *G. kola* and kolaflavanones are major components of kolaviron. *G. kola* is used in folklore remedies for the treatment of various infections caused by pathogens.

Garcinia kola (commonly known as bitter kola) is a medicinal plant widely recognized for its diverse pharmacological and therapeutic properties. Extensive research has been carried out on the **seeds** of *Garcinia kola*, establishing their antimicrobial, antioxidant, anti-inflammatory, and other bioactive potentials. However, while the seeds have received significant scientific attention, the **leaf** of the plant remain largely underexplored despite their traditional use in herbal medicine by local communities.

This research gap poses a limitation in fully understanding the plant’s phytochemical diversity and therapeutic potential. The leaves may harbor unique or complementary bioactive compounds that contribute to the overall medicinal value of *Garcinia kola*. Without adequate investigation into this plant part, opportunities for novel drug discovery, improved antimicrobial agents, and expanded pharmacological applications may be overlooked.

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 Adil et al. (2015) 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

DNA Extraction using ZR Bacterial DNA Miniprep

Two milliliters (2 ml) of the bacterial cells were added to the broth in a ZR Bashing™ 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™ 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™ 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™ 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 leaves 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 °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).

Results

Table 1: Phytochemical constituents of ethanol extracts of *Garcinia kola* leaf.

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 leaf extracts of *Garcinia kola*

Sample	**Spot**	**Observed Color**	**Distance Travelled by Compound (cm)**	**Distance Travelled by Solvent Front (cm)**	**Rf Value**
Leaf	A	Greenish-brown	3.50	5.20	0.67
	B	Yellow-brown	4.50	5.20	0.87

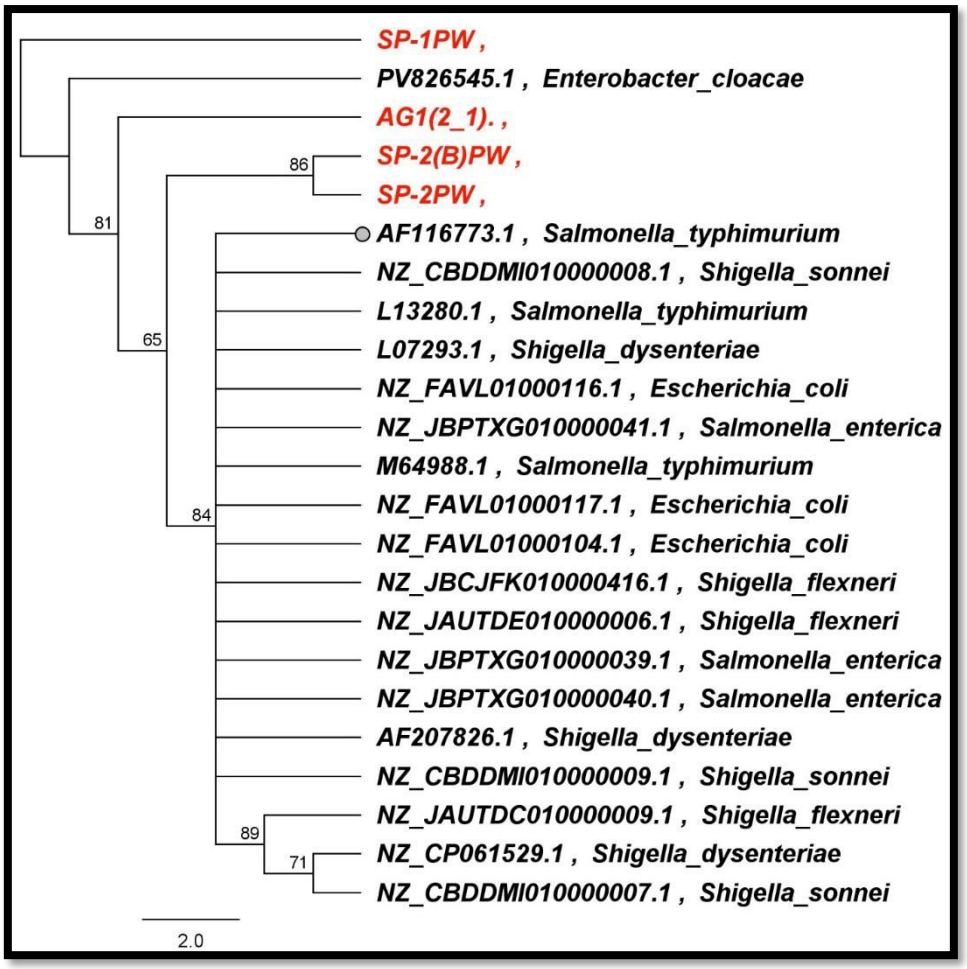
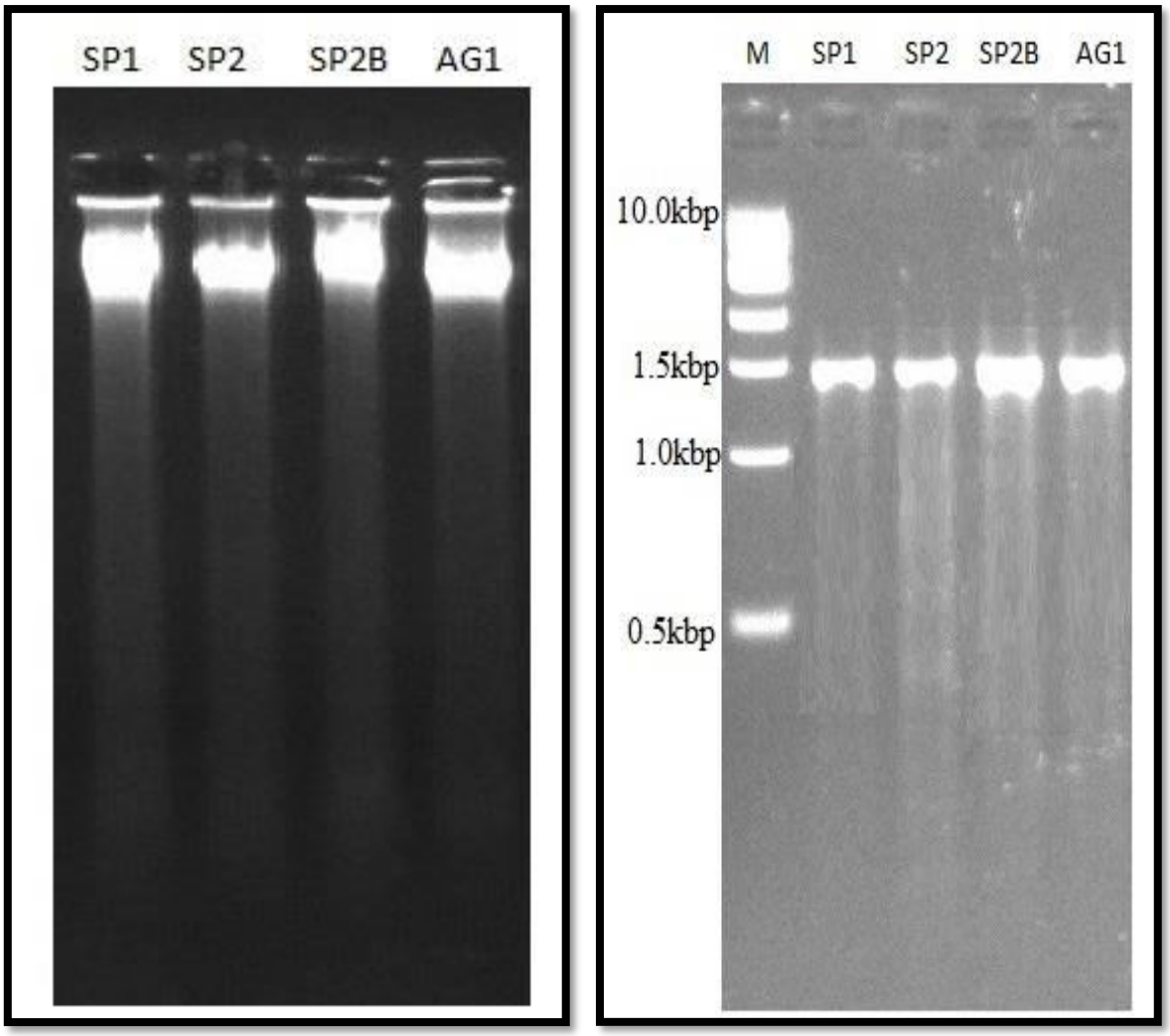


Figure 1: The phylogenetic tree showing the evolutionary relationships among *Salmonella enterica*, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei*, *Escherichia coli* and the four query isolates under study (SP-1PW, SP-2(B)PW, SP-2PW, and AG1).



(a) **(b)**

Figure 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 fermentationtes

Table 4: Sensitivity Profile of Commercial Antibiotics against the isolates

Bacterial Isolate	C (30 µg)	AK (30 µg)	RD (5 µg)	AMC (30µg)	LEV (5 µg)	TE (10 µg)	CN (10 µg)	F (100 µg)	NA (30 µg)	CIP (5 µg)	SXT (5 µg)	TZP (36 µg)
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

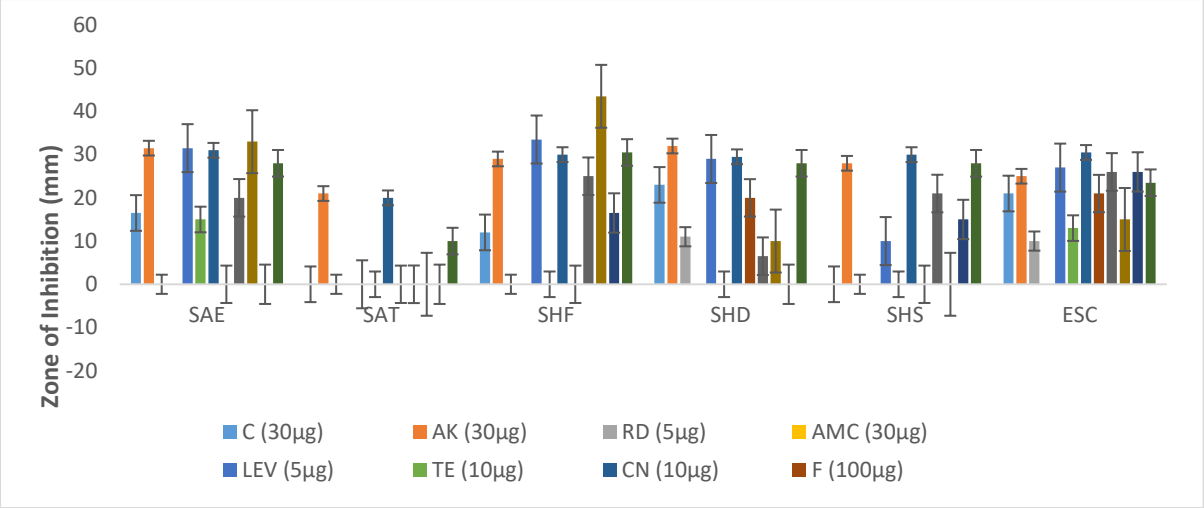


Figure 3: Sensitivity Profile of Commercial Antibiotics against the isolates

Table 5: Sensitivity and zone of inhibition of Ethanol extract of bitter kola leaf (LF) 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	18.00 ± 2.00	15.50 ± 0.50	14.50 ± 0.50	0.00 ± 0.00
SAT	20.00 ± 0.00	12.00 ± 0.00	11.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
SHF	31.00 ± 1.00	12.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SHD	43.50 ± 1.50	19.00 ± 1.00	11.00 ± 1.00	10.00 ± 0.00	0.00 ± 0.00
SHS	40.00 ± 0.00	20.00 ± 2.00	11.00 ± 1.00	15.00 ± 0.00	0.00 ± 0.00
ESC	24.50 ± 0.50	0.00 ± 0.00	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*

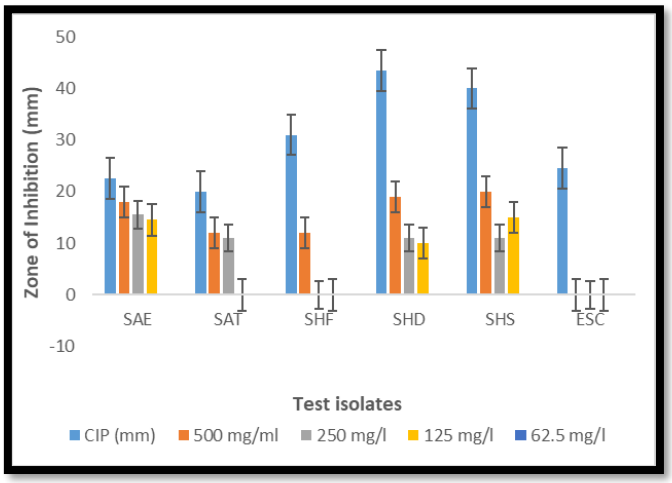


Figure 4: Sensitivity and zone of inhibition of Ethanol extract of bitter kola leaf (LF) on the isolate

Table 6: Minimum inhibitory concentration (MIC) of ethanol leaf extract of Bitter kola on the isolates (ƛ=420 nm)

Bacterial Isolate	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
SAE	0.08 ± 0.00	0.04 ± 0.01	0.10 ± 0.01	0.26 ± 0.01
SAT	0.07 ± 0.00	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.00
SHF	0.13 ± 0.00	0.12 ± 0.00	0.21 ± 0.00	0.23 ± 0.01
SHD	0.11 ± 0.01	0.15 ± 0.01	0.17 ± 0.00	0.14 ± 0.00
SHS	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.10 ± 0.01
ESC	0.03 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.05 ± 0.00

Values expressed as Mean± Stanardard deviation, n=2

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

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

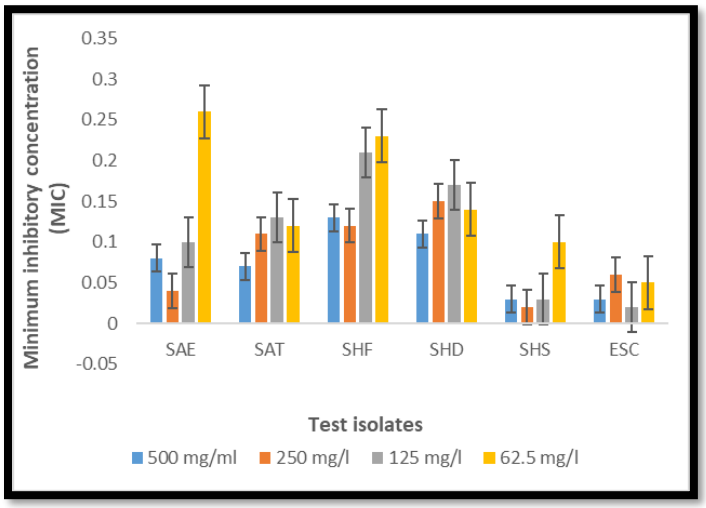


Figure 5: Minimum inhibitory concentration (MIC) of ethanol leaf extract of Bitter kola on the isolates (λ=420 nm)

Table 7: Minimum bactericidal concentration (MBC) of ethanol leaf extract of Bitter kola on the isolates

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

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*

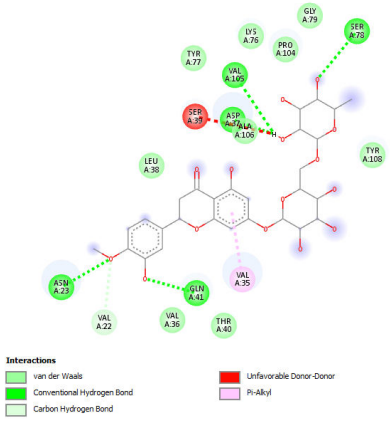
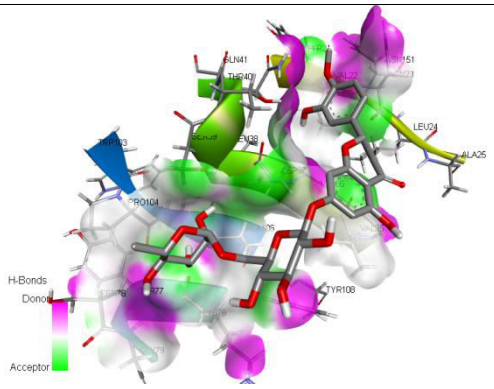
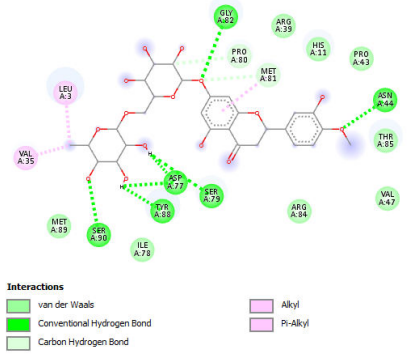
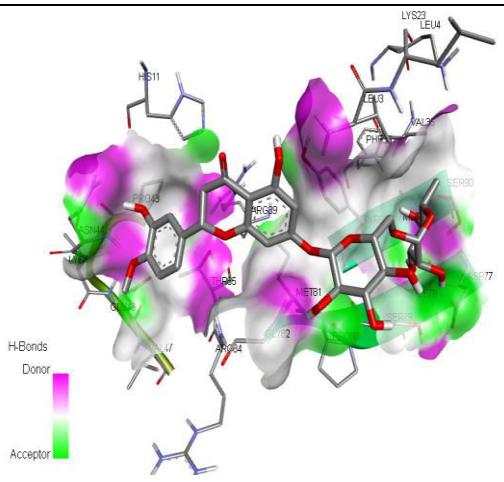
Reference Drugs	**PubChem CID**	**Binding Affinities (Kcal/mol)**		
		4XO8	**5V2W**	**2RG7**
Rifampicin	135398735	**-11.8**	**-11.8**	**-20.6**
Levofloxacin	149096	-5.9	-6.2	-8.2
Ciprofloxacin	2764	-5.9	-5.8	-8.7
Amoxicillin	33613	-5.8	-5.8	-7

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* Ethanolic Leaf extract with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae*

Compounds	**PubChem CID**	**Binding Affinities (Kcal/mol)**		
		4XO8	**5V2W**	**2RG7**
Hesperidin	10621	**-7.5**	**-7.7**	**-10**
2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol	1203	-5.5	-6.1	-7.8
Chlorogenic acid	1794427	-6.1	-6.2	-8.1
Caffeine	2519	-4.6	-4.5	-6.4
Eugenol	3314	-4.6	-4.7	-6.1
Gallic acid	370	-5.2	-5.3	-6.1
Naringenin	439246	-5.8	-6.1	-8.2
Cinnamic acid	444539	-4.4	-5	-5.8
Farnesol	445070	-4.7	-4.9	-6.1
Ferulic acid	445858	-5.1	-5.1	-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	-5.7	-8.9
Kaempferol	5280863	-5.9	-6.1	-8.2
Erucic acid	5281116	-3.9	-3.7	-5.7
Beta-caryophyllene	5281515	-4.8	-5.2	-6
Fisetin	5281614	-5.9	-6.2	-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

Docosenoic acid	6433893	-3.7	-3.6	-5.5
Camphene	6616	-4.3	-4.3	-4.5
Artemisinin	68827	-5.4	-6	-7.4
Caffeic acid	689043	-5.3	-5.1	-6.5
Benzofuran	9223	-4.2	-4.9	-5.9
Curcumin	969516	-6.2	-6.7	-9.2
L-citrulline	9750	-4.3	-4.6	-6.3

Complex	**2D binding Interaction**	**3D binding interaction**
E.coli with Hesperidin		
Salmonella typhimurium with Hesperidin		

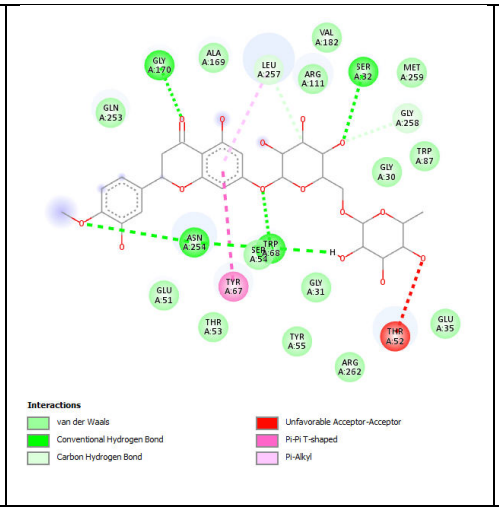
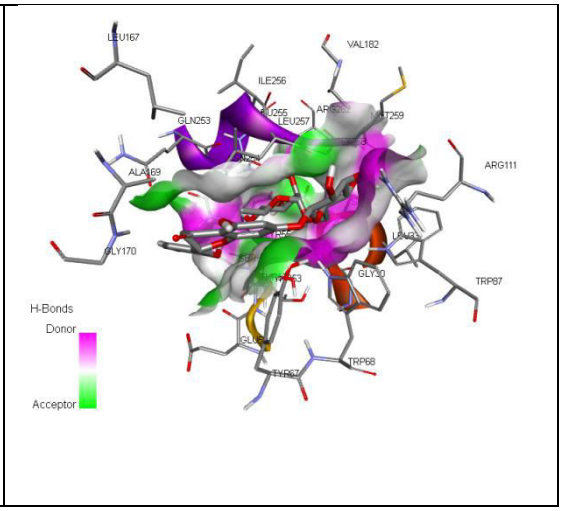
Shigella dysenteriae with Hesperidin		

Figure 6: 2D and 3D Binding interactions of the best docked bioactive compounds in *Garcinia kola* Ethanolic Leaf extract with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteria*

Discussion

Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs. This study screened the bioactive components of *Garcinia kola* against clinical isolates from gastrointestinal tract. Phytochemical screening of the plant part revealed the presence of important phytochemicals such as; saponins, alkaloids, phenols, tannins, anthranoids, anthraquinones, phlobatannins and cardiac glycosides.

The findings from this study are in line with the reports of Chea et al. (2021) who reported the presence of phenols, anthraquinones, tannins, alkaloids and saponins from ethanol and aqueous extracts of *Garcinia kola.* Similarly, Bosso and Innalegwu (2018) reported the presence of alkaloids, phenols, tannins, saponins, cardiac glycosides, phlobatannins, anthraquinones, steroids, terpenes and flavonoids from methanol extract of *Garcinia kola.* In contrast, Bukar et al. (2019) reported the absence of alkaloids, tannins and anthraquinones in n – hexane and chloroform extracts of *Garcinia kola* seeds but the presence of alkaloids, tannins and anthraquinones were reported with ethyl acetate, acetone and methanol extracts.

The Thin Layer Chromatography (TLC) analysis of *Garcinia kola* extracts from various plant parts revealed multiple distinct spots, indicating the presence of diverse bioactive compounds with varying polarities. The leaf extract exhibited Rf values of 0.67 and 0.87, indicating moderately polar to non-polar compounds. These observations are consistent with previous reports that identified flavonoids, tannins, saponins, and other phytochemicals in *Garcinia kola* extracts, with Rf values typically ranging from 0.43 to 0.84 (Adegbaju et al., 2021; Okoye & Onyekwelu, 2022). The presence of these compounds supports the plant's traditional medicinal uses,

including antimicrobial, anti-inflammatory, and antioxidant activities (Olayemi et al., 2020; Eze et al., 2021). Compared to other medicinal plants, *Garcinia kola* exhibits a broader spectrum of bioactive compounds, highlighting its potential as a valuable source for pharmaceutical applications (Abdullahi et al., 2023).

The TLC bioautography results demonstrated that plant-derived spots exhibited varying degrees of antibacterial activity against the tested clinical isolates, with distinct selectivity across different plant parts and fractions. Spots labeled "A" generally displayed the strongest inhibition zones, with the Leaf–A showing consistent effects against *Salmonella enterica*, *Shigella flexneri*, and *Shigella dysenteriae*. Leaf–A was particularly notable against *Shigella sonnei* (18.00 ± 2.83 mm).

These findings are consistent with contemporary studies employing TLC-bioautography to identify antibacterial fractions in complex botanical matrices. Similar reports indicate that Gram-negative bacteria such as *Escherichia coli* and *Salmonella* species are susceptible to specific plant-derived compounds, though sensitivity often varies across fractions (Komape et al., 2024; Wang et al., 2023).

For instance, Rahman et al. (2023) reported inhibitory TLC bands from garlic and ginger extracts effective against multidrug-resistant *E. coli* and *Salmonella*, corroborating the present results. Furthermore, reviews emphasize effect-directed HPTLC as a valuable front-line tool for screening and isolation of antimicrobial metabolites, supporting the significance of the spot-dependent activity observed in this study (Abreu et al., 2021; Shi et al., 2025; Zhang et al., 2025).

The phylogenetic tree shows 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). The clustering pattern observed is consistent with existing genomic and taxonomic knowledge within the *Enterobacteriaceae* family (Adeolu et al., 2016).

One important observation is the close association between *Shigella* species and *E. coli*, which is consistent with previous findings. Several genomic studies have shown that *Shigella* is not a distinct genus in the traditional taxonomic sense but rather a specialized lineage within the broader *E. coli* species complex (Lan & Reeves, 2002; Chen, J.-S et al., 2015). These groups share a high degree of nucleotide identity and exhibit conserved synteny, where gene order remains largely unchanged across their genomes which is confirmed by distance matrix of the samples being studied. This relationship is reflected in our results, where multiple *E. coli* strains (e.g NZ_FAVL01000116.1 and NZ_FAVL01000117.1) cluster together with *Shigella dysenteriae* and *Shigella sonnei*. This supports the hypothesis of relatively recent divergence which is likely due to environmental pressures such as host adaptation,

acquisition of virulence factors or genome reduction commonly attributed to obligate pathogenic lifestyles.

Among the query isolates, SP-1PW seems to be the most evolutionarily divergent, based on its position near the root of the tree and its proximity to the inferred common ancestor of all the strains. However, SP-2(B)PW and *Shigella dysenteriae* AF207826.1 form a sister clade that is basal to the remaining strains which is suggestive of early divergence from the common ancestor of the rest of the samples. This basal clade further splits into two distinct sub-clades. The first sub-clade includes *Shigella sonnei* NZ_CBDDMI010000007.1 and *Shigella flexneri* NZ_JAUTDE010000006.1, supported by a bootstrap value of 77, while the second consists of SP-2PW_(1) and AG1(2_1), with a bootstrap value of 69. Several other strains branch directly from the common ancestor to become independent lineages with unique ecological adaptations.

A total of six (6) gastrointestinal tract organisms comprising *Salmonella enterica, Salmonella typhimurium, Shigella flexneri, Shigella dysenteriae, Shigella sonnei* and *Escherichia coli* were used in this study. Findings from the result showed that the test organisms were resistant to commercial antibiotics such as rifampicin, amoxicillin, septrin and tetracycline. Gentamycin was the most effective antibiotics followed by Amikacin and piperacillin.

The Antibacterial activity screening of the ethanol leaf extract showed better antibacterial activities against the test organisms.

Similarly, Okonkwo et al. (2017) revealed that the *Garcinia kola* extract yielded the highest inhibition zone diameter of 21 mm and 20mm at 100mg/ml and 50mg/ml in ethanol against *Staphylococcus aureus* while in *Candida albicans* at 100mg/ml and 50mg/ml recorded (20mm) and (20mm) in ethanol solvent. The findings showed *Garcinia kola* nut was best extracted with ethanol solvent because it also enhances the bioactive compound or component of the plants.

The result of the minimum bactericidal concentrations of the plant part extracts revealed that bactericidal effect recorded ranged from 250 mg/ml to 500 mg/ml. The zone of inhibition recorded ranged from 25 mm to 32 mm with 500 mg/ml, 16 mm to 25 mm with 250 mg/ml, 10 mm to 18 mm with 125 mg/ml and 8 mm to 18 mm with 62.5 mg/ml. Similar report has been given by Amalu et al. (2014) who reported minimal zones of inhibition started from 40% ethanol and diameter of inhibition increases with increase in ethanol concentration for *E. coli,* while *Staphylococcus aureus* started from 20% ethanol increasing in diameter as the concentration of ethanol increases. *Candida albicans* showed no zone of inhibition.

The molecular interaction of the bioactive compounds in *Garcinia kola* leaf was investigated against *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae*. The results were also compared to some selected conventional antibiotics drugs which served as positive control.

Table 4.23 shows the binding affinities of the bioactive compounds in *Garcinia kola* Ethanolic leaf extract with *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae.* The result showed that Hesperidin had the highest sensitivity of -7.8 Kcal/mol for *E.coli*, -7.7 Kcal/mol for *Salmonella typhimurium* and -10 for *Shigella dysenteriae*. This high binding score reported was higher than that of the 12 conventional antibiotics except for Rifampicin. However, the binding score of Hesperidin is as a result of the molecular interaction of with the amino acid residues at the active site of the disease targets shown in the figure 4.18 and this also suggests that Hesperidin potentially inhibits the growth of the selected bacteria with higher sensitivity on *Shigella dysenteriae.*

Conclusion

The findings from this study revealed that bitter kola leaf extract had antibacterial activities against gastrointestinal tract organisms. From the result, most of the organisms that showed resistant to commercial antibiotics were susceptible to bitter kola leaf. The ethanol extracts showed bactericidal activities against the test organisms. The molecular docking revealed that the bioactive compounds identified had higher binding affinities compared to the twelve (12) conventional antibiotics except for Rifampicin. The result also showed the antibacterial activities is as result of the abundant phyto-active compounds in the leaves. However, Hesperidin, Proanthocyanidin, Columbin, Cynaroside, Sanguinarine, Silymarin, (22E)-Stigmasta-4,22-dien-3-ol, Siphonaxanthin triacetate, Desmosterol acetate, Stigmasterol methyl ether, Stigmasterol, Desmosterol were reported to have higher binding interactions with the disease targets. 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.*

Conflict of Interest

The authors declare no conflict of interest.

Ethics and Consent to Participate Declaration: Not applicable

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