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ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS AGAINST DIARRHOEA CAUSING PATHOGENS

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Abstract

Traditional preparations of medicinal plants with antimicrobial activities have been extensively used in the West African regions. This study was carried out to identify the antimicrobial properties and synergistic effects of the bioactive compounds of selected medicinal plants against diarrhea causing pathogens.Using cold maceration methods, extracts from *Vernonia amygdalina* (22.86g), *Acacia nilotica* (22.80g),,

Keywords: Diarrhea Pathogens, Plant Extracts, Antibacterial activity.

INTRODUCTION

Background to the Study

Diarrhoea is one of the leading causes of morbidity and mortality worldwide, especially in developing countries in sub–Saharan Africa and south East Asia (Cohen *et al*., 2022). According to the report of the global enteric multicentre study, secretory diarrhoea is a historically known serious health problem around the world which primarily originates through pathogenic microorganisms including *Escherichia coli, Shigella* species, *Salmonella* species, *Proteus* species, *Yersinia* species, *Vibrio cholerae and Campylobacter* species. These are some of the common infectious agents which cause enteric disease rather than immunological or genetic disorders (WHO, 2020).

Anogeissus leiocarpus (22.87g), *Carica papaya* (19.05g),, *Piliostigma thoningi* (22.80g), and *Khaya senegalensis* (13.94g) .The highest extract yield was obtained in *A. leiocarpus* The plant crude extracts exhibited varying degree of antibacterial activities at various concentrations against *Vibrio cholerae, Klebsiella pneumoniae* and *Salmonella enterica* serovar, with *A. leiocarpus* (32.33±0.73, 34.00±1.15f, 36.33±1.17) and *K. senegalensis* (36.00±0.81f, 19.00±0.98, 22.00±0.69) having the highest antibacterial activity as they showed significantly higher zones of inhibition at all concentrations against all the test organisms. The minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC) was observed and recorded at 1.02±0.12, 1.04±0.29 and 1.92±0.23 mg/mL concentrations. The most active extracts were fractioned by column chromatography and, n-Hexane fraction of the most active crude extracts *A. leiocarpus* (0.64g) and *K. senegalensis* (0.46g) produced the highest yields followed by the aqueous and ethyl acetate fractions. Significant difference was observed in the antibacterial activity of *A. leiocarpus* and *K. senegalensis* fractions. All fractions of *A. leiocarpus (*aquous, and n-Heaxane) fractions had higher activity against *all test organisms (*36.67±1.00, 32.00±0.23 and 31.00±1.56*)* while Ethylacetate fraction of *K. senegalensis* showed activity against *K. pneumoniaee* and *V. cholarae* while no activity against *S. enterica* serovar *Kentucky*. The synergistic effect of column chromatography fractions of *A. leiocarpus* and *K. senegalensis* was concentration dependent as one of inhibition increase with increasing concentration of the fractions. However, there was no significant difference between all experimental animals from all groups when compared to the control. Significant difference was observed in some liver parameters including a decrease in Alanine aminotransferasae (ALT) and aspartate aminotransferasease (AST) and increase in Total protein and albumin values. Significant differences were also observed in lipid parameters, kidney function and haematological parameters. Histological analysis of the liver and kidney of infected mice administered with *A. leiocarpus* and *K. senegalensis* fractions showed largely preserved architecture signifying that the plant extracts did not cause any detrimental changes and had no toxic effect. Therefore, the plant crude and fractions are recommended for the treatment of diseases caused by the test organism based on traditional medicine rules and regulations..

Most diarrhoea-associated morbidities and mortalities occur in low income and medium-income countries, usually in rural areas as well as in the suburbs and slums of urban areas (Mohammed *et al*., 2014). In these settings, the incidence is further fuelled by the vicious cycles of poverty,

ignorance, malnutrition, and endemic infectious diseases. Obviously, issues directly or remotely connected to socio-environmental factors such as sanitation and quality of water, unhygienic feeding practices (including hand hygiene), suboptimum breastfeeding, zinc deficiency, and barriers to appropriate and affordable health care exist as catalysts for diarrheal disease burden among under-fives in these parts of the world (WHO, 2018)

Large numbers of plant species have been documented for the treatment of various ailments and serve as remedies for human diseases, because they contain chemical components of therapeutic values (Ibrahim *et al.,* 2016). Nigeria is blessed with a large number of plant species such as *Acacia nitolitica, Carica papaya, Khaya senegalenses, Ficus sycomorus and Piliostigma thonningii,* some of which have been in use for centuries to diagnose, prevent and treat various ailments. The exploration of newer antimicrobial agents in plants brings about a different approach in minimizing antibiotic resistance and thus offer potential benefits (Kowero *et al*., 2015). The medicine quest focuses on the drug of the future that will be derived from natural products (Eng *et al*., 2015). The search for unfamiliar plants in the wild with potential value as human and animal food as well curative medicine is gathering momentum (Okorondu *et al.,* 2015). The derivatives of these plants are claimed to have several medicinal and other desirable properties, Furthermore, the nontoxic nature of most chemicals in plants, positive healthy properties, consumer perception and acceptance of their use has been well demonstrated. There are estimated 250,000–500,000 species of plants on Earth. A relatively small percentage (1–10%) of these is consumed as food by both humans and animal species (Chomini *et al*., 2021). It is possible that a greater number are used for medicinal purposes. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants. Currently, antimicrobial plant extracts are of special interest to chemists and microbiologists due to growing public awareness of the negative effects of the over-use of antibiotics and disinfectants.

MATERIALS AND METHODS

Study Area

The study was carried out in General Hospital Minna, Niger State, Nigeria. Which is the major hospital attended by the populace. Minna is a city in Middle belt Nigeria, consisting of 2 Major ethnic groups: Nupe and Gwari. Minna Lies between Latitude 9.58360 N and Longitude 6.54630 E at an altitude of 256m above sea level and has a land area of about 88km2 (www.minna.climatetemps.com/map.php).

Study Population

This study involved diarrhoea patients attending General Hospital Minna, Niger State.

Inclusion Criteria

All diarrhoea patients attending General Hospital Minna, Niger State, who have not started receiving the treatmrnt.

Exclusion Criteria

Diarrhoea patients admitted or already receiving treatment were excluded.

Ethical Consideration

Ethical approval was obtained from the Ethics and Research Committee of General Hospital Minna, Niger State.

Sample Size Determination

The sample size was calculated using the Fisher's formula:

$$n = \frac{z^2 pq}{d^2}$$

Where n = Sample size

z = the standard normal deviate which corresponds to 95% confidence interval (normally set at 1.96)

p = the prevalence of diarrhoea as obtained from a similar study = 13.4% (Omole *et al*., 2019).

q = 1 − p = 0.866.

d = degree of precision (0.05)

$$n = \frac{z^2 pq}{d^2}$$

$$n = \frac{(1.96)^2(0.134)(0.866)}{(0.05)^2}$$

$$\boldsymbol{n = 178.3}$$

The sample size was increased to 500

Sources of Media, Solvents, and Reagents

All chemicals and reagents used for the analysis were of analytical grade (alanar) manufactured by British Drug House Limited. The media were Oxoid products.

Sample Collection

Stool samples from diarrhoea patients were collected in sterile sample bottles, packaged in icepacks and transported to the microbiology laboratory at the Center

for genetic engineering and biotechnology, Federal university of technology, Minna.

Isolation of Diarrhoea causing Pathogens

Samples collected were inoculated onto MacConkey agar, Eosine methylene blue (EMB) agar and Thiosulfate citrate bile-salts sucrose (TCBS) agar and incubated at 37^0C for 24 hours. Distinct colonies were sub-cultured repeatedly to obtain pure cultures of diarrhoea causing pathogens, which were stored on nutrient agar slant for further use.

Identification of Diarrhoea causing Pathogens

Suspected bacterial diarrhoea causing pathogens obtained were identified using colony morphology and conventional biochemical tests including Gram staining, Oxidase test, Voges proskauer test, Indole test, Methyl red, Citrate test, Catalase, Urease and Motility test.

Gram Staining

A thin smear of the pure 24 hours old culture was prepared on clean grease-free slide; it was allowed to dry and fixed by passing over gentle flame. The smear was then stained by adding 2 drops of crystal violet (Primary stain) solution for 60 seconds and then rinsed with water. The smear was again flooded with Lugol's iodine (Mordant) for 30 seconds and rinsed with water before decolourizing with 70% alcohol. The smear was then counter stained with Safranin for 30 seconds before rinsing with water, and then allowed to air dry. The smear was mounted on a microscope and observed under oil immersion objective lens (x100). Gram negative cells appeared pink or red while gram positive organisms appeared purple.

Citrate Utilization Test

Appropriate amount of Simon citrate agar was prepared according to manufacturer's standard (24 g - 1000 mL), dispensed into test tubes (5 mL each) and autoclaved at1210C and 15 psi for 15 minutes. The autoclaved test tubes are allowed to cool and gel in a slanted position. The tubes are then inoculated with the test organism and incubated at 37 0C for 24-48 hours. Observe for colour change from green to blue indicating a positive result and a negative result showed no change in colour.

Indole Test

Appropriate amount of peptone broth was prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture

of the test organisms (18-24 hrs) is aseptically inoculated into the broth and incubated for 24 hours at 37 0C. After incubation about 0.5 ml of kovac's reagent is added to the broth culture and observed for the presence or absence of coloured ring at the top of the broth. A positive indole test is indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer remains yellow or be slightly cloudy.

Methyl Red Test

Appropriate amount of Nutrient broth was prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture of the test organisms (18-24 hours) is aseptically inoculated into the broth and incubated for 24 hours at 370C. After incubation about 5 drops of the methyl red solution is added to the broth culture and observed for colour change. Positive result is indicated by a bright red colour and no change is observed for negative results.

Oxidase Test

A piece of filter paper was placed on a clean Petri dish and 3 drops of oxidase reagent was dropped on it. The test organism was smeared on it. An oxidase positive organism produces a blue-purple colour in 10 seconds, while an oxidase negative organism produced no colouration.

Voges - Proskauer Test

Appropriate amount of Nutrient broth was prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture of the test organisms (18-24 hours) was aseptically inoculated into the broth and incubated for 24 hours at 37^0C. After incubation about One milliliter of 40% KOH and 3 ml of 5 % alpha-naphthol was added and shacked well. Colour formation was be observed. Organism positive for Voges Proskauer gives a pink colour within 2-5 minutes.

Plant Sample collection and identification.

Fresh leaves of five medicinal plants *Anogeissus leiocarpus* (Marke) (KSUSTA/PSB/H/84DC), Guill & Perr., *Carica papaya* (KSUSTA/PSB/H/109C), *Khaya senegalenses* (Madaci) (KSUSTA/PSB/H/61A), *Acacia nilotica* (Bagaruwa) Wild. (KSUSTA/PSB/H/284). *Vernonia amygdalina* (Shuwaka) (KSUSTA/PSB/H/42B) and *Piliostigma thonningii*(Kalgo)(KSUSTA/PSB/H/109L) was collected in Minna, Niger State. The plants were identified by Plant taxonomist, Prof, Dharmendrar Singh from the Department of Plant science and

Biotechnology faculty of Life Sciences, Kebbi state University of science and Technology Aliero, Kebbi state, Nigeria.

Extraction of the Crude Extracts

The plant extraction procedure was carried out according to the method described by AOAC (2010). The different parts of the plant were dried under shade at room temperature for at least 7 days, segregate and pulverized by mechanical grinder to form coarse powder. 100g of powder samples was weighed and macerated into 500ml of methanol for 72 h in the ratio of 1:5 (w/v) respectively. The supernatant obtained was filtered using Whatman No 1 filter paper and evaporated until dred under reduced pressure (204 mbar) at the temperature of 40^0C.

Antimicrobial Assay

Antibacterial activity of the selected plant extracts was tested using agar well diffusion technique on Muller Hinton agar according to method described by Agarry *et al*. (2005). Suspension of the test organisms was prepared in peptone water to the turbidity of 0.5 Macfarland standard and inoculated onto Mueller Hinton agar. Agar wells with diameter of 8mm were created aseptically using sterile cork borer. Each extract solution at desired concentration (50, 70 and 100mg/ml) was introduce into each well and then incubate at 37^0C for 24 hours. Antimicrobial activity of the extracts was measure according to the Association of Official Analytical Chemists (AOAC, 2010).

Determination of Minimum Inhibitory Concentration (MBC) and Minimum Bactericidal Concentration (MBC)

Based on the results of the antibacterial testing, the most efficient extract was chosen to identify its MIC and MBC. The Broth tube dilution method as described by Srikacha and Ratananikom (2020) was used. Serial dilution was adopted to prepare various concentrations of the selected extracts in test tubes, which was inoculated with standardized number of organisms and incubated at 37^0C for 24 hours. The lowest concentration (highest dilution) of the extract with no visible growth of the test organism was considered to be the MIC. The MBC was determined by plating the contents of the MIC tubes onto nutrient agar plates. The lowest concentration of extract with minimum growth of the test organism on the agar plate was considered the MBC.

Determination of Synergism among Plant Fractions

Two-point five (2.5mg/ml, 25mg/ml and 50mg/ml) concentration was mixed from aqueous fraction of *Anogessus leocarpus* and *Ethyl acetates of khaya* senegalensis to identify the synergistic effect between the plant fraction, the activity was determined as described by (NCCLS, 2002).

Column Chromatography

Ten grams (10g) of aqueous and 5g of n-hexane of *Anogessus leocarpus* and 5g of Ethyl acetate fraction of *khaya senegalensis* was subjected to column chromatography to separate the extract into its component fractions. A 120g Silica gel for column chromatography (60-120 mesh) was used as the stationary phase, and the solvent system, n-hexane: ethyl acetate: methanol as mobile phase. The plant extracts were loaded on top of the packed column. The elution of the extract was done using the solvent system; hexane: ethyl acetate: methanol (100:0:0 % v/v) to (0:80:20 % v/v) respectively 100% each. The eluent was collected into the sterile sample bottles (Davies and Johnson, 2007).

UV Analysis

After column chromatography the eluates were scanned using Double beam Shimadzu UV visible spectrophotometer (UV-1800 series) in order to ascertain the eluates with the same compounds, as to bulk them together (Sarker *et al.,*2006) The eluates bulked together were properly labelled as fractions. The fractions were air-dried using water bath at 45 ^{o}C, and the dried fractions were weighed, later was screened against the test bacteria before confirming the compounds responsible for the activities using GC-FID.

***In vivo* Toxicity Studies**

Acute oral toxicity test was carried out according to Lorkes' method (1983). It involved two phases (Phase 1 and 2) of three stages each. The first phase involved the oral administration of the extract with the highest activity, to the three groups of the Wistar albino rats, each group comprising three Wistar albino rats, group one received 10ml/kg bodyweight 100mg/kg body weight and 1000ml/kg bodyweight after an overnight fasting of the animals and control group received 1ml respectively.

Phase 2 animals in their respective group receive doses of 1600, 2000, 5000, depending on the result obtained from Phase 1 experiment. The side cage observation was done within the first 6 hours and mortality rate was monitored for 48 hours.

Acute Toxicological Test

SAMPLES	NO OF ANIMAL TO BE USE	DOSES	TOXICITY SIGNS
		PHASE 1	
EXTRACT X	3	10mg/kgbw	
	3	100mg/kgbw	
	3	1000mg/kgbw	
		PHASE 2	

EXTRACT X	1	1600mg/kgbw
	1	2000mg/kgbw
	1	5000mg/kgbw

Route of Administration: Oral

Sub-Acute Toxicity Studies

The sub-acute toxicity studies were carried out according to Dekant and Vanvakas (2005), the dose of the extract with the highest activity to be administered in sub-acute toxicity studies and this was depend on the result obtained from the acute toxicity test.

However, rats were grouped into six, each Group having four rats, Group four and five was negative and positive control while group six was normal respectively, the extracts with the highest activity was administered (after overnight fasting) to the remaining group once in a day for twenty-eight (28) days. All clinical sign, mortality and morbidity was recorded. At the end of the experiment the animals were euthanized under s mild chloroform and the blood samples was collected from jugular vein for haematological and biochemical analysis using EDTA and samples bottles respectively.

***In-vivo* Antibacterial Susceptibility of Aqueous *Anogeissus leiocarpus* and Ethyl Acetate *Khaya senegalensis* Fractions on experimental Animals.**

Adult Wistar rats of both sexess with weight ranging between 120 and 130 g were bought from Olatunji farms in Ogbomosho, Oyo State Nigeria and were used for the study. The rats were kept in plastic cages in a conventional laboratory setting of 37^{o}C and relative humidity of 40-45 ^{o}C. The animals were fed with finisher (Vital feeds Nigeria) and tap water for three weeks as they were allowed to acclimatize to the settings above ahead of the research.

Animal Grouping

The rats were divided into ten (10) groups each with three rats and were administered with various proportion f the plant fractions

Group 1 was administered 100 mg/kgbw aqueous fractions
Group 2 was administered 300mg/kgbw Ethyl acetate fractions
Group 3 was administered 100mg/kgbw aqueous fractions
Group 4 was administered 300mg/kgbw ethyl acetates fractions
Group 5 was administered 100mg/kgbw aqueous fractions
Group 6 was administered 300mg/kgbw Ethyl acetates fractions
Group 7 was administered 100mg/kgbw aqueous fractions
Group 8 was administered 300mg/kgbw Ethyl acetates fraction

Group 9 was administered ciprofloxacine 100mg/ml/kgbw as a positive control. Group 10 received no treatment; this was used as a negative control.

Infection of Animals

The three rats from each group were infected with the test organisms using method described by Pan *et al.* (2014). Two millilitre (2ml) of saline solution (0.9% NaCl) containing $1.5x10^8$ CFU of the test organism was injected into the rats in each group, injection of the solution was done intrapritoneally, After 72 hours blood culture was collected through the tail (jugular method) from each group into already prepared 5 ml nutrient broth in test tubes which was then inoculated unto Petri dishes containing nutrient agar and incubated for 24 hours. After which the growth of microorganisms was observed, on the plates that the animals was infected.

Administration of the plant fractions

Aqueous and ethyl acetate plant fractions of 100 and 300mg/kgbw were used to treat the animals for seven days (7) the concentrated fractions were choose based on the previous litratures Muhammad *et al*,.2015 The fractions were administered twice a day,one 1ml in the and 1ml in the evening alongside feeding but the animal in group ten (10) received no treatment, they were only fed and given water, During the course of treatment the weight of the animals was taken eac day,their feaces was observed daily and behaviour toward eating was observed on daily basis.

Animals sacrifices and blood culture

Microbial blood culture was carried out from each of the animals before they were sacrificed to ascertain the level of activity of the plants fractions in treating their infections. Blood samples were collected using jugular methods, dropped on Petri dishes soap and mixed with 20ml of nutiernt agar immediately. This was incubated at 37^0C for 24hours and the colonies on the plates were identified, counted with the compared result of the blood culture done before the treatment, which indicated the efficacy of plant fractions after the treatment.

Statistical Analysis

The data was evaluated using analysis of variance (ANOVA) and was presented as the mean value ± SEM (standard error of mean). Differences among the means for the groups was assessed using the Duncan's Multiple Range Test to determine which mean values was significantly different at $p<0.05$

Results

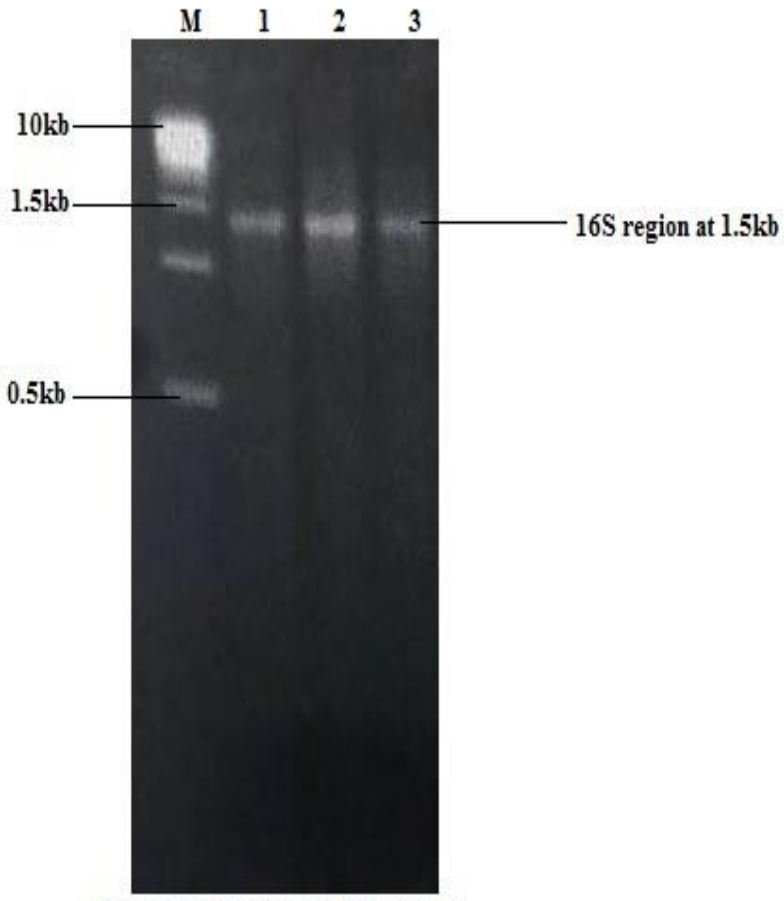
Isolation of Test Organisms

Diarrhea stool samples were collected in sterile sample bottles. The samples were inoculated onto MacConkey agar and incubated at 37^0C for 24 hours. Distinct

bacterial colonies were sub-cultured repeatedly to obtained pure cultures of diarrhea causing pathogens, which was stored on nutrient agar slant battles for further use.

Molecular Identification of Test isolates

Identification of bacterial isolates was carried out using primer pair 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA. The result of pulsed field gel electrophoresis (PFGE) of selected isolates is shown in Plate I. The sequences obtained were queried in GenBank of the National Centre for Biotechnology information (NCBI). Basic local alignment search tool (BLAST) results showed that the test organisms were similar to *Klebsiella pneumoniaee* strain IAUK 8738 with a 98.57% pairwise identity which has NCBI accession number MK571203.1, *Salmonella* enterica subsp. enterica serovar Kentucky strain Medellin Colombian with a 97.40% pairwise identity which has NCBI accession number MH445517.1 and finally *Vibrio choleraee* strain RP01 with 100.00% pairwise identity which has NCBI accession number MH174981.1 (Table 4.1). The phylogenetic tree of the test isolates (Figure 1) showed the relations between the organisms and their origin using the NCBI database while the result of the sequence analysis is presented in appendix.1



Gel image showing amplification of the 16S region at 1.5kb
M is 1kb Ladder NEB.

Plate I: PCR amplification of the DNA region of the bacterial isolates.

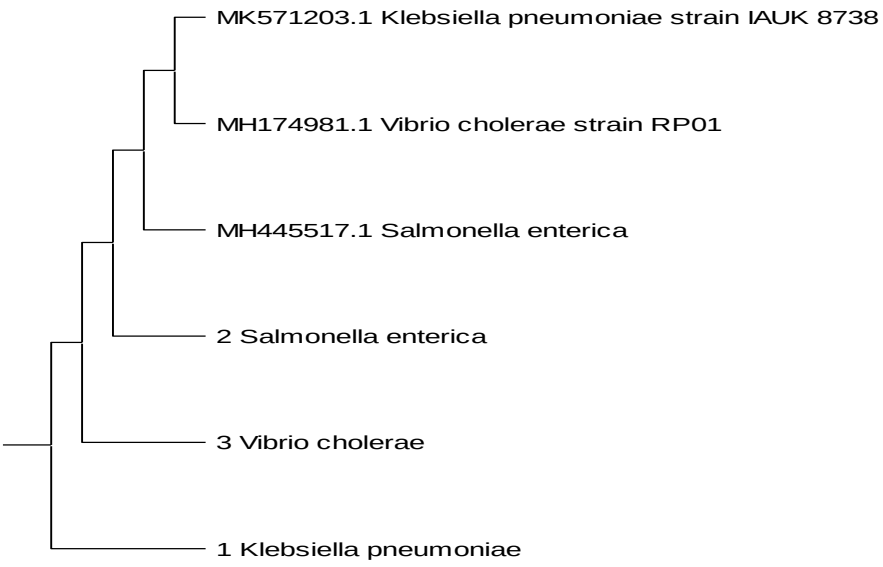


Figure 1: Phylogentic tree showing the relations among the bacterial isolates

Table 4.1: Identity of the test organisms

SAMPLE ID	ISOLATE CODE	SCIENTIFIC NAME	E VALUE	PER. IDENT	ACCESSION
1	KP	*Klebsiella pneumoniaee* strain IAUK 8738	9e-171	98.57%	MK571203.1
2	ST	*Salmonella enterica* subsp. enterica serovar Kentucky strain Medellin Colombian	0	97.40%	MH445517.1
3	VC	*Vibrio choleraee* strain RP01	0	100.00%	MH174981.1

Extraction Yields of Plants

The extraction yield of plant used in this study is shown in Table 4.2. *Anogeissus leiocarpus* (22.87g) had the highest yield followed by *Vernonia amygdalina* (22.86g), *Poliostigma thoningii* (22.80g), *Carica papaya* (19.05g), *Acasia nolitica* (18.67g) and the lowest yield was observed in *Khaya senegalensis* (13.94g).

Table 4.2: Yield of Extract from Plants used.

PLANTS	EXTRACTION YIELD (G)
VERNONIA AMYGDALINA	22.86
ACASIA NOLITICA	18.67
ANOGEISSUS LEIOCARPUS	22.87

CARICA PAPAYA	19.05
POLIOSTIGMA THONINGII	22.80
KHAYA SENEGALENSIS	13.94

Antibacterial Activity of Crude Plant Extracts against Test Organisms

The Antibacterial activity of the six different plant extracts used in this study against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* is shown in Table 4.3. It was observed that all plant extracts used in this study exhibited a varying degree of antibacterial activity against test organisms. *Anogeissus leiocarpus at* 300mg/ml (32.33±0.73, 34.00±1.15f, 36.33±1.17) and *Khaya senegalensis* at 300mg/ml (36.00±0.81f, 19.00±0.98, 22.00±0.69) were the most active among the six plant extracts tested, as they showed significantly higher zones of inhibition at all concentrations against all the test organisms. The other plant extracts also showed activity at all concentrations against the test organisms except in the case of *Carica papaya* and *Poliostigma thoningii* against *Vibrio cholerae* Table 4.3.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Crude Extracts

The MIC and MBC of the crude extracts of *Anogeissus leiocarpus* and *Khaya senegalensis* is shown in Table 4.4. The Minimum Inhibitory Concentration (MIC) of *Anogeissus leiocarpus* against *Vibrio cholerae, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* was observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL respectively, while the minimum bactericidal concentration (MBC) was observed at 1.09±0.87, 0.10±0.05 and 1.80±0.17mg/mL respectively. For *Khaya senegalensis* MIC was observed at 0.96±0.04, 0.96±0.05 and 1.80±0.17 while MBC was recorded at 1.02±0.12, 1.04±0.29 and 1.92±0.23 Table 4.3.

Table 4.3: Effects of Methanolic Crude Extracts Against Bacteria (mm).

EXTRACT	CONCENTRATION (MG/ML)	*VIBRIO CHOLERAEE*	*KLEBSIELLA PNEUMONIAEE*	*SALMONELLA ENTERICA SEROVAR KENTUCKY*
VERNONIA AMYGDALINA	200	24.00±1.15^{c}	16.33±0.60^{cd}	12.00±0.58^{bc}
	250	28.00±0.58^{d}	18.00±1.15^{d}	14.33±0.60^{cd}
	300	29.00±0.15^{d}	24.00±1.44^{e}	16.50±0.29^{de}
ACASIA NOLITICA	200	11.00±0.58^{b}	22.00±0.87^{e}	14.00±0.58^{c}
	250	25.33±0.60^{c}	31.50±0.87^{f}	11.00±0.58^{ab}
	300	29.33±1.17^{d}	31.50±0.29^{f}	9.00±1.15^{a}
ANOGEISSUS LEIOCARPUS	200	28.00±0.58^{d}	18.00±1.15^{d}	28.17±0.60^{h}
	250	32.00±1.15^{e}	22.00±0.58^{e}	32.00±1.73^{i}
	300	32.33±0.73^{e}	34.00±1.15^{f}	36.33±1.17^{j}

CARICA PAPAYA	200	0.00±0.00[a]	9.00±1.15[a]	18.00±0.58[ef]
	250	0.00±0.00[a]	17.00±1.15[d]	26.33±0.60[h]
	300	0.00±0.00[a]	24.00±0.58	28.33±1.17[h]
POLIOSTIGMA THONINGII	200	0.00±0.00[a]	12.50±0.29[b]	8.50±0.29[a]
	250	0.00±0.00[a]	14.00±0.58[bc]	10.00±0.29[ab]
	300	0.00±0.00[a]	24.00±1.15[e]	12.00±0.58[bc]
KHAYA SENEGALENSIS	200	29.00±0.87[d]	12.00±0.58[b]	20.00±0.29[fg]
	250	32.50±0.29[e]	16.20±1.27[cd]	20.00±1.15[fg]
	300	36.00±0.81[f]	19.00±0.98[d]	22.00±0.69[g]
CONTROL	8mg/ml	24.21±0.82	12.03±13	32.07±1.43

Keys: Values are presented as mean ± standard error of mean (SEM) of triplicates
Values with different superscript in a column are significantly different at $p < 0.05$.

Table 4.4: Minimum Inhibitory and Minimum Bactericidal Concentrations of *Anogeissus leiocarpus and Khaya senegalensis* plant crude extracts

Plant Extract	MIC (mg/mL)			MBC (mg/mL)		
	Vibrio choleraæ	*Klebsiella pneumoniaæ*	*Salmonella* enterica serovar Kentucky	*Vibrio choleraæ*	*Klebsiella pneumoniaæ*	*Salmonella* enterica serovar Kentucky
Anogeissus leiocarpus	0.96±0.03	0.19±0.00	0.96±0.06	1.09±0.87	0.20±0.05	1.04±0.17
Khaya senegalensis	0.96±0.04	0.96±0.05	1.80±0.17	1.20±0.12	1.40±0.29	1.920±0.23
sControl	0.005	0.005	0.020	0,005	0.005	0.020

Values are presented as mean ± standard error of mean (SEM) of triplicates
MIC = Minimum Inhibitory; MBC = Minimum Bactericidal Concentrations

Partition Yields of Active crude Extracts *Anogeissus leiocarpus and Khaya senegalensis.*

N-hexane of *A. leocarpus* yields (0.64g) followed by aqueous (0.57g) and then ethyl-acetate (0.25g). Similarly, n-hexane had the highest yield (0.46g) in *Khaya senegalensis* followed by ethyl-acetate (0.28g) and then aqueous (0.25g). Table 4.5

Table 4.5: Partition Yields of Active crude extracts of *Anogeissus leiocarpus and Khaya senegalensis.*

PLANT EXTRACT	FRACTIONS	YIELD (G)
ANOGEISSUS LEIOCARPUS	n-Hexane	0.64
	Aqueous	0.57
	Ethyl acetate	0.25

KHAYA SENEGALENSIS	n-Hexane	0.46
	Aqueous	0.25
	Ethyl acetate	0.28

Antibacterial Activity of Partitioned Fractions of *Anogeissus leiocarpus* and *Khaya senegalensis*

Antibacterial Activity of n-hexane of *Anogeissus leiocarpus* was observed the highest (36.67±1.00) against *Vibrio cholera,* and the lowest activities of ethylacetate of the same plants (*A. leiocarpus*) shows no activities against the same organism at the same concentrations (0.00±0.00). *Khaya senegalensis* fractions against showed no activities against *Salmonella enterica* serovar *Kentucky,* but it was observed against *Vibrio cholerae,* and *Klebsiella pneumoniaee* and at 300mg/ml.(24.00±0.69, 19.33±1.11). While against *Salmonella enterica* serovar *Kentucky* highest activity was observed in the aqueous fraction of *Anogeissus leiocarpus.* None of the *Khaya senegalensis* fraction was active against *Salmonella.*

Yields of *Khaya senegalensis* Ethyl acetate Column Chromatography fractions

Column Chromatography yields of *Khaya senegalensis* Ethyl acetate fraction is shown in Table 4.7. Fraction 7 (0.8987g) had the highest yield followed by Fraction 2 (0.7610g) and Fraction 5 (0.5616g). Lowest yield was observed in Fraction 3 (0.1442g).

Table 4.6: Effect of Partitioned fractions of active extracts at (300mg/ml)

PLANT EXTRACT	FRACTION 300MG/ML	*VIBRIO CHOLERA*	*KLEBSIELLA PNEUMONIAE*	*SALMONELLA ENTERICA* SEROVAR *KENTUCKY*
ANOGEISSUS LEIOCARPUS	n-Hexane	23.93 ± 1.33^{c}	20.00 ± 1.15^{a}	19.00 ± 0.87^{b}
	Aqueous	36.67 ± 1.00^{b}	32.00 ± 0.23^{b}	31.00 ± 1.56^{d}
	Ethyl acetate	0.00 ± 0.00^{a}	22.00 ± 0.75^{a}	22.00 ± 1.04^{c}
KHAYA SENEGALENSIS	n-Hexane	24.00 ± 0.69^{c}	19.33 ± 1.11^{a}	0.00 ± 0.00^{a}
	Aqueous	24.00 ± 1.85^{c}	21.00 ± 1.15^{a}	0.00 ± 0.00^{a}
	Ethyl acetate	24.00 ± 1.73^{c}	24.00 ± 1.56^{c}	00.00 ± 0.00^{a}
CONTROL	8mg/ml	32.50±0.64	34.00±0.16	35.40±0.08

Key: Values are presented as mean ± standard error of mean (SEM) of triplicates Values with different superscript in a column are significantly different at $p < 0.05$.

Antibacterial Activity of the *Khaya senegalensis* Ethylacetate Column chromatography fractions

Antibacterial Activity of *Khaya senegalensis* ethylacetate column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* is shown in Table 4.8. Antibacterial activity was concentration dependent as inhibition zones increase with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio cholerae* was observed in fraction 7 (30.00±1.15) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 5 (24.00±1.73) at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 6 (32.00±2.31) at 200mg/mL concentrations.

Yields of *Anogeissus leiocarpus* n-Hexane Column chromatography fractions

Column Chromatography yields of *Anogeissus leiocarpus* n-Hexane fraction is shown in Table 4.9. Fraction 4 (1.9384g) had the highest yield followed by Fraction 3 (0.8726g), Fraction 1 (0.8673g) and lastly Fraction 2 (0.07682g).

Antibacterial Activity of *Anogeissus leiocarpus* n-hexane Column Chromatography Fractions

Antibacterial Activity of *Anogeissus leiocarpus* n-Hexane Column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* is shown in table 4.10. Significant difference was observed in the zones of inhibition. Antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio choleraee* was observe in fraction 2 (22.67±0.75) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 2 (24.00±0.58), fraction 3 (25.67±1.11), and fraction 4 (24.00±1.73) all at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 1 (24.00±1.39, 24.00±0.75 and 26.00±1.33) at 50, 100 and 200mg/mL concentrations and fraction 4 (24.00±1.27) at 200ml/ml concentration.

Table 4.7: Column Chromatography yields of *Khaya senegalensis* Ethyl acetate fractions

FRACTIONS	YIELD (G)
1	0.0407
2	0.7610
3	0.1442
4	0.02367

5	0.5616
6	0.23965
7	0.8987

Table 4.8 Effect of *Khaya senegalensis Ethyl acetate* Column chromatography fractions on the test organisms.

FRACTIONS	CONCENTRATIONS (MG/ML)	*VIBRIO CHOLERAE*	*KLEBSIELLA PNEUMONIAE*	*SALMONELLA* ENTERICA SEROVAR KENTUCKY
F1	50	16.00±1.15^{cd}	15.00±0.58^{b}	0.00±0.00^{a}
	100	20.00±1.73^{ef}	15.00±0.87^{bc}	15.00±0.58^{b}
	200	24.00±1.44^{g}	14.00±0.58^{cd}	14.00±0.75^{bc}
F2	50	15.47±0.73^{c}	18.00±1.15^{ef}	14.00±0.87^{bc}
	100	18.00±0.69^{de}	20.00±0.92^{fg}	18.00±1.73^{cde}
	200	20.00±1.15^{ef}	23.00±1.73^{gh}	20.00±0.58^{de}
F3	50	0.00±0.00^{a}	0.00±0.00^{a}	15.00±0.58^{bc}
	100	10.00±1.15^{b}	0.00±0.00^{a}	18.00±1.73^{cde}
	200	14.00±0.58^{c}	14.00±1.15^{cd}	18.00±1.44^{cde}
F4	50	14.00±0.58^{c}	18.00±0.87^{ef}	16.00±1.15^{cd}
	100	18.00±0.58^{de}	20.33±1.17^{fg}	18.00±1.15^{cde}
	200	20.00±1.15^{ef}	22.00±0.58^{gh}	20.00±0.00^{de}
F5	50	20.00±1.15^{ef}	20.00±1.73^{fg}	22.00±1.15^{e}
	100	24.00±1.73^{g}	22.00±1.15^{gh}	27.00±1.73^{f}
	200	24.00±1.15^{g}	24.00±1.73^{h}	29.00±1.27^{fg}
F6	50	18.00±1.15^{de}	15.00±0.58^{bc}	28.00±1.73^{f}
	100	22.00±1.15^{fg}	16.00±1.15^{de}	30.00±1.50^{fg}
	200	24.00±1.73^{g}	18.00±1.15^{ef}	32.00±2.31^{g}
F7	50	24.00±1.15^{g}	18.00±0.58^{ef}	22.00±0.58^{e}
	100	28.00±1.73^{h}	22.00±0.58^{gh}	26.00±1.15^{f}
	200	30.00±1.15^{h}	22.00±1.73^{gh}	28.00±1.15^{f}
CONTROL	8mg/ml	33.67±33	30.67±0.33	32.67±0.33

Table 4.9: Yields of *Anogeissus leiocarpus n-hexane* Column chromatography fractions

FRACTIONS	YIELD (G)
1	0.8673
2	0.07682
3	0.8726
4	1.9384

Yields of *Anogeissus leiocarpus Aqueous* Column Chromatography Fractions

Column Chromatography yields of *Anogeissus leiocarpus* aqueous fraction is shown in table 4.11. Fraction 9 (2.2355g) had the highest yield followed by fraction 8 (1.3687g), fraction 6 (1.2371g). Lowest yield was observed in fraction 3 (0.0446g) and fraction 2 (0.0761g).

Table 4.10: Effects of *Anogeissus leiocarpus n-hexane* Column Chromatography fraction on the test organisms.

FRACTIONS	CONCENTRATIONS (MG/ML)	*VIBRAE CHOLERAE*	*KLEBSIELLA PNEUMONIAEE*	*SALMONELLA ENTERICA* SEROVAR *KENTUCKY*
1	50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	100	0.00 ± 0.00^{a}	15.00 ± 0.81^{b}	22.00 ± 1.15^{c}
	200	0.00 ± 0.00^{a}	15.00 ± 1.10^{bc}	22.00 ± 0.87^{c}
2	50	0.00 ± 0.00^{a}	14.00 ± 0.58^{c}	24.00 ± 1.39^{cd}
	100	18.00 ± 1.04^{c}	18.33 ± 0.83^{de}	24.00 ± 0.75^{cd}
	200	22.67 ± 0.75^{d}	24.00 ± 0.58^{fg}	26.00 ± 1.33^{d}
3	50	15.33 ± 0.38^{b}	21.00 ± 0.58^{e}	0.00 ± 0.00^{a}
	100	14.00 ± 1.27^{b}	22.00 ± 1.15^{ef}	0.00 ± 0.00^{a}
	200	14.00 ± 1.15^{b}	25.67 ± 1.11^{g}	0.00 ± 0.00^{a}
4	50	0.00 ± 0.00^{a}	14.00 ± 0.58^{c}	14.00 ± 1.15^{b}
	100	18.00 ± 1.21^{c}	18.00 ± 1.04^{d}	22.00 ± 1.15^{c}
	200	20.00 ± 1.33^{c}	24.00 ± 1.73^{fg}	24.00 ± 1.27^{cd}
CONTROL	8mg/ml	34.00±00	30.64±0.67	31.00±0.55

Values are presented as mean ± standard error of mean (SEM) of triplicates

Values with different superscript in a column are significantly different at $p < 0.05$.

Table 4.11: *Anogeissus leiocarpus* Aqueous Column Chromatography Fraction Yield

FRACTIONS	YIELD(G)
1	0.551
2	0.076
3	0.044
4	0.976
5	0.394
6	1.237
7	0.552
8	1.368

9	2.235

Antibacterial Activity of the *Anogeissus leiocarpus* Aqueous Column Chromatography Fractions

Antibacterial Activity of *Anogeissus leiocarpus* aqueous Column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniae* and *Salmonella enterica* serovar *Kentucky* is shown in Table 4.12. Significant difference ($P \leq 0.05$) was observed in the zones of inhibition. Antibacterial activity was concentration dependent as inhibition zones increase with increasing concentration of the extract. Fraction 1-4 showed no activity against *Vibrio cholerae*. Significantly high activity against *V. cholerae* was observed in fraction 6 (42.00±1.27) at 200mg/mL concentration. Against *Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* Fraction 1-5 showed no significant activity. Highest significant activity against *Klebsiella pneumoniae* was observed in fraction 6 (32.00±1.15), fraction 7 (32.00±1.15) and fraction 9 (32.00±1.15) all at 200mg/mL concentration. While for *Salmonella enterica* fraction 8 (32.00±1.15) at 200mg/mL was the highest observed.(Table 4.10.).

Synergistic Effect of Column Chromatography Fractions of *Anogeissus leiocarpus* and *Khaya senegalensis*

The synergistic antibacterial effect of the column chromatography fractions of *Anogeissus leiocarpus* and *Khaya senegalensis* against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* is shown in table 4.13 and 4.14. Observed antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. Significantly highest antimicrobial activity against for all test organisms was observed at 100mg/mL concentration.

Table 4.12: Effects of Column Fractions of aqueous fraction of *Anogeissus leiocarpus* on the test organisms.

FRACTIONS	CONCENTRATIONS (MG/ML)	*VIBRIO CHOLERAEE*	*KLEBSIELLA PNEUMONIAEE*	*SALMONELLA ENTERICA* SEROVAR *KENTUCKY*
1	50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	100	0.00±0.00^{a}	15.00±0.58^{b}	22.00±0.75^{de}
	200	0.00±0.00^{a}	15.33±0.38^{b}	22.00±1.15^{de}
2	50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	100	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	200	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
3	50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	100	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	200	0.00±0.00^{a}	0.00±0.00^{a}	15.00±0.64^{b}
4	50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	100	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

	200	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	100	22.00±1.15^{c}	0.00±0.00^{a}	0.00±0.00^{a}
	200	24.00±1.50^{cd}	0.00±0.00^{a}	0.00±0.00^{a}
6	50	23.00±1.73^{c}	18.00±0.58^{c}	19.00±0.75^{c}
	100	28.00±0.75^{ef}	30.00±1.27^{fg}	20.00±1.15^{cd}
	200	42.00±1.27^{i}	32.00±1.15^{g}	22.00±0.69^{de}
7	50	26.00±1.15^{de}	20.00±2.02^{cd}	22.00±1.15^{de}
	100	28.00±1.04^{ef}	22.00±0.75^{de}	24.00±1.50^{ef}
	200	29.00±1.15^{f}	32.00±1.15^{g}	26.00±0.87^{fg}
8	50	32.00±1.15^{g}	22.00±1.50^{de}	28.00±1.15^{gh}
	100	36.00±1.21^{h}	24.00±1.39^{e}	30.00±1.85^{hi}
	200	36.00±0.75^{h}	28.00±1.27^{f}	32.00±1.15^{i}
9	50	18.00±0.58^{b}	18.00±1.27^{c}	15.00±1.15^{b}
	100	26.00±1.73^{de}	30.00±1.85^{fg}	18.00±0.58^{c}
	200	36.00±1.67^{h}	32.00±1.15^{g}	20.00±1.39^{cd}
CONTROL	8mg/ml	30.67±0.33	38.33±0.33	32.67±0.13

Values are presented as mean ± standard error of mean (SEM) of triplicates
Values with different superscript in a column are significantly different at $p < 0.05$.

Table 4.13: Effect of the Synergistic of Aqueous Column Chromatography Fractions of *Anogeissus leiocarpus against test organisms.*

AQUEOUS COLUMNS CHROMATOGRAPHY FRACTIONS	CONCENTRATIONS (MG/ML)	ISOLATES/ZONES OF INHIBITION(MM)		
		Vibrio choleraee	***Klebsiella pneumoniaee***	***Salmonella enterica* serovar *Kentucky***
F6 + F8	25	27.00±1.73	30.00±1.15	28.00±0.58
	50	28.00±2.31	32.00±1.15	30.00±1.73
	100	30.00±173	24.00±2.31	32.00±1.15
CONTROL	8	32.20±0.50	37.80±1.56	34.33±0.88

Values are presented as mean ± standard error of mean (SEM) of triplicates

Table 4.14: Effects of the Synergistic of Ethyl acetate column chromatography fractions of *Khaya senegalensis* on the test organisms.

ETHYL ACETATE COLUMNS CHROMATOGRAPHY FRACTIONS	CONCENTRATIONS (MG/ML)	ISOLATES/ZONES OF INHIBITION(MM)		
		Vibrio choleraee	***Klebsiella pneumoniaee***	***Salmonella enterica* serovar *Kentucky***
F5 + F7	25	28.00±2.31	36.00±1.15	28.00±0.87
	50	24.00±1.73	28.00±1.44	26.00±1.15
	100	24.00±2.31	28.00±1.73	28.00±0.58
CONTROL	8	34.60±6.19	33.67±0.33	31.67±0.48

Values are presented as mean ± standard error of mean (SEM) of triplicate

Ultra Violet Spectroscopic Analysis of fractions of *Anogeissus leiocarpus* and *Khaya senegalensis.*

UV spectroscopy of fractions of *Anogeissus leiocarpus* and *Khaya senegalensis* was done at a range of 200 to 700nm. The UV absorption spectrum and Peak values of the fractions is shown in Appendix c (Tigure 2-21 and Table 4.15-4.34).

FTIR Spectroscopy of *Anogeissus leiocarpus* aqueous fractions and ethylacetate *Khaya senegalensis*

The FTIR spectra of *Anogeissus leiocarpus* aqueous fractions and *Khaya senegalensis* ethylacetate fractions are shown in Appendix D (Figures 22-25); these enabled the identification of possible functional groups of the active components present in the fractions of *Anogeissus leiocarpus* and *Khaya senegalensis.* The identified functional groups are shown in Tables 4.15 – 4.18. Functional groups identified include hydroxyl group (OH), Cyclo alkane, Phenol rings, Methyl group (CH_3-), Aromatic ring, Ester carboxyl, Alkanes, C-O-C group.

Table 4.15: FTIR spectral peak valves and functional groups obtained from F6 aqueous fraction of *Anogeissus leiocarpus*

WAVELENGHT (CM^{-1})	FUNCTIONAL GROUP
3287.5	OH Hydroxyl group
2918.5	Cyclo alkane
2844.0	Carboxyl acid
1563.6	Phenol ring
1412.7	C-O/C-H bonding
1015.7	C-O-C group
903.9	C-H

Table 4.16: FTIR spectral peak valves and functional groups obtained from F8 aqueous fraction of *Anogeissus leiocarpus*

WAVELENGHT (CM^{-1})	FUNCTIONAL GROUP
3272.6	OH
2929.7	Methyl group (CH_3-)
1599.0	Phenol ring
1433.2	Aromatic ring
1168.5	C-O-C group
1019.4	C-O-C group
875.9	C-H

Table 4.17: FTIR spectral peak valves and functional groups obtained from F5 ethylacetate fraction of *Khaya senegalensis*

WAVELENGHT (CM^{-1})	FUNCTIONAL GROUP
2922.2	CH_3 (Methyl group)
2855.1	Cyclo alkane
1727.6	Carboxyl compounds
1459.3	Phenol ring

1162.9	Aromatic ring
1039.9	C-O-C group
715.6	C-H

Table 4.18: FTIR spectral peak valves and functional groups obtained from F7 ethylacetate fraction of *Khaya senegalensis*

WAVELENGHT (CM^{-1})	FUNCTIONAL GROUP
3362.1	OH Hydroxyl
3004.2	Methyl group
2926.0	Cyclo alkane
1720.7	Carboxyl compounds
1408.9	Alkane - CH_3-
1241.2	Ester carboxyl
1004.5	C-O-C group
950.5	C-O-C group
700.7	C-S Linkage

In-vivo Antibacterial Susceptibility of Aqueous *Anogeissus leiocarpus* and ethyl acetate *Khaya senegalensis* Fractions

In-vivo Antibacterial activity of the Aqueous *Anogeissus leiocarpus* and Ethyl acetate *Khaya senegalensis* fractions is shown in Table 4.35. The in vivo assay showed that the ethyl acetate fraction of *Khaya senegalensis* had higher activity than aqueous fraction of *Anogeissus leiocarpus*. The in vivo activity was dose concentration depedents with 300mg/ml having the highest colonies reduction for all the test organisms.

Effect of Plant Fractions on Body Weight Gain of infected mice

The effect of plant fractions on body weight gain of infected mice is shown in Table 4.36. There was no significant difference between the bodyweights of all experimental animals from all groups when compared to the control.

Table 4.35: In-vivo Antibacterial Susceptibility of mice following ($1.5x10^8$ CFU).

EXTRACTS	CONCENTRATIONS (MG/ML)	*VIBRIO CHOLERAEE* (±SD) CFU/P	*KLEBSIELLA PNEUMONIAE* (±SD) CFU/P	*SALMONELLA ENTERICA* SEROVAR KENTUCKY (±SD) CFU/P
ETHYL ACETATE *KHAYA SENEGALENSIS* FRACTION	100	59.00±4.62	24.00±3.46	12.10±1.54
	300	48.00±3.46	21.33±1.86	8.00±1.43
AQUEOUS *ANOGEISSUS*	100	71.33±1.86	68.67±2.33	25.50±3.14
	300	47.00±4.04	50.00±1.73	17.00±2.32

LEIOCARPUS FRACTION				
POSITIVE CONTROL	100	29.33±3.18	12.00±2.56	43.00±4.62
NEGATIVE CONTROL	NS	180.67±4.41	124.67±5.87	157.67±6.39

Key: AS: *Anogeissus leiocarpus;* KS: *Khaya senegalensis*

Effect of Plant Fractions on Body Weight Gain of infected mice

The effect of plant fractions on body weight gain of infected mice is shown in Table 4.36. There was no significant difference between the bodyweights of all experimental animals from all groups when compared to the control.

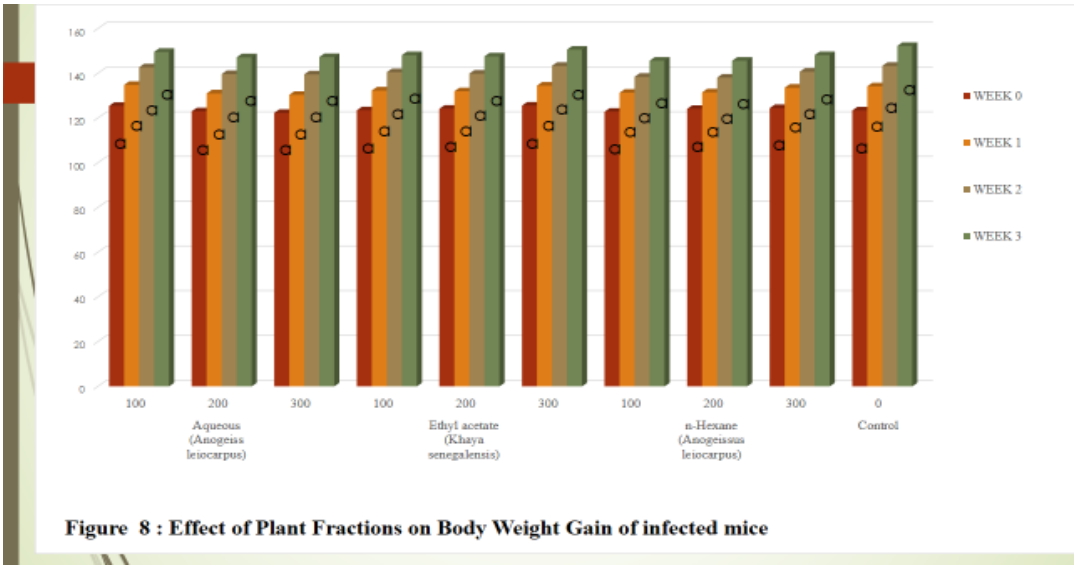


Figure 8 : Effect of Plant Fractions on Body Weight Gain of infected mice

Effect of Plant Fractions on Liver Function of Infected Mice

The effect of plant fractions on liver function of infected mice is shown in Table 4.37. Significant difference was observed in some liver parameters. Significant decrease in Alanine transaminase (ALT) was observed in mice treated with aqueous fraction of *Anogeissus leiocarpus* at 300 mg/kgbw (17.89±1.00) when compared to the control. In the case of Aspartate transaminase (AST) significant decrease was observed in all experimental groups at all concentrations when compared to the control. Similarly, significant decrease in alkaline phosphatase value was noted in some experimental groups, while an increase was observed in total protein and albumin values.

Effect of Plant Fractions on Lipid Profile of Infected Mice

The effect of plant fractions on lipid profile of infected mice is shown in Table 4.38. Significant differences were observed in lipid parameters when compared to the control. There was significant increase in the total cholesterol (TC) value in experimental animals treated with n-Hexane fraction of *Anogeissus leiocarpus* at

all concentration, while decrease in total cholesterol (TC) value was observed only in animals treated with ethyl acetate fraction of *Khaya senegalensis* at 300 mg/kgbw concentration. No significant change in Triglycerides value was observed in all treatment groups except for animals treated with ethyl acetate fraction of *Khaya senegalensis* at 200 and 300 mg/kg bw where a decrease in Triglycerides values was noted. Significant increase was observed in the High-density lipoprotein (HDL) values of some treatment groups, while no significant differences was observed in low density lipoprotein (LDL) in all groups when compared to the control.

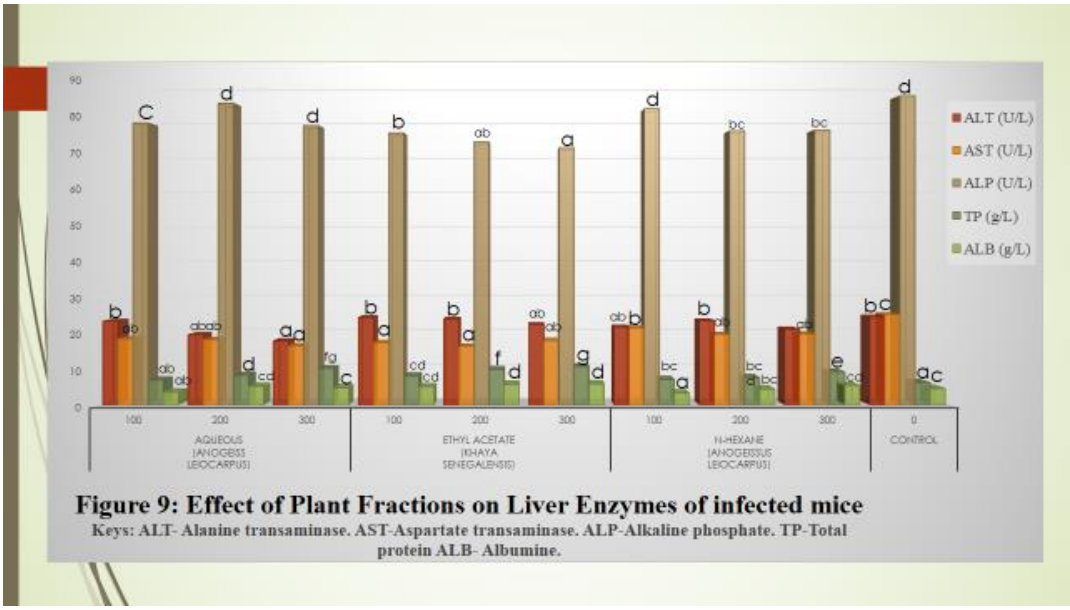
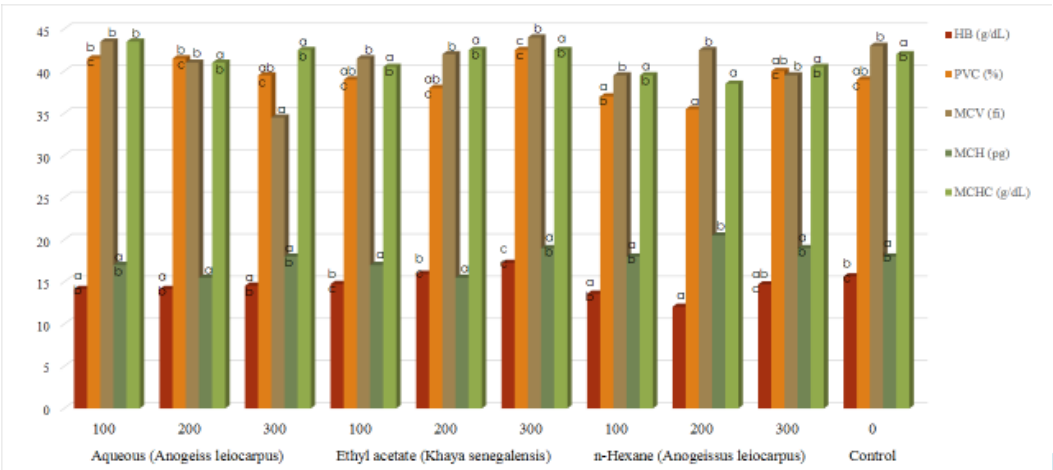


Figure 9: Effect of Plant Fractions on Liver Enzymes of infected mice

Keys: ALT- Alanine transaminase. AST-Aspartate transaminase. ALP-Alkaline phosphate. TP-Total protein ALB- Albumine.



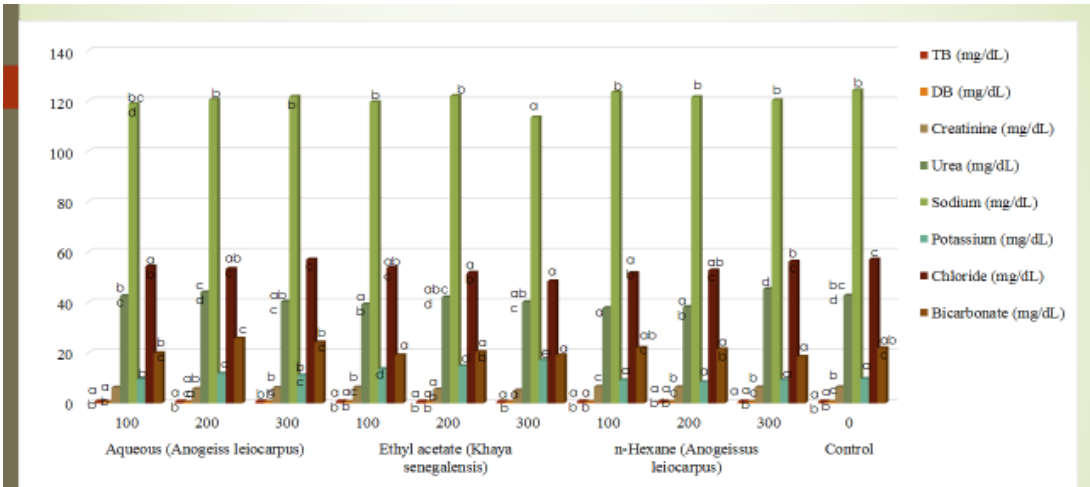
Keys: HB –Haemoglobin PVC – Packed cell volume MCV – Mean cell volume MCH –Mean cell haemoglobin MCHC –Mean cell haemoglobin concentration.

Values are presented as mean ± standard error of mean (SEM) of three replicates.

Figure 12: Effect of Plant Fractions on haematological Indices of infected mice

Effect of Plant Fractions on Kidney Function of Infected Mice

The effect of plant fractions on kidney function of infected mice is shown in Table 4.39. Significant difference was observed in all parameters for evaluating kidney function of experimental animals except for total bilirubin (TB), direct bilirubin (DB) and bicarbonate whose values remained similar to that of the control group. Decrease in creatinine (5.12±0.14) and sodium (113.44±1.68) level was observed in animals treated with ethyl acetate fraction of *Khaya senegalensis* at 300mg/kgbw concentration. Significant increase in urea value (37.76±0.85) was observed in animals with n-Hexane fraction of *Anogeissus leiocarpus* at 100mg/kgbw concentration. Potassium levels significantly increased in animals treated with aqueous and ethyl acetate fraction of *Anogeissus leiocarpus* and *Khaya senegalensis* respectively. Finally, Chloride level decreased in animals treated with Ethyl acetate fraction of *Khaya senegalensis* at all concentration and n-Hexane fraction of *Anogeissus leiocarpus* at 100mg/kgbw concentration.



Keys: TB –Total Bilirubin DB –Direct Bilirubin
Values are presented as mean ± standard error of mean (SEM) of three replicates.

Figure 11: Effect of Plant Fractions on Kidney Function of Infected Mice

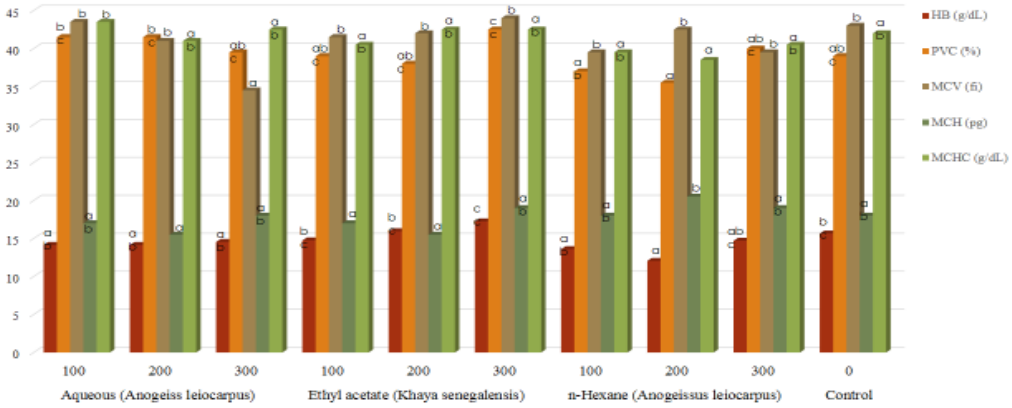
Effect of Plant Fractions on Haematological Parameters of Infected Mice

The effect of plant fractions on Haematological parameters of infected mice is shown in Table 4.40 and 4.41. Analysis of haematological parameters (Packed Cell Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration) in test animals treated with various concentrations of Aqueous and n-Hexane fraction of *Anogeissus leiocarpus* and Ethyl acetate fraction of *Khaya senegalensis* did not differ significantly from those of the control animals. Significant difference was observed for other parameters, including a significant decrease in Haemoglobin level (12.05±1.07) in animals treated with n-Hexane fraction of *Anogeissus leiocarpus* at 200mg/kg bw, decrease was also observed in Mean Cell Volume (34.50±1.44), Platelet Count (131.50±1.44), and Leukocytes

(33.00±1.73) in animals treated with Aqueous fraction of *Anogeissus leiocarpus* at 300, 300 and 100mg/kg bw concentration respectively. An increase in Neutrophils levels (46.00±1.73) and Total White Blood Cell Count (8.00±0.12) was observed in animals treated with ethylacetate fraction of *Khaya senegalensis*.

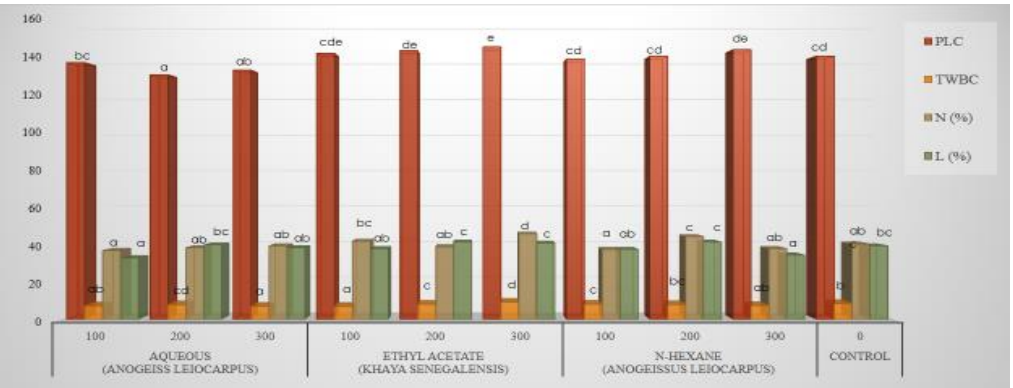
Histological Analysis of the Effect of Plant Extracts on the Liver and Kidney of Mice

The histology of the liver and kidney of infected mice administered with *Anogeissus leiocarpus* and *Khaya senegalensis* fractions at 100, 200 and 300 mg/kg/bw dosages are shown in plate II - VII. The liver sections of the all the experimental animals show largely preserved architecture, composed of cords of normal hepatocytes, normal portal tracts and central vein. As such no features of acute or chronic damage were observed. Similarly, the kidney showed renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. There are no features of acute or chronic damage.



Keys: HB –Haemoglobin PVC – Packed cell volume MCV – Mean cell volume MCH –Mean cell haemoglobin MCHC –Mean cell haemoglobin concentration.
Values are presented as mean ± standard error of mean (SEM) of three replicates.

Figure 12: Effect of Plant Fractions on haematological Indices of infected mice



Keys: PLC – TWBC – Total white blood cell N –Neutrophil L-Lymphocytes
Values are presented as mean ± standard error of mean (SEM) of three replicates.

Figure 13: Effect of Plant Fractions on WBC Indices of Infected Mice

Histophatology results

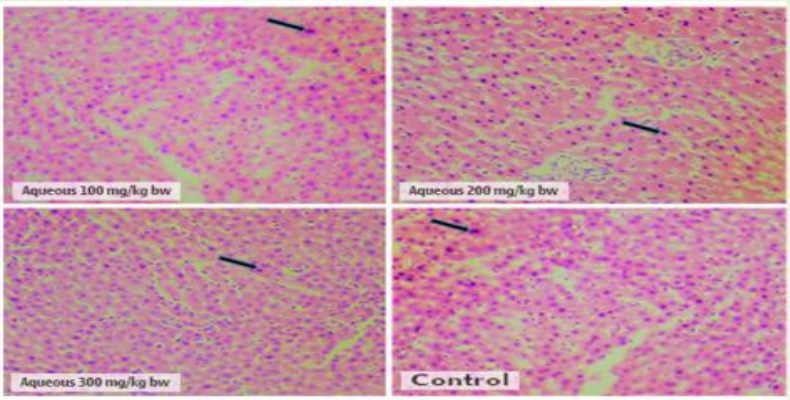


Plate I: Photomicrograph of kidney section of infected mice treated with aqueous fraction of *Anogeissus leiocarpus* at 100, 200 and 300mg/kg bw concentrations. All sections shows renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. There are no features of acute or chronic damage.

Black Arrow; Glomeruli, Blue Arrow; Capsular space

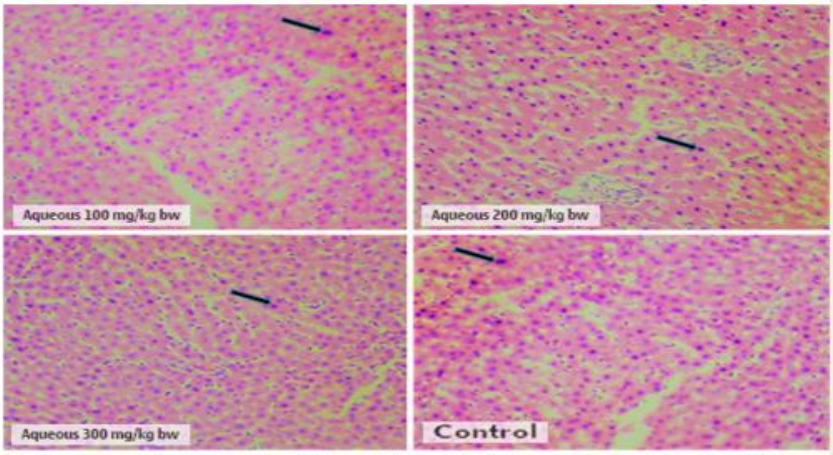


Plate II: Photomicrograph of a liver section of infected mice treated with aqueous fraction of *Anogeissus leiocarpus* at 100, 200 and 300mg/kg bw concentrations. All sections show hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. There are no features of acute or chronic damage.

Black Arrow; Hepatocyte

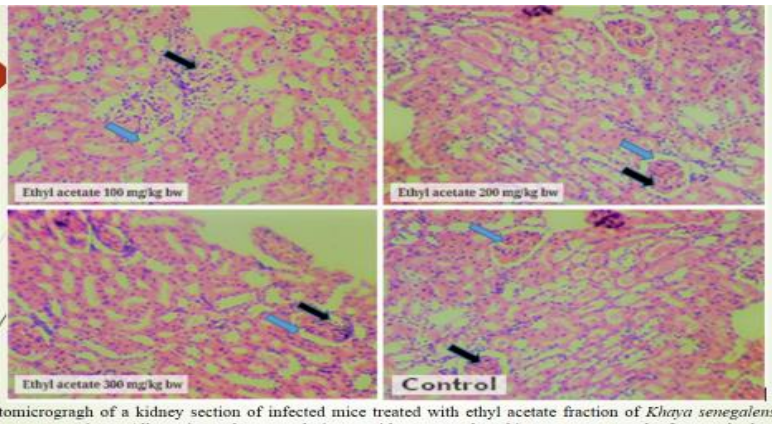


Plate III: Photomicrograph of a kidney section of infected mice treated with ethyl acetate fraction of *Khaya senegalensis* at 100, 200 and 300mg/kg bw concentrations. All sections show renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. There are no features of acute or chronic damage.

Black Arrow; Glomeruli,

Blue Arrow; Capsular space

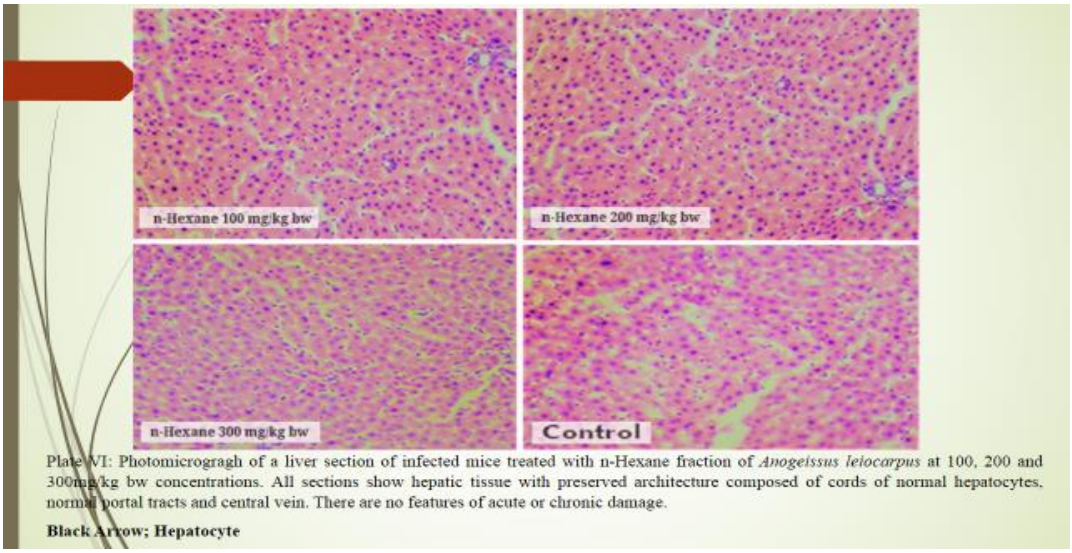


Plate VI: Photomicrograph of a liver section of infected mice treated with n-Hexane fraction of *Anogeissus leiocarpus* at 100, 200 and 300mg/kg bw concentrations. All sections show hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. There are no features of acute or chronic damage.

Black Arrow; Hepatocyte

DISCUSSION

Antibacterial Activity of crude plant extracts against Test Organisms

Anogeissus leiocarpus, *Vernonia amygdalina*, *Poliostigma thoningii*, *Carica papaya*, *Acasia nolitica* and *Khaya senegalensis* are well-known medicinal plants with health benefit against many ailments (Ahmad and Wudil, 2013; Ibrahim and Islam, 2013.) Extracts from these plants used in this study exhibited varying degree of antibacterial activity against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky*. *Anogeissus leiocarpus* and *Khaya senegalensis* were noted to be the most active among the six plant extracts tested, as they showed significantly higher zones of inhibition at all concentrations against all the test organisms.

The efficacy of the plant extracts evaluated was concentration dependent; as the antimicrobial activity increases with increasing concentration of the plant extracts, indicated by the increasing diameter of each inhibition zone. The observed inhibitory activity of the plant extracts against these diarrhoea causing pathogens validates the use of the plant for the treatment of diarrhoea in traditional medicine. This result is consistent with findings reported by Doughari (2012), Mann *et al*. (2014), Kuta *et al*. (2015), Abdallah *et al*. (2016), Ali *et al*. (2017), Chukwunonye *et al*. (2017), , Dayok *et al*. (2018), Evbuomwan *et al*. (2018), Musah, (2019) and Chomini *et al*., (2020) with few exceptions. They all reported varying ranges of inhibitory effect of these plant extracts against various bacterial pathogens at various concentrations. According to Kuta *et al*. (2015) leaf and stem extracts of *Khaya senegalensis* showed inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Escherichia coli* at concentrations ranging from 400 to 1000mg/mL.

Similarly, Abdallah *et al*. (2016) reported leafs and stem extracts of *Khaya senegalensis* to have inhibitory activity against diarrheal/stool isolates (*E. coli*, *Shigella* spp. and *Salmonella* sp.). It has been observed that matured leaf extract of *Poliostigma thoningii* showed antibacterial activity against *E. coli* and *S. enterica Typhi* at 10 and 5mg/cm3 concentrations but had no activity against *P. aeruginosa*. From the study of Evbuomwan *et al*. (2018) *Vernonia amygdalina* leaf extract showed significant antibacterial activity against multidrug resistant bacterial isolated namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at various concentrations ranging from 25 to 200mg/mL.

Mann *et al*. (2014) and Ali and Bukar (2018) both observed that stem bark extract of *Anogeissus leiocarpus* showed inhibitory effect against multidrug resistant *Staphylococcus aureus* and food pathogen of *Hibiscus sabdariffa calxy* (Zobo) drink respectively. Antimicrobial activity of these plant extracts is usually attributed to the phytochemical constituent they possess. Although presence and roles of phytochemicals were not investigated in this study, previous studies have shown Tannins, Flavonoids, Saponins and Alkaloids which are usually reported to be present in these plants to have antibacterial properties (Maria *et al*., 2009; Bakri *et al*., 2010; Mohamed et al., 2010; Harouna *et al*., 2012; Mann *et al*., 2014; Abalaka *et al*., 2016; Salih *et al.,* 2020; Dayok *et al*., 2018; Evbuomwan *et al*., 2018; Eguchi *et al*., 2019).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of Crude Extracts of *Anogeissus leiocarpus* and *Khaya senegalensis* against test isolates

The minimum inhibitory concentration (MIC) is the smallest concentration that visibly inhibits growth. The MIC is useful in determining the smallest effective dosage of a substance against an organism (Kuta *et al*., 2015). The MIC of *Anogeissus leiocarpus* against *Vibrio choleraee, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* was observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL respectively, while the minimum bactericidal concentration (MBC) was observed at 1.09±0.87, 0.96±0.05 and 1.09±0.17mg/mL respectively. For *Khaya senegalensis* MIC was detected at 0.96±0.04, 0.96±0.05 and 1.80±0.17 mg/mL concentration, while MBC was recorded at 1.02±0.12, 1.40±0.29 and 1.92±0.23 mg/mL concentration. Plant extract are considered to have a good inhibitory activity, if they present MIC value ≤ 100mg/mL, a moderate inhibitory activity when MIC ranges between 100 to 500mg/mL, a weak inhibitory activity if MIC value ranges between 500 to 1000mg/mL and no inhibitory activity when > 1000mg/mL.

Considering this report, the MIC and MBC values recorded from the antibacterial activity of the present study showed good inhibitory activity. The current findings lend credence to the traditional use of this plant as medicines for infectious diseases particularly those caused by the test organisms susceptible to the extracts. However, this result is contrary to the findings of Ugoh *et al*. (2014), Kuta *et al*. (2015), Abdallah *et al*. (2016), Ali *et al*. (2017), and Sadiku *et al*. (2020), who all reported much higher MIC and MBC values against various bacterial pathogens. Ugoh *et al*. (2014) observed methanolic and ethanolic stem bark extracts of *Khaya senegalensis* to have an MIC value of 250 and 200mg/mL respectively against *Salmonella enterica* subsp. enterica serovar Typhi. MIC value of 25mg/ml was reported by Sadiku *et al*. (2020) for methanolic stem bark extract of *Khaya senegalensis* against both *Klebsiella pneumoniae* and a carbapenem resistant strain of *Escherichia coli.*

In Kuta *et al*. (2015) studies, aqueous and ethanolic leaf extract of *Khaya senegalensis* had MIC values of 400 and 200mg/mL respective against *Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Escherichia coli.* The study also reported MBC values 0f 800 and 400mg/mL for aqueous and ethanolic leaf extract of *Khaya senegalensis* against same organisms respectively. Ali *et al.* (2017) studied the antimicrobial activity of methanolic stems bark extracts of *Anogeissus leiocarpus* against several bacterial isolates, the study reported MIC and MBC values of 10 and 40 mg/mL for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus feacalis* and *Corynebacterium ulcerans.* While for *Staphylococcus aureus* MIC and MBC was detected at 10 and 40 mg/mL. In Dayok *et al*. (2018) studies on the antimicrobial activity of *Anogeissus leiocarpus* leaf extract on clinical isolates.

For the aqueous extract MIC was detected at 100, 200 and 100 mg/mL, while the ethanolic extract had MIC values at 200 400 and 200mg/mL against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus mutans* respectively. While the MBC was detected at 400mg/mL for both aqueous and ethanolic extract against all the isolates. The variation in the MIC and MBC values reported in these studies could be due to the phytochemical composition of their respective extracts which is usually dependent on the polarity of the solvents used for extraction, as solvents type tend to influence the kind of bioactive compound released from the plant materials. Also, variation in results could be attributed to the genetic make-up of each test organisms used. Different organisms have been shown to respond differently to different and same concentrations of a specific antimicrobial substance (Altemimi *et al*., 2017; Sadiku *et al.,* 2020).

Yields of partitioned fractions of Active Crude Extracts

Fractionation of *Anogeissus leiocarpus* and *Khaya senegalensis* extracts using different solvents had varying yields. For *A. leiocarpus* the highest yield was

observed in n-hexane (0.64g) followed by aqueous (0.57g) and then ethyl-acetate (0.25g). Similarly, n-hexane had the highest yield (0.46g) in *Khaya senegalensis* followed by ethyl-acetate (0.28g) and then aqueous (0.25g). Similar result was reported by Senjobi *et al*. (2017) who also observed from their study that Hexane extracts demonstrated highest extraction yield. From the result it is evident that the recovery yield is dependent on the type of solvent used and its polarity. In the current research three solvents with different polarity were used, and they can be arranged as follows starting from the more nonpolar solvent (n-Hexane < Ethyl-acetate <Ethanol). Obtained results showed that yield generally increased with decreasing polarity of solvents.

Antibacterial Activity of *Anogeissus leiocarpus* and *Khaya senegalensis* fractions

Significant difference was observed in the antibacterial activity of *Anogeissus leiocarpus* and *Khaya senegalensis* fractions at 300mg/ml. All *Khaya senegalensis* fractions (74.00±1.73 74.00±1.56) and *Anogeissus leiocarpus* n-Hexane fraction (74.00±0.69 19.33±1.11) had significantly higher activity against *Vibrio cholerae,* while the *Anogeissus leiocarpus in* Ethyl acetate fraction showed no activity. Against *Klebsiella pneumoniaee* significant activity was observed for *Khaya senegalensis* Ethyl acetate fraction. While against *Salmonella enterica* serovar *Kentucky* highest activity was observed in the aqueous fraction of *Anogeissus leiocarpus* and none of the *Khaya senegalensis* fraction was active against *Salmonella.* The variation in activity of these plant extracts could be attributed to the differences in phytochemical constituents present and concentration of the extract used (Doughari, 2012). Angienda *et al*. (2010), also stated that each bacteria specie could have difference in susceptibility levels to particular inhibiting compounds due to them possessing certain structures and properties such capsules and efflux pumps and reduced cell permeability. Normally the cell envelopes of gram-negative bacteria are more complex than the cell wall of gram-positive bacteria. Gram-negative bacteria are composed of two layers that protect the cell and provide rigidity. Similar finding was reported by Abdallah *et al*. (2016) who worked on the Antibacterial activity and phytochemical screening of leaf and stem (bark) extract of *Khaya senegalensis* against Diarrhoeal stool isolates.

Antibacterial Activity of the *Khaya senegalensis* ethylacetate Column chromatography fractions

The antibacterial Activity of *Khaya senegalensis* ethyl acetate column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* was concentration dependent as inhibition

zones increases with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio choleraee* was observe in fraction 7 (30.00±1.15) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 5 (24.00±1.73) at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 6 (32.00±2.31) 200mg/mL concentrations. The variation in antibacterial activity of these fractions could be attributed to the differences in active component present in each fraction and and the concentration of fractions. Also, difference in susceptibility of each bacteria to the fractions could contribute to such variations (Angienda *et al*., 2010).

Antibacterial Activity of the *Anogeissus leiocarpus* n-hexane Column chromatography fractions

Antibacterial Activity of *Anogeissus leiocarpus* n-Hexane Column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* is showed significant difference in the zones of inhibition. Antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio choleraee* was observe in fraction 2 (22.67±0.75) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 2 (24.00±0.58), fraction 3 (25.67±1.11), and fraction 4 (24.00±1.73) all at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 1 (24.00±1.39, 24.00±0.75 and 26.00±1.33) at 50, 100 and 200mg/mL concentrations and fraction 4 (24.00±1.27) at 200ml/ml concentration. Variation in antibacterial activity of these fractions could mostly be attributed to the differences in active component present in each fraction and the difference in susceptibility of each bacteria to the fractions (Angienda *et al.,* 2010).

Antibacterial Activity of the *Anogeissus leiocarpus* Aqueous Column chromatography fractions

Significant difference was observed in the zones of inhibition. Antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. Fraction 1-4 showed no activity against *Vibrio cholerae*. Significantly highest activity against *V. cholerae* was observed in fraction 6 (42.00±1.27) at 200mg/mL concentration. Against *Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* Fraction 1-5 showed no significant activity. Highest significant activity against *Klebsiella pneumoniaee* was observed in fraction 6 (32.00±1.15), fraction 7 (32.00±1.15) and fraction 9 (32.00±1.15) all at 200mg/mL concentration. While for *Salmonella enterica*

fraction 8 (32.00±1.15) at 200mg/mL was the highest observed. Variation in antibacterial activity of these fractions could mostly be attributed to the differences in active component present in each fraction and the difference in susceptibility of each bacteria to the fractions (Angienda *et al*., 2010).

Synergistic effect of aqueous column chromatography fractions

The synergistic antibacterial effect of the aqueous column chromatography fractions of *Anogeissus leiocarpus* and *Khaya senegalensis* against *Vibrio cholerae, Klebsiella pneumoniaee* and *Salmonella enterica* serovar *Kentucky* showed significant activity. The observed antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. Significantly highest antimicrobial activity against for all test organisms was observed at 200mg/mL concentration. According to Karmegam *et al*. (2012) plant extracts in combination of two or more exhibit effective **antimicrobial activity** against a wide range of microorganisms including drug resistant bacteria. The increase antibacterial activity is mostly pinned to the combine effect of the phytochemical and active components of the plant extracts against the test organisms (Rachuonyo *et al*., 2016)

Fourier Transformed Infrared Spectroscopy

FTIR analysis of the fractions of the F6 Aqueous fraction of *Anogeissus leiocarpus* and Ethyl Acetate *Khaya senegalensis* showed varioius functional groups to be present at varying peaks. The result of the analysis showed the extracts contains phytochemicals with different functional groups including OH hydroxyl group, Cyclo alkane, Phenol rings, Methyl group (CH_3), Aromatic ring, Ester carboxyl, Alkanes, C-O-C group. A similar study by Usman *et al*., (2020) who worked on the phytochemical and antimicrobial studies of stem-bark extracts of *Anogeissus leiocarpus* found in dutsin-ma, katsina-nigeria. They reported that FTIR spectroscopic analysis of the plant extract showed characteristic peaks of alcohols, ethers, esters, carboxylic acids, aldehydes, ketones and amines groups. The presence of various functional groups such as those observed in this study has been corroborated by various authours including Adigun and Kelly, (2001), Rao *et al*., (2016) and Oladele *et al*., (2021). This presences of these fuctional groups usually corroborates the presence of phytochemical compounds in the extract.

***In-vivo* Antibacterial Susceptibility of Aqueous *Anogeissus leiocarpus* and ethyl acetate *Khaya senegalensis* Fractions**

The in vivo assay showed that the ethylacetate fraction of *Khaya senegalensis* had higher activity than aqueous fraction of *Anogeissus leiocarpus*. The dose

concentration of 300mg/ml of the extracts had the highest colonies reduction for all the test organisms, suggesting that as the dose of the extracts increases the numbers of colonies reduced. There was no significant difference when concentrations used was compared, this could be attributed to the fact that antimicrobial activities of substance is a function of active compounds reaching an organism (Yunana *et al*., 2018). When treatment groups were compared with negative control, there was significant difference between the treatment groups and the negative control suggesting, that plant extracts may have antibacterial activity and could be employed in the treatment of infectious diseases.

Effect of Plant Fractions on Body Weight Gain of infected mice

Change in bodyweight is a sensitive and predictive toxicity marker (Reduan *et al*., 2021). Monitoring the body weight during treatment provides a fair index of the general health status of experimental animals (Daniel *et al.,* 2020). The obtained results for the effect of *Anogeissus leiocarpus* and *Khaya senegalensis* fractions on bodyweight of experimental animals indicates that there was no significant difference ($p < 0.05$) in bodyweight of all test animals when compared to the control after exposure to various doses of the extracts. Similar finding was observed by Onu *et al*. (2013), Oyelowo *et al*. (2015). Changes in bodyweight have often been used as an indicator of toxicity of substances; increase in bodyweight is an indication of inflammation, while reduction in the same parameter can be attributed to cellular constriction (Adomi *et al*., 2017). As such, lack of any significant change in bodyweight as observed in this study mean that the animals were in good physical state which is a likely indication that the extracts were non-toxic.

Effect of Plant Fractions on Liver function of infected mice

The liver is a major target organ for xenobiotic metabolism (Liman and Atawodi, 2015). Liver integrity is often determined by measuring serum biochemical parameters such as Total protein, Albumin, Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) (Mobolade *et al*., 2019). Significant difference ($p<0.05$) was observed in liver parameters in this study. Aspartate transaminase (AST) value decreased in all experimental animals at all concentrations while Alanine transaminase (ALT) only decreased in experimental animals treated with aqueous fraction of *Anogeissus leiocarpus* at 300mg/kg bw. Significant decrease was also observed in alkaline phosphatase (ALP) values in some experimental groups when compared to control, while an increase was observed in total protein and albumin values.

Aminotransferases (ALT and AST) are markers of hepatocellular damage and injury to the liver, which is signified by their increase in serum concentrations

(Aragon and Younossi, 2010; Ojiako *et al.,* 2017; Elhusain *et al.,* 2019). Similarly, alkaline phosphatase is also an enzyme found in hepatocytes, it is involved in the transportation of ions across membranes and hydrolysis of phosphate. It is a biomarker of the integrity of plasma membranes the cells and endoplasmic reticulum (Biobaku *et al.,* 2014; Salau *et al*, 2018). The decreased ALP levels observed in the present study may be attributed to inhibition and/or *in situ* inactivation as a result of the presence of secondary metabolites in the extract, prominent among which may be saponins and tannins; which are known to chelate metal ions and precipitate proteins (Akanji *et al*., 2013). The reduction in ALP levels may also be attributed to increased plasma clearance of the enzyme (Salau *et al,* 2018). In this study the observed decrease in serum enzymes AST, ALT and ALP values could indicate that the extract had no negative effect on liver cells, which is further confirmed by the normal liver histology observed in experimental animals.

In fact, various studies have reported extracts of *Anogeissus leiocarpus* and *Khaya senegalensis* to have hepatoprotective properties, mainly due to the antioxidant property of its high content of flavonoids, tanins, sterols and triterpens (Latha *et al*., 2003; Barku *et al.,* 2013, Ali *et al*., 2019 and Elhusain *et al*., 2019). Similar finding was reported by Ahmad and Wudil, (2013) in their study on the toxicological effect of aqueous stem bark extract of *Anogeissus leiocarpus* in rats; they reported significant decrease in AST, ALT and ALP values. Similarly, Salau *et al* (2018) who researched on the effect of root extract of *Anogeissus leiocarpus* on liver indices of male rats also observed a decrease in all these parameters. Adamu *et al*. (2022) reported a decline in serum ALT level in rats treated with methanol stem bark extracts of *Anogeissus leiocarpus*. Biobaku *et al*. (2014) also observed significant decrease in liver enzymes in experimental animals treated with aqueous extract of *Khaya senegalensis.*

The decrease in ALT, and AST levels could be attributed to decreased enzyme synthesis, enhanced inhibition of the enzyme molecules, and/or increased plasma clearance. This may adversely affect amino acid and energy metabolism, because both processes are linked via the aminotransferases (Salau *et al*, 2018). Levels of total protein and albumin in the serum can also be used as biomarkers of synthetic function of the liver (Yakubu and Musa, 2012; Salau *et al*., 2015). Albumin and proteins involved in secondary haemostasis and fibrinolysis are exclusively synthesized by the liver, and their plasma concentrations are, therefore, used as indirect indicators of liver synthetic function (Oduola *et al*., 2018). A significant increase ($p < 0.05$) in plasma albumin and total protein were observed in rats administered with *Anogeissus leiocarpus* and *khaya senegalensis* extracts when compared to the control group. This observation corroborates findings from other studies, Salau *et al*. (2018) reported significant increase in

Total protein values while Albumin level were not affected. Elevated concentration of total protein and albumin may indicate impaired synthetic function of the liver (Owolarafe *et al*., 2017).

Effect of Plant Fractions on Lipid Profile of infected mice

Significant differences (P<0.05) was observed in the total cholesterol (TC) levels of experimental animals treated, increase in (TC) was observed in animals treated with n-Hexane fraction of *Anogeissus leiocarpus* at all concentration, while a decrease was observed only in animals treated with Ethyl acetate fraction of *Khaya senegalensis* at 300 mg/kg bw concentration. The observed decrease in total cholesterol values suggests that *Khaya senegalensis* extracts may have affected cholesterol biosynthesis which resulted to reduction in the level of cholesterol in the blood. Similar findings were reported by Muhammad *et al*. (2015). No significant change in Triglycerides value was observed in all treatment groups except in groups treated with Ethylacetate fraction of *Khaya senegalensis* at 200 and 300 mg/ kg bw where a decrease in Triglycerides values was noted. Significant increase (P<0.05) was observed in High density lipoprotein (HDL) values of some treatment groups, while no significant change (P<0.05) was observed in low density lipoprotein (LDL) in all treatment groups when compared to the control.

Effect of Plant Fractions on Kidney Function of infected mice

Albumin and bilirubin concentrations indicate the secretory and synthetic functions of the liver and can be used to ascertain types of liver damage. Bilirubin is the major breakdown product from physiologic destruction of senescent red blood cells. It is removed from the blood by the liver; hence it is a good indicator of liver function. Bilirubin concentration is elevated in the blood either due to increased production or decreased liver uptake. Result from this study showed a significant ($p < 0.05$) decrease in the total and conjugated bilirubin concentrations in rats administered with Aqueous and n-Hexane fraction of *Anogeissus leiocarpus* and Ethyl acetate fraction of *Khaya senegalensis* at all concentrations when compared with the control. This observation corroborates the findings of Ojo *et al.* (2013) and suggests that the extract had no adverse effect on the liver.

Effect of Plant Fractions on Haematological parameters of infected mice

Analysis of haematological parameters (Packed Cell Volume,(PCV) Mean Cell Haemoglobin, (MCH) and Mean Cell Haemoglobin Concentration,(MCHC) in test animals treated with various concentrations of Aqueous and n-Hexane fraction of *Anogeissus leiocarpus* and Ethyl acetate fraction of *Khaya senegalensis* did not differ significantly from those of the control animals. Significant difference was

observed for other parameters, including a significant decrease in Haemoglobin level (12.05±1.07) in animals treated with n-Hexane fraction of *Anogeissus leiocarpus* at 200mg/kg bw, decrease was also observed in Mean Cell Volume (34.50±1.44), Platelet Count (131.50±1.44), and Leukocytes (33.00±1.73) in animals treated with Aqueous fraction of *Anogeissus leiocarpus* at 300, 200 and 100mg/kg bw concentration respectively. Findings akin to those in this study were also reported by Agaie *et al*. (2007), Kolawole *et al.* (2011) and Cyril-Olutayo *et al.* (2013). Kolawole *et al.* (2011) In their study, they reported that the active principles of plant extracts could cause toxic effects. This is especially so when it is administered repeatedly as in the present study. They suggested that prolong use of the plant extract could result in hemolytic effect. An increase in Total White Blood Cell Count (8.00±0.12) and Neutrophils levels (46.00±1.73) was also observed in animals treated with Ethylacetate fraction of *Khaya senegalensis.* The white blood cells and neutrophils, constitute part of the defense mechanism of the body system. Increase in leukocyte production is indicative of either infection, injury or toxic substance in the body (Adeyemo-Salami and Ewuola, 2015).

Histological Analysis of the Effect of Plant Extracts on the Liver and Kidney of Mice

The histology of the liver and kidney of infected mice administered with *Anogeissus leiocarpus* and *Khaya senegalensis* fractions at 100, 200 and 300mg/kg/bw dosages show largely preserved architecture, composed of cords of normal hepatocytes, normal portal tracts and central vein. No features of acute or chronic damage were observed. Similarly, the kidney showed renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. Similar to the liver no features of acute or chronic damage. Similar finding was reported by Agaie *et al*. (2007), Arbab, (2014), Elagib *et al*. (2014) and Motto *et al*. (2020) who all report that histopathological sections of the live and kidney presented a normal morphological appearance. This signifies that the plant extracts did not cause any detrimental changes and had no toxic effect at doses used in the study.

GC-FID Analysis of the Most Active Column Chromatogram Fraction (Fraction 6) of Acqeous *A. leiocarpus* against Diarrhoea causing Pathogens

The result of GC-FID analysis of Acqeous cholumn chromatogram fractions of *Anogeissus leiocarpus* identified thirteen (13) compounds with significant spectrum of activities against tested organisms (i.e *Vibrio choleraee, Klebsiella pneumoniaee* and *Salmonella enterica* serovar). The various compounds identifies

are: Ellagic acid with retention time 3.933, area of 543.1070 and height of 94.964. Gallic Acid with retention time 4.233, area of 1427.3470 and height of 99.019. Flavellagic Acid with retention time 5.266, area of 512.9120 and height of 36.945. Ampelopsin with retention time 5.500, area of 3757.9410 and height of 56.481. Quercetin with retention time 7.783, area of 1434.3990 and height of 71.907. Colilagin with retention time 9.183, area of 358.1350 and height of 15.976. Anoli gnan A with retention time 9.666, area of 7492.0870 and height of 624.169. Anogeissinin with retention time 10.283, area of 4850.6325 and height of 289.059. Rutin with retention time 10.816, area of 4139.0310 and height of 73.372. Castalagin with retention time 11.850, area of 4434.4080 and height of 59.427. Pinosylvin with retention time 13.950, area of 1095.6320 and height of 23.537 and lastly Punicalagin with retention time 14.916, area of 320.0500 and height of 9.395. The compounds identified were arranged in increasing order of their retention time, effective from lowest to the highest. The compound with highest retention time is Punicalagin with retention time 14.916, and the compound with the least retention time is Ellagic acid with retention time 3.933. The antibacterial activity of the tested *Anogeissus leiocarpus* fraction aginst tested organisms may not be unconnected with the presence of the identified compounds

GC-FID Analysis of the Most Active Column Chromatogram Fraction (Fraction 8) of Acqeous *A. leiocarpus* against Diarrhoea causing Pathogens

The result of GC-FID analysis of aqeous cholumn chromatogram fraction of *Anogeissus leiocarpus* identified twelve (12) compounds with significant spectrum of activity against tested organisms (i.e *Vibrio choleraee, Klebsiella pneumoniaee* and *Salmonella enterica* serovar). The various compounds identifies are: Ellagic Acid with retention time 3.933, area of 522.3270 and height of 92.283. Gallic Acid with retention time 4.233, area of 950.1220 and height of 81.645. Flavellagic Acid Acid with retention time 5.266, area of 423.0820 and height of 32.681. Ampelopsin with retention time 5.500, area of 2192.2490 and height of 52.922. Quercetin with retention time 7.783, area of 790.5830 and height of 68.282. Colilagin with retention time 9.183, area of 331.6100 and height of 15.212. Anolignan A with retention time 9.666, area of 6825.2820 and height of 611.492. Anogeissinin with retention time 10.283, area of 3378.1435 and height of 251.660. Rutin with retention time 10.816, area of 613.6120 and height of 24.169. Castalagin with retention time 11.883, area of 1301.9240 and height of 23.098. Pinosylvin with retention time 13.950, area of 209.7960 and height of 6.258. Punicalagin with retention time 14.916, area of 153.3210 and height of 7.388. The active compounds identified in this fraction 8 were arranged in an increasing order of their retention time, effective from the compound with least retention time to the one with the

highest retention time. The compound with highest retention time here is Punicalagin with retention time 14.916 the later with the least retention time is Ellagic Acid with retention time 3.933

GC-FID Analysis of the Most Active Column Chromatogram Fraction (Fraction 5) of Ethylacetate of *K. Senegalenses* against Diarrhoea causing Pathogens

The result of GC-FID analysis of Ethylacitate of *Khaya senegalenses* identified thirteen (13) compounds with significant spectrum of activity against tested organisms (i.e *Vibrio choleraee, Klebsiella pneumoniaee* and *Salmonella enterica* serovar). The various compounds identifies are: Scopoletin with retention time 3.300, area of 1866.7590 and height of 50.892. Scoparone with retention time 4.033, area of 971.0510 and height of 44.520. Khayanolide A with retention time 5.016, area of 9219.9410 and height of 558.807. Khayalactol with retention time 6.450, area of 1266.9690 and height of 35.565. Khayanone with retention time 7.150, area of 1423.7230 and height of 147.642. Khayanoside with retention time 7.533, area of 4546.1560 and height of 240.555. Khayanolide B with retention time 7.816, area of 4955.4895 and height of 333.290. 2-Hydroxyseneganolide with retention time 8.600, area of 5297.6940 and height of 435.588. Seneganolide with retention time 9.200, area of 19589.3280 and height of 1814.355. Oleuropein with retention time 11.016, area of 457.6980 and height of 29.167. Aesculetin with retention time 11.483, area of 1217.7470 and height of 77.232. Beta-Gurjunene with retention time 12.550, area of 195.5790 and height of 13.366. Calicedrin with retention time 14.566, area of 166.3655 and height of 15.596. The compounds identified were arranged in increasing order of their retention time, effective from lowest to the highst. The compound with highest retention time is Calicedrin with retention time 14.566, and the compound with the least retention time is Scopoletin with retention time 3.300. The antibacterial activity of the tested Ethylacetate of *K. Senegalenses* against Diarrhoea causing Pathogens may not be unconnected with the presence of the idntified compounds

GC-FID Analysis of the Most Active Column Chromatogram Fraction (Fraction 7) of Ethylacetate of *K. Senegalenses* against Diarrhoea causing Pathogens

The result of GC-FID analysis of Ethylacetate of *Khaya senegalenses* identified thirteen (13) compounds with significant spectrum of activity against tested organisms (i.e *Vibrio choleraee, Klebsiella pneumoniaee* and *Salmonella enterica* serovar). The various compounds identifies are: Scopoletin with retention time 3.300, area of 727.4040 and height of 25.173. Scoparone with retention time 4.033, area of 241.8820 and height of 12.606. Khayanolide A with retention time 5.016,

area of 5796.0560 and height of 518.588. Khayalactol with retention time 6.450, area of 193.8350 and height of 10.860. Khayanone with retention time 7.150, area of 1080.0365 and height of 125.736. Khayanoside with retention time 7.533, area of 3843.1435 and height of 213.776. Khayanolide B with retention time 7.816, area of 3715.6255 and height of 302.876. 2-Hydroxyseneganolide with retention time 8.600, area of 4763.1435 and height of 422.149. Seneganolide with retention time 9.200, area of 17970.0910 and height of 1785.996. Oleuropein with retention time 11.016, area of 286.9850 and height of 22.165. Aesculetin with retention time 11.483, area of 571.2110 and height of 62.241. Beta-Gurjunene with retention time 12.550, area of 147.6840 and height of 12.220. Calicedrin with retention time 14.566, area of 136.7030 and height of 14.558. The compound with highest retention time is Calicedrin with retention time 14.566, and the compound with the least retention time is Scopoletin with retention time 3.300. The antibacterial activity of the tested Ethylacetate of *K. Senegalenses* against Diarrhoea causing Pathogens may not be unconnected with the presence of the identified compounds

Conclusion

Various diarrhea causing pathogens were isolated from stool samples of patient attending General Hospital, Minna. They include *Salmonella enterica* serovar *Kentucky* strain Medellin Colombian, *Klebsiella pneumoniaee* strain IAUK 8738 and *Vibrio choleraee* strain RP01.

Crude extract of the six plants showed varying degree of antimicrobial activity against the test organisms, with *Anogeissus leiocarpus* and *Khaya senegalensis* been the most active.Various active fractions were identified from the partitions of *Anogeissus leiocarpus* and *Khaya senegalensis*. The active fractions showed varying degree of antibacterial activity at various concentrations. Synergy of the four most active column fractions showed significant activity against the test organisms.

The FTIR spectra of *Anogeissus leiocarpus* aqueous fractions and Khaya senegalensis ethyl acetate fractions are showed various functional groups present including the OH hydroxyl group, Cycloalkane, Phenol rings, Methyl group (CH3-), Aromatic ring, C-O-C group, Carboxyl compounds, Alkanes etc. The in-vivo Antibacterial activity of the Aqueous *Anogeissus leiocarpus* and Ethyl acetate Khaya senegalensis fractions showed significant activity on the test organisms. Therefore, the results of toxicological studies showed that *Anogeissus leiocarpus* and Khaya senegalensis fractions had no detrimental effect on the test animals.

Recommendation

- The studied medicinal plants are recommended for use as antimicrobial agents in the treatment of infectious diseases caused by dirrhoea causing pathogens.

- However, uncontrollable use of the extracts without adequate dose regulation should be discouraged.
- Further studies are required to assess bioactive compounds in the studied plants against other dirrhoea causing pathogens.
- There is also a need to study the in-vivo antifungal activity of these plants using laboratory animals, for reassurance in medical used.
- Special toxicity studies such as teratogenic and genotoxic are also important to be carried out for reassurance in medical uses

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